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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/141,769	01/08/2019	10174113	3338.0330001/ELE/C-K	6926

115018 7590 12/19/2018

Sterne, Kessler, Goldstein & Fox P.L.L.C.
1100 New York Avenue NW
Washington, DC 20005

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 212 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

Arvin YANG, Princeton, NJ;
Bristol-Myers Squibb Company, Princeton, NJ

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

Amgen Exhibit 1002
Amgen v. BMS

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Alexandria, Virginia 22313-1450
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/141,769	04/28/2016	Arvin YANG	3338.0330001/ELE/C-K	6926

115018 7590 11/20/2018

Sterne, Kessler, Goldstein & Fox P.L.L.C.
1100 New York Avenue NW
Washington, DC 20005

EXAMINER
OUSPENSKI, ILIA I

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
11/20/2018	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ckim@sternekessler.com
e-office@sternekessler.com
pduffy@sternekessler.com

UNITED STATES DEPARTMENT OF COMMERCE
U.S. Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450

APPLICATION NO./ CONTROL NO.	FILING DATE	FIRST NAMED INVENTOR/ PATENT IN REEXAMINATION	ATTORNEY DOCKET NO.
15/141,769	04/28/2016	YANG, Arvin	3338.0330001/ELE/

	EXAMINER	
Steme, Kessler, Goldstein & Fox P.L.L.C. 1100 New York Avenue NW Washington, DC 20005	ILIA I OUSPENSKI	
	ART UNIT	PAPER
	1644	20181115

DATE MAILED: _____

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner for Patents

Applicant's IDS filed on 10/19/2018 is acknowledged and has been considered.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ILIA I OUSPENSKI whose telephone number is (571)272-2920. The examiner can normally be reached 8:30 AM – 6 PM.
If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel Kolker can be reached at 571-272-3181. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.
Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ILIA I OUSPENSKI/ Primary Examiner, Art Unit 1644	

PTO-90C (Rev.04-03)

Approved for use through 07/31/2016. OMB 0651-0031
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Substitute for form 1449/PTO

THIRD SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG; Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author(in CAPITAL LETTERS),title of the article(when appropriate), title of the item (book,magazine,journal,serial,symposium,catalog,etc.),date,page(s),volume-issue number(s),publisher, city and/or country where published.	T[2]
	NPL47	GETTINGER, S.N., et al., "Overall Survival and Long-Term Safety of Nivolumab (Anti-Programmed Death 1 Antibody, BMS-936558, ONO-4538) in Pateints with Previously treated Advanced Non-Small-Cell Lung Cancer," Journal of Clinical Oncology 33(18): 2004-2012, American Society of Clinical Oncology, United States (June 2015).	☐
	NPL48	JOHNSON, D. B., et al., "Immune Checkpoint Inhibitors in NSCLC," Current Treatment Option in Oncology 15(4):658-669, SpringerLink, United States (2014).	☐
	NPL49	Office Action mailed October 3, 2018 in United States Patent Application No. 15/141,772, Yang, A et al., filed April 28, 2016, 17 pages.	☐
	NPL50	ZHAO, X., et al., "Assessment of nivolumab benefit-risk profile of a 240-mg flat dose relative to a 3-mg/kg dosing regimen in patients with advanced tumors," Annals of Oncology: Official Journal of the European Society for Medical Oncology 28(8):2002-2008, Oxford Journals, England (2017).	☐

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Examiner Signature	/ILIA I OUSPENSKI/	Date Considered	11/15/2018

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. 1 Applicant's unique citation designation number (optional). 2 Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /I.I.O/

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Substitute for form 1449/PTO

THIRD SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG; Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author(in CAPITAL LETTERS),title of the article(when appropriate), title of the item (book,magazine,journal,serial,symposium,catalog,etc.),date,page(s),volume-issue number(s),publisher, city and/or country where published.	T[2]
	NPL47	GETTINGER, S.N., et al., "Overall Survival and Long-Term Safety of Nivolumab (Anti-Programmed Death 1 Antibody, BMS-936558, ONO-4538) in Pateints with Previously treated Advanced Non-Small-Cell Lung Cancer," Journal of Clinical Oncology 33(18): 2004-2012, American Society of Clinical Oncology, United States (June 2015).	☐
	NPL48	JOHNSON, D. B., et al., "Immune Checkpoint Inhibitors in NSCLC," Current Treatment Option in Oncology 15(4):658-669, SpringerLink, United States (2014).	☐
	NPL49	Office Action mailed October 3, 2018 in United States Patent Application No. 15/141,772, Yang, A et al., filed April 28, 2016, 17 pages.	☐
	NPL50	ZHAO, X., et al., "Assessment of nivolumab benefit-risk profile of a 240-mg flat dose relative to a 3-mg/kg dosing regimen in patients with advanced tumors," Annals of Oncology: Official Journal of the European Society for Medical Oncology 28(8):2002-2008, Oxford Journals, England (2017).	☐

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Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. 1 Applicant's unique citation designation number (optional). 2 Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Electronic Patent Application Fee Transmittal

Application Number:	15141769
Filing Date:	28-Apr-2016
Title of Invention:	Treatment of PD-L1-Negative Melanoma Using an Anti-PD-1 Antibody and an Anti-CTLA-4 Antibody
First Named Inventor/Applicant Name:	Arvin YANG
Filer:	Ji Eun Kim/Kim Perry
Attorney Docket Number:	3338.0330001/ELE/C-K

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
UTILITY APPL ISSUE FEE	1501	1	1000	1000

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
SUBMISSION- INFORMATION DISCLOSURE STMT	1806	1	240	240
		Total in USD ($)		**1240**

Electronic Acknowledgement Receipt	
EFS ID:	34065578
Application Number:	15141769
International Application Number:	
Confirmation Number:	6926
Title of Invention:	Treatment of PD-L1-Negative Melanoma Using an Anti-PD-1 Antibody and an Anti-CTLA-4 Antibody
First Named Inventor/Applicant Name:	Arvin YANG
Customer Number:	115018
Filer:	Ji Eun Kim/Kim Perry
Filer Authorized By:	Ji Eun Kim
Attorney Docket Number:	3338.0330001/ELE/C-K
Receipt Date:	19-OCT-2018
Filing Date:	28-APR-2016
Time Stamp:	19:51:31
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$1240
RAM confirmation Number	102218INTEFSW19523500
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Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	NPL47_Gettinger.pdf	1601889 136763fe2485526ad40a6e684ccb64c3e86a3689	no	12
Warnings:					
Information:					
2	Non Patent Literature	NPL48_Johnson.pdf	1140923 75afa8216d8599e136d8fbabefcfe06f663b0134	no	12
Warnings:					
Information:					
3	Non Patent Literature	NPL49_OA_15141772_3Oct2018.pdf	673592 6fd84be109c61536ee9e28f0ea8eb87fde159cc4	no	17
Warnings:					
Information:					
4	Non Patent Literature	NPL50_Zhao.pdf	1231762 a9588b85034dfed813dfad2fa7369dd1912ce728	no	7
Warnings:					
Information:					
5	Issue Fee Payment (PTO-85B)	2018-10-19-Issue-Fee-3338-0330001.pdf	90497 c95250b6821d75f4dea117dd1f96b8fd5ffdc99b	no	1
Warnings:					
Information:					
6	Transmittal Letter	2018-10-19-3rd-Supp-IDS-3338-0330001.pdf	138282 e52177ae3b14b98d04631164627ff1cf10a5ec50	no	3
Warnings:					
Information:					

7	Information Disclosure Statement (IDS) Form (SB08)	2018-10-19-PTO-SB08-3338-0330001.pdf	120068 df555d7f855da5773481bf50f9d8fbc236a33dce	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
8	Fee Worksheet (SB06)	fee-info.pdf	32419 c1d82b559e9a50c5298061a0170e386347418e7a	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	5029432	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

115018 7590 10/19/2018
Sterne, Kessler, Goldstein & Fox P.L.L.C.
1100 New York Avenue NW
Washington, DC 20005

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/141,769	04/28/2016	Arvin YANG	3338.0330001/ELE/C-K	6926

TITLE OF INVENTION: Treatment of PD-L1-Negative Melanoma Using an Anti-PD-1 Antibody and an Anti-CTLA-4 Antibody

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1000	$0.00	$0.00	$1000	01/22/2019

EXAMINER	ART UNIT	CLASS-SUBCLASS
OUSPENSKI, ILIA I	1644	424-142100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 Sterne, Kessler, Goldstein & Fox P.L.L.C.
2
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: Bristol-Myers Squibb Company

(B) RESIDENCE: (CITY and STATE OR COUNTRY): Princeton, NJ

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ☒ Corporation or other private group entity ❑ Government

4a. Fees submitted: ☒ Issue Fee ❑ Publication Fee (if required) ❑ Advance Order - # of Copies _____

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

☒ Electronic Payment via EFS-Web ❑ Enclosed check ❑ Non-electronic payment by credit card (Attach form PTO-2038)

☒ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. 19-0036

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29

❑ Applicant asserting small entity status. See 37 CFR 1.27

❑ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /Ji-Eun Kim/ Date October 19, 2018

Typed or printed name Ji-Eun Kim Registration No. 68504

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor: Arvin YANG	Confirmation No.: 6926
Applicant: Bristol-Myers Squibb Company	Art Unit: 1644
Application No.: 15/141,769	Examiner: OUSPENSKI, Ilia I.
Filing Date: April 28, 2016	Atty. Docket: 3338.0330001/ELE/C-K

Title: **TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY**

Third Supplemental Information Disclosure Statement

Mail Stop AF

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Commissioner:

Listed on accompanying IDS Form PTO/SB/08b equivalent are documents that are submitted under 37 C.F.R. §1.56 and in compliance with the duty of disclosure requirements of 37 C.F.R. §§ 1.97 and 1.98. The numbering on this Third Supplemental Information Disclosure Statement is a continuation of the numbering in Applicant's Second Supplemental Information Disclosure Statement filed on **September 20, 2018** in connection with the above-captioned application.

Applicant has listed publication dates on the attached IDS Forms based on information presently available to the undersigned. However, the listed publication dates should not be construed as an admission that the information was actually published on the date indicated.

Applicant reserves the right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered.

This statement should not be construed as a representation that a search has been made, or that information more material to the examination of the present patent application does not exist. The Examiner is specifically requested not to rely solely on the material submitted herewith.

This is a filing under 37 C.F.R. § 1.97(d). This Information Disclosure Statement is being filed more than three months after the U.S. filing date and after the mailing date of a Notice of Allowance, but on or before payment of the Issue Fee. No item of information contained in this information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in § 1.56(c) more than three months prior to the filing of the information disclosure statement. 37 C.F.R. § 1.97(e)(2). The required fee is provided through online credit card payment authorization in the amount of $240.00 in payment of the fee under 37 C.F.R. § 1.17(p).

Copies of documents **NPL47-NPL50** are submitted.

It is respectfully requested that the Examiner initial and return a copy of the enclosed IDS Forms, and indicate in the official file wrapper of this patent application that the documents have been considered.

In accordance with the Federal Circuit decision in *Dayco Prods., Inc. v. Total Containment, Inc.* 329 F.3d 1358 (Fed. Cir. 2003), Applicants submit herewith an Office Action from the co-pending U.S. Patent Application No. 15/141,772, filed April 28, 2017, as document **NPL49**. The

identification of this Office Action is not to be construed as a waiver of secrecy as to those applications now or upon issuance of the present application as a patent.

The Examiner is respectfully requested to consider the cited applications and the art cited therein during examination.

It is respectfully requested that the Examiner initial and return a copy of the enclosed IDS Forms, and indicate in the official file wrapper of this patent application that the documents have been considered.

The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 19-0036.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

/Ji-Eun Kim/

Ji-Eun Kim
Attorney for Applicant
Registration No. 68,504

Date: October 19, 2018

1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

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UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

115018 7590 10/19/2018

Sterne, Kessler, Goldstein & Fox P.L.L.C.
1100 New York Avenue NW
Washington, DC 20005

EXAMINER	
OUSPENSKI, ILIA I	
ART UNIT	PAPER NUMBER
1644	

DATE MAILED: 10/19/2018

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/141,769	04/28/2016	Arvin YANG	3338.0330001/ELE/C-K	6926

TITLE OF INVENTION: Treatment of PD-L1-Negative Melanoma Using an Anti-PD-1 Antibody and an Anti-CTLA-4 Antibody

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1000	$0.00	$0.00	$1000	01/22/2019

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

115018 7590 10/19/2018
Sterne, Kessler, Goldstein & Fox P.L.L.C.
1100 New York Avenue NW
Washington, DC 20005

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/141,769	04/28/2016	Arvin YANG	3338.0330001/ELE/C-K	6926

TITLE OF INVENTION: Treatment of PD-L1-Negative Melanoma Using an Anti-PD-1 Antibody and an Anti-CTLA-4 Antibody

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1000	$0.00	$0.00	$1000	01/22/2019

EXAMINER	ART UNIT	CLASS-SUBCLASS
OUSPENSKI, ILIA I	1644	424-142100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. Fees submitted: ❑Issue Fee ❑Publication Fee (if required) ❑Advance Order - # of Copies _____

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

❑ Electronic Payment via EFS-Web ❑ Enclosed check ❑ Non-electronic payment by credit card (Attach form PTO-2038)

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29

❑ Applicant asserting small entity status. See 37 CFR 1.27

❑ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/141,769	04/28/2016	Arvin YANG	3338.0330001/ELE/C-K	6926

115018 7590 10/19/2018

Sterne, Kessler, Goldstein & Fox P.L.L.C.
1100 New York Avenue NW
Washington, DC 20005

EXAMINER	
OUSPENSKI, ILIA I	

ART UNIT	PAPER NUMBER
1644	

DATE MAILED: 10/19/2018

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 15/141,769	Applicant(s) YANG, Arvin	
	Examiner ILIA I OUSPENSKI	Art Unit 1644	AIA Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to 09/20/2018.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are See Continuation Sheet . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file areply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☑ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☐ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/ILIA I OUSPENSKI/
Primary Examiner, Art Unit 1644

Continuation of 3. The allowed claim(s) is/are: 1-2,8,14,16-18,20-21,25,27-28,32,34,36,46 and 48-51

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/141,769	YANG, Arvin
	Examiner	Art Unit
	ILIA I OUSPENSKI	1644

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
Priority application	02/27/2018	io
WEST: attached	02/27/2018	io
ProQuest: same as WEST	02/27/2018	io
Inventor search	02/27/2018	io
WEST and Inventor searches updated	06/18/2018	io
WEST and Inventor searches updated	10/16/2018	io

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
See search notes		10/16/2018	io

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/141,769	YANG, Arvin
	Examiner	**Art Unit**
	ILIA I OUSPENSKI	1644

CPC							
Symbol						**Type**	**Version**
C07K	/	16	/	2818		F	2013-01-01
A61K	/	39	/	39558		I	2013-01-01
A61K	/	45	/	06		I	2013-01-01
G01N	/	33	/	5743		I	2013-01-01
G01N	/	33	/	577		I	2013-01-01
A61K	/	2039	/	507		A	2013-01-01
A61K	/	2039	/	545		A	2013-01-01
A61K	/	2039	/	55		A	2013-01-01
C07K	/	2317	/	21		A	2013-01-01
C07K	/	2317	/	92		A	2013-01-01
G01N	/	2333	/	70503		A	2013-01-01

CPC Combination Sets							
Symbol					**Type**	**Set**	**Ranking**	**Version**
	/		/					

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	20	
/ILIA I OUSPENSKI/ Primary Examiner, Art Unit 1644	16 October 2018	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

U.S. Patent and Trademark Office — Part of Paper No.: 20181016

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/141,769	YANG, Arvin
	Examiner	**Art Unit**
	ILIA I OUSPENSKI	1644

INTERNATIONAL CLASSIFICATION				
CLAIMED				
C07K	/	16	/	28
A61K	/	39	/	395

NON-CLAIMED				
	/		/	

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**

CROSS REFERENCES(S)						
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	20	
/ILIA I OUSPENSKI/ Primary Examiner, Art Unit 1644	16 October 2018	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

U.S. Patent and Trademark Office — Part of Paper No.: 20181016

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/141,769	YANG, Arvin
	Examiner	**Art Unit**
	ILIA I OUSPENSKI	1644

☑ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	1		10		19	12	28		37	16	46				
2	2		11	8	20		29		38		47				
	3		12	9	21		30		39	17	48				
	4		13		22		31		40	18	49				
	5	4	14		23	13	32		41	19	50				
	6		15		24		33		42	20	51				
	7	5	16	10	25	14	34		43						
3	8	6	17		26		35		44						
	9	7	18	11	27	15	36		45						

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 20	
/ILIA I OUSPENSKI/ Primary Examiner, Art Unit 1644 (Primary Examiner)	16 October 2018 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure NONE

U.S. Patent and Trademark Office — Part of Paper No.: 20181016

WEST Search History

Hide Items | Restore | Clear | Close

DATE: Tuesday, October 16, 2018

Hide?	Set Name	Query	Hit Count
	DB=PGPB,USPT; THES=ASSIGNEE; PLUR=YES; OP=ADJ		
☐	L6	L5 and L1.clm. and L2.clm. and melanoma.clm.	4
☐	L5	L3 and (yang-a$.in. or Bristol$.as. or Bristol$.aanm.)	29
☐	L4	L3 and @pd>20180716	32
☐	L3	(L1 with L2 with melanoma$)	234
☐	L2	(CTLA4$ or ctla-4$ or cd152$ or anti-ctla4$ or anti-ctla-4$ or anti-cd152$ or ".alpha.ctla$4" or ".alpha.ctla-4$" or ".alpha.cd152$")	23034
☐	L1	("PD-L1$" or pdL1$ or pdL-1$ or b7-h1$ or b7h1$ or b7-4$ or cd274$ or "anti-pd-l1$" or anti-pdl1$ or anti-b7-h1$ or anti-b7h1$ or anti-b7-4$ or anti-cd274$ or ".alpha.pd-l1$" or ".alpha.pdl1" or ".alpha.b7-h1$" or ".alpha.b7h1" or ".alpha.b7-4$" or ".alpha.cd274$")	6335

END OF SEARCH HISTORY

Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Substitute for form 1449/PTO

SECOND SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 2

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG; Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	US47	US-20160022814-A1	01-28-2016	PETIT; R et al.	
	US48	US-20150290316-A1	10-15-2015	GRAZIANO; R.F. et al.	
	US49	US-20090217401-A1	08-27-2009	KORMAN; A.J. et al.	

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author(in CAPITAL LETTERS),title of the article(when appropriate), title of the item (book,magazine,journal,serial,symposium,catalog,etc.),date,page(s),volume-issue number(s),publisher, city and/or country where published.	T[2]
	NPL38	Kuenen, B., *et al*. "A Phase I Pharmacologic Study of Necitumumab (IMC-11 F8), a Fully Human IgG1 Monoclonal Antibody Directed Against EGFR in Patients with Advanced Solid Malignancies," *Clin Cancer Res*., 16(6):1915-23, United States (2010).	☐
	NPL39	Wang, D.D., *et al.* "Fixed Dosing Versus Body Size-Based Dosing of Monoclonal Antibodies in Adult Clinical Trials," J Clin Pharmacol., 49(9):1012-24, United States (2009)	☐
	NPL40	Office Action mailed May 2, 2018, in United States Patent Application No. 15/311,409, Feltquate, D. *et al*., filed November 15, 2016, 13 pages.	☐
	NPL41	Office Action mailed June 13, 2018, in United States Patent Application No. 15/210,612, Manekas, D. *et al*., filed July 14, 2016, 16 pages.	☐
	NPL42	BAI, S., et al., "A Guide to Rational Dosing of Monoclonal Antibodies," Clin Pharmacokinetics 51(2):119-135, Springer-Business, United States (February 2012).	☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /I.I.O/

Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

SECOND SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 2 of 2

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG; Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author(in CAPITAL LETTERS),title of the article(when appropriate), title of the item (book,magazine,journal,serial,symposium,catalog,etc.),date,page(s),volume-issue number(s),publisher, city and/or country where published.	T[2]
	NPL43	GULER, E., et al., "A review of the fixed douse use of new oral anticoagulants in obese patients: Is it really enough?," Anatol J Cardiol 15:1020-1029, Turkish Society of Cardiology, Turkey (December 2015).	☐
	NPL44	PAN, S-D., et al., "Weight-based dosing in medication use: what should we know?," Patient Preference and Adherence 10:549-560, Dove Medical Press, United Kingdom (April 12, 2016).	☐
	NPL45	OPDIVO (nivolumab) [package insert], 32 pages; Bristol-Myers Squibb: Approved by U.S. Food and Drug Administration, United States; (August 2018).	☐
	NPL46	Advisory Action mailed August 3, 2018, in United States Patent Application No. 15/141,772, YANG, Arvin. *et al*., filed April 28, 2016, 3 pages.	☐

10069743_1.docx

Examiner Signature	/ILIA I OUSPENSKI/	Date Considered	10/16/2018

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. 1 Applicant's unique citation designation number (optional). 2 Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /I.I.O/

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BIB DATA SHEET

CONFIRMATION NO. 6926

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
15/141,769	04/28/2016 RULE	424	1644	3338.0330001/ELE/C-K

APPLICANTS
Bristol-Myers Squibb Company, Princeton, NJ

INVENTORS
Arvin YANG, Princeton, NJ;

**** CONTINUING DATA ***************************
This appln claims benefit of 62/153,973 04/28/2015

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****
05/06/2016

Foreign Priority claimed ❑ Yes ☑ No 35 USC 119(a-d) conditions met ❑ Yes ☑ No Verified and Acknowledged /ILIA I OUSPENSKI/ Examiner's Signature	❑ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		NJ	6	20	3

ADDRESS

Sterne, Kessler, Goldstein & Fox P.L.L.C.
1100 New York Avenue NW
Washington, DC 20005
UNITED STATES

TITLE

Treatment of PD-L1-Negative Melanoma Using an Anti-PD-1 Antibody and an Anti-CTLA-4 Antibody

FILING FEE RECEIVED	FEES: Authority has been given in Paper	❑ All Fees
1740	No._____ to charge/credit DEPOSIT ACCOUNT	❑ 1.16 Fees (Filing)
	No._____ for following:	❑ 1.17 Fees (Processing Ext. of time)
		❑ 1.18 Fees (Issue)
		❑ Other _____
		❑ Credit

PTO/SB/30EFS (02-18)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL
(Submitted Only via EFS-Web)

Application Number	15/141,769	Filing Date	2016-04-28	Docket Number (if applicable)	3338.0330001/ELE/C-K	Art Unit	1644
First Named Inventor	Arvin YANG			Examiner Name	OUSPENSKI, Ilia I.		

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. The Instruction Sheet for this form is located at WWW.USPTO.GOV

SUBMISSION REQUIRED UNDER 37 CFR 1.114

Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

- ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
- ☐ Other _____

☒ Enclosed

- ☒ Amendment/Reply
- ☒ Information Disclosure Statement (IDS)
- ☐ Affidavit(s)/ Declaration(s)
- ☐ Other _____

MISCELLANEOUS

☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____
(Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

☐ Other _____

FEES

☒ **The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.**
The Director is hereby authorized to charge any underpayment of fees, or credit any overpayments, to Deposit Account No 190036

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

☒ Patent Practitioner Signature

Applicant Signature

PTO/SB/30EFS (02-18)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Signature of Registered U.S. Patent Practitioner			
Signature	/Ji-Eun Kim/	Date (YYYY-MM-DD)	2018-09-20
Name	Ji-Eun Kim	Registration Number	68504

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450.

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Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

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7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor: Arvin YANG	Confirmation No.: 6926
Applicant: Bristol-Myers Squibb Company	Art Unit: 1644
Application No.: 15/141,769	Examiner: OUSPENSKI, Ilia I.
Filing Date: April 28, 2016	Atty. Docket: 3338.0330001/ELE/C-K

Title: **TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY**

Amendment and Reply Under 37 C.F.R. § 1.114

Mail Stop RCE

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Commissioner:

In reply to the Final Office Action dated June 22, 2018, Applicant submits the following Amendment and Remarks.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks and Arguments begin on page 6 of this paper.

It is not believed that extensions of time are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any additional fees required to continue prosecution or appeal of this application (including issue fee, fees for net addition of claims or forwarding to appeal) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Currently Amended) A method for treating a melanoma tumor in a human patient in need thereof comprising:

(i) identifying a patient having a PD-L1-negative melanoma tumor; and

(ii) administering to the patient:

(a) about 1 mg/kg of an antibody or an antigen-binding portion thereof that binds specifically to a human PD-1 ("an anti-PD-1 antibody") and a dose of an antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4 ("an anti-CTLA-4 antibody") every three weeks for about 4 doses; followed by

(b) a ~~flat~~ dose of the anti-PD-1 antibody,

wherein the dose in (b) is a flat dose of about 240 mg ~~every two weeks~~ or about 480 mg ~~every four weeks~~.

2. (Currently Amended) A method for treating a melanoma tumor in a human patient in need thereof comprising administering to the patient:

(a) about 1 mg/kg of an antibody or an antigen-binding portion thereof that binds specifically to a human PD-1 ("an anti-PD-1 antibody") and a dose of an antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4 ("an anti-CTLA-4 antibody") every three weeks for about 4 doses; followed by

(b) a ~~flat~~ dose of the anti-PD-1 antibody,

wherein the dose in (b) is a flat dose of about 240 mg ~~every two weeks~~ or about 480 mg ~~every four weeks~~.

3-7. (Cancelled)

8. (Previously Presented) The method of claim 2, wherein the patient is characterized by (i) extended progression-free survival for over 8 months following the administration, (ii) tumor size reduction at least about 10% compared to the tumor size prior to the administration, or (iii) both (i) and (ii).

9-13. (Cancelled)

14. (Previously Presented) The method of claim 2, further comprising measuring a PD-L1 expression on the melanoma tumor prior to the administration.

15. (Cancelled)

16. (Previously Presented) The method of claim 14, wherein the measuring comprises providing a test tissue sample obtained from the patient, the test tissue sample comprising tumor cells and/or tumor-infiltrating inflammatory cells.

17. (Original) The method of claim 16, wherein the measuring further comprises assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface.

18. (Previously Presented) The method of claim 17, wherein the test tissue sample is a formalin-fixed paraffin-embedded (FFPE) tissue sample, and wherein the presence of PD-L1 is determined using an automated IHC assay.

19. (Cancelled)

20. (Previously Presented) The method of claim 18, wherein the IHC assay is performed using an anti-PD-L1 monoclonal antibody that specifically binds to the PD-L1 and wherein the anti-PD-L1 monoclonal antibody comprises a variable heavy region comprising the amino acid sequence set forth in SEQ ID NO: 1 and a variable light region comprising the amino acid sequence set forth in SEQ ID NO: 2.

21. (Previously Presented) The method of claim 2, wherein less than about 5% of tumor cells show binding to an anti-PD-L1 antibody or an antigen-binding portion thereof.

22-24. (Cancelled)

25. (Previously Presented) The method of claim 2, wherein the anti-PD-1 antibody is nivolumab.

26. (Cancelled)

27. (Currently Amended) The method of claim 2, wherein the ~~flat~~ dose of the anti-PD-1 antibody in (b) is about 240 mg administered once every two weeks.

28. (Currently Amended) The method of claim 2, wherein the ~~flat~~ dose of the anti-PD-1 antibody in (b) is about 480 mg administered once every four weeks.

29-31. (Cancelled)

32. (Previously Presented) The method of claim 2, wherein the anti-CTLA-4 antibody is ipilimumab.

33. (Cancelled)

34. (Previously Presented) The method of claim 2, wherein the dose of the anti-CTLA-4 antibody is from 0.1 mg/kg to 10.0 mg/kg.

35. (Cancelled)

36. (Previously Presented) The method of claim 34, wherein the dose of the anti-CTLA-4 antibody is 3 mg/kg.

37-45. (Cancelled)

46. (Previously Presented) The method of claim 2, further comprising administering an anti-cancer agent.

47. (Canceled)

48. (Currently Amended) The method of claim 1, wherein the ~~flat~~ dose of the anti-PD-1 antibody in (b) is about 240 mg administered once every two weeks.

49. (Currently Amended) The method of claim 1, wherein the ~~flat~~ dose of the anti-PD-1 antibody in (b) is about 480 mg administered once every four weeks.

50. (Previously Presented) The method of claim 1, wherein the dose of the anti-CTLA-4 antibody is 3 mg/kg.

51. (New) The method of claim 1, wherein the anti-PD-1 antibody is nivolumab.

Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 1, 2, 8, 14, 16-18, 20, 21, 25, 27, 28, 32, 34, 36, 46, and 48-51 are pending in the application, with claims 1 and 2 being the independent claims. Claims 1, 2, 27, 28, 48 and 49 are sought to be amended. Claim 47 is sought to be cancelled, and claims 3-7, 9-13, 15, 19, 22-24, 26, 29-31, 33, 35, and 37-45 were previously cancelled without prejudice to or disclaimer of the subject matter therein. Applicant reserves the right to pursue the cancelled subject matter in a continuing application.

Support for the amendments to the claims can be found throughout the specification as filed. In particular, claims 1, 2, 27, 28, 48 and 49 are amended for clarity and for antecedent basis.

New claim 51 is sought to be added. Support for new claim 51 can be found throughout the specification as filed, including at, *inter alia*, paragraphs [0087] and [0097] and original claim 25.

These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendments and the following remarks, Applicant respectfully requests that the Examiner reconsider all outstanding rejections and that they be withdrawn.

Rejections under 35 U.S.C. § 112(a)

The Examiner rejected claims 1, 2, 8, 14, 16-18, 20, 21, 25, 27, 28, 32, 34, 36, and 46-50 under 35 U.S.C. § 112(a) for allegedly failing to comply with the written description requirement. Office Action, pp. 2-5. In particular, the Examiner contends that the specification as filed allegedly does not support "two newly added" elements: (i) recitation of "followed by a flat dose" in claims 1

and 2; and (ii) "240 mg every two weeks" recited in claims 1, 2, and 48. *Id.* at pp. 2-3. The Examiner notes that this is a New Matter Rejection. Applicant respectfully disagrees.

However, solely to advance prosecution, claim 47 is cancelled, rendering the Examiner rejection of claim 47 moot. In addition, claim 1 is amended to recite a method for treating a melanoma tumor in a human patient in need thereof comprising: (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient: (a) about 1 mg/kg of an anti-PD-1 antibody and a dose of an anti-CTLA-4 antibody every three weeks for about 4 doses; followed by (b) a dose of the anti-PD-1 antibody, wherein the dose in (b) is a flat dose of about 240 mg or about 480 mg. Similarly, claim 2 is amended to recite a method for treating a melanoma tumor in a human patient in need thereof comprising administering to the patient: (a) about 1 mg/kg of an anti-PD-1 antibody and a dose of an anti-CTLA-4 antibody every three weeks for about 4 doses; followed by (b) a dose of the anti-PD-1 antibody, wherein the dose in (b) is a flat dose of about 240 mg or about 480 mg. The specification as filed discloses each element of amended claims 1 and 2, and therefore amended claims 1 and 2 contain no new matter and meet the written description requirement.

In particular, the specification provides:

> In certain embodiments, a regimen comprises: (i) an induction dosing schedule comprising **combined administration of the anti-PD-1 and anti-CTLA-4 antibodies** at a dosing frequency of at least once about every 2 weeks, at least once about every 3 weeks, or at least once about every 4 weeks, or at least about once a month, for at least 2, 4, 6, 8 or 10 doses, **followed by administration of the anti-PD-1 antibody alone** at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 12 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of at least about once every 8 weeks, once about every 12 weeks, or once about every 16 weeks, or at least once a quarter, for at least 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical

> benefit is observed, or until unmanageable toxicity or disease progression occurs.

Specification, paragraph [0110]. The specification further provides that "[i]n some embodiments, the anti-PD-1 is administered at a dosage of **about 1 mg/kg** at a dosing frequency of **once about every 3 weeks** for up to 48 doses." *Id.* at paragraph [0108]. In addition, the specification provides that "[i]n one particular embodiment, the dosing schedule comprises administering **about 1 mg/kg of an anti-PD-1 antibody followed by an anti-CTLA-4 antibody** (*e.g.*, about 3 mg/kg anti-CTLA-4 antibody) **on the same day every 3 weeks for 4 doses**, followed by about 3 mg/kg of an anti-PD-1 antibody every 2 weeks." *Id.* at paragraph [0110]. Accordingly, the specification provides for administering to a patient: (a) about 1 mg/kg of an anti-PD-1 antibody and a dose of an anti-CTLA-4 antibody every three weeks for about 4 doses (*id.* at paragraphs [0108] and [0110]; followed by (b) administration of the anti-PD-1 antibody alone (*id.* at paragraph [0110]) at least once every two weeks.

The specification further provides that the anti-PD-1 antibody can be "administered at a flat dose regardless of the weight of the patient." *Id.* at paragraph [0115]. In particular, the disclosure specifically states that "[i]n some embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of **about 240 mg**." *Id.* at paragraph [0118]. It would have been readily apparent to a person of ordinary skill in the art at the time the application was filed that the disclosure of "the anti-PD-1 antibody" that can be "administered at a dose of about 240 mg" refers to the "dose" of "the anti-PD-1 antibody" in (b), as recited in amended claims 1 and 2.

The specification therefore provides for administering to a patient: (a) about 1 mg/kg of an anti-PD-1 antibody and a dose of an anti-CTLA-4 antibody every three weeks for about 4 doses;

followed by (b) administration of the anti-PD-1 antibody, wherein the dose in (b) is a flat dose of about 240 mg, *e.g.*, at least once every two weeks.

Applicant respectfully disagrees with the Examiner that selecting a flat dose of 240 mg requires a skilled artisan to pick one dose out of "31 different flat doses." Office Action, p. 4. As the excerpt from paragraph [0118], shown above, illustrates, a flat dose of 240 mg was specifically called out in the specification as filed. Thus, a skilled artisan would not have to select from 31 different flat doses, but rather would recognize immediately that a flat dose of 240 mg is specifically disclosed.

Furthermore, while amended claims 1 and 2 have been revised to expedite examination, Applicant nonetheless disagrees with the Examiner's assertion that, in order to arrive at a dosing regimen of 240 mg administered once every two weeks, a skilled artisan would be required to select from "10 different regimens of administration, ranging from once a week to once every 10 weeks." Office Action, p. 4. Rather, the specification clearly provides at paragraph [0122] that "[i]n some embodiments, the composition is administered by intravenous infusion about once per week, about **once every 2 weeks**, about once every 3 weeks, or about once a month." Specification, paragraph [0122]. Thus, it would have been immediately clear to a person of ordinary skill in the art that the inventors had possession of administering a flat dose of an anti-PD-1 antibody once every two weeks in view of the specification as filed.

In addition, the specification is replete with embodiments of dosing regimens that include administering the anti-PD-1 antibody alone once every two weeks following the administration of about 1 mg/kg of an anti-PD-1 antibody and the anti-CTLA-4 antibody. *See, e.g.*, *id.* at paragraphs [0110], [0112], and [0130]. Rather than selecting from multiple lists, as the Examiner contends, a

skilled artisan would have recognized that the specification teaches that a weight based dose disclosed therein can be substituted by a flat dose, s*ee, e.g.*, *id.* at paragraph [0115], and the specification specifically teaches a flat dose 240 mg of an anti-PD-1 antibody, *see, e.g.*, *id.* at paragraph [0118].

For at least these reasons, it would have been clear to a person of ordinary skill in the art at the time the application was filed that the inventors were in possession of and adequately disclosed the full scope of amended claims 1 and 2. Therefore, amended claims 1 and 2 recite no new matter and satisfy the written description requirement.

Claims 8, 14, 16-18, 20, 21, 25, 27, 28, 32, 34, 36, 46, and 48-50 depend directly or indirectly from claim 1 or 2 and thus include every element of claim 1 of 2. Therefore, insofar as the Examiner rejected claims 8, 14, 16-18, 20, 21, 25, 27, 28, 32, 34, 36, 46, and 48-50 based on their decency on claim 1 or 2, claims 8, 14, 16-18, 20, 21, 25, 27, 28, 32, 34, 36, 46, and 48-50 also recite no new matter and satisfy the written description requirement. As the Examiner has provided no other grounds for rejection, Applicant respectfully requests that the Examiner reconsider the rejections under 35 U.S.C. §112, first paragraph, and allow the present claims. *See* MPEP 2163.06.

Conclusion

All of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding rejections and that they be withdrawn. Applicant believes that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

/Ji-Eun Kim/

Ji-Eun Kim
Attorney for Applicant
Registration No. 68,504

Date: September 20, 2018

1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

9858692_1.docx

Electronic Patent Application Fee Transmittal

Application Number:	15141769
Filing Date:	28-Apr-2016
Title of Invention:	Treatment of PD-L1-Negative Melanoma Using an Anti-PD-1 Antibody and an Anti-CTLA-4 Antibody
First Named Inventor/Applicant Name:	Arvin YANG
Filer:	Ji Eun Kim/Jeanette Yando
Attorney Docket Number:	3338.0330001/ELE/C-K

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
RCE- 1ST REQUEST	1801	1	1300	1300
		Total in USD ($)		**1300**

Electronic Acknowledgement Receipt	
EFS ID:	33781575
Application Number:	15141769
International Application Number:	
Confirmation Number:	6926
Title of Invention:	Treatment of PD-L1-Negative Melanoma Using an Anti-PD-1 Antibody and an Anti-CTLA-4 Antibody
First Named Inventor/Applicant Name:	Arvin YANG
Customer Number:	115018
Filer:	Ji Eun Kim/Jeanette Yando
Filer Authorized By:	Ji Eun Kim
Attorney Docket Number:	3338.0330001/ELE/C-K
Receipt Date:	20-SEP-2018
Filing Date:	28-APR-2016
Time Stamp:	18:03:00
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$1300
RAM confirmation Number	092118INTEFSW18032300
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	NPL38_Kuenen.pdf	1529252 de5697225578b69392a4dbc7be37415f39407085	no	11
Warnings:					
Information:					
2	Non Patent Literature	NPL39_Wang.pdf	1458994 7f42d62ed8055d2513b4e60ea5589008572f64dc	no	13
Warnings:					
Information:					
3	Non Patent Literature	NPL40_OA.pdf	450227 9630816dcc26f86464c4672efe5a04bbed1f87c6	no	13
Warnings:					
Information:					
4	Non Patent Literature	NPL41_OA.pdf	621960 148639406ced6aeffb8ab2368f6be082ebe758f9	no	16
Warnings:					
Information:					
5	Non Patent Literature	NPL42_Bai.pdf	456409 4d3f708a63f5976997829b2c0288ce45153e4597	no	17
Warnings:					
Information:					
6	Non Patent Literature	NPL44_Pan_.pdf	266197 40c17bd896ba0ce01ca20cf42ee83dabca01a3be	no	12
Warnings:					
Information:					

7	Non Patent Literature	NPL45_pi_opdivo.PDF	699943 acfaddc7fac841050bee8bfa6d198f001acf5c0f	no	32
Warnings:					
Information:					
8	Non Patent Literature	NPL46_AA_15141772.pdf	172835 9cd2c7951884c27f88e68bfff6bbdde44b0271de	no	3
Warnings:					
Information:					
9	Transmittal Letter	2018-09-20-2nd-Supp-IDS-3338-0330001.pdf	110909 6c3d9d22e2b29bc63a03bb97528ebd8039705e92	no	3
Warnings:					
Information:					
10	Information Disclosure Statement (IDS) Form (SB08)	2018-09-SB08A-B-3338-0330001.pdf	117720 676db9c7831b89f24b9d2ecf3c5f58fa359b91c9	no	2
Warnings:					
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This is not an USPTO supplied IDS fillable form					
11	Request for Continued Examination (RCE)	2018-09-20-RCE-3338-0330001.pdf	1350129 a677ac635ce1c04fc122f903f38c00f7619c7bab	no	3
Warnings:					
Information:					
12		2018-09-20-Amendment-114-3338-0330001.pdf	134811 a44e7b9943223f4b3b7a273093b542c86dafe6a2	yes	11

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment Submitted/Entered with Filing of CPA/RCE	1	1
	Claims	2	5
	Applicant Arguments/Remarks Made in an Amendment	6	11

Warnings:					
Information:					
13	Non Patent Literature	NPL43_Guler.pdf	124198 e09f955f5d0ef668dd78c86c8f7ba290f0971a7b	no	10
Warnings:					
Information:					
14	Fee Worksheet (SB06)	fee-info.pdf	30550 74f1fb9e046d058e7657bf8897df311f63f2c89c	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	7524134	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor: Arvin YANG	Confirmation No.: 6926
Applicant: Bristol-Myers Squibb Company	Art Unit: 1644
Application No.: 15/141,769	Examiner: OUSPENSKI, Ilia I.
Filing Date: April 28, 2016	Atty. Docket: 3338.0330001/ELE/C-K

Title: **TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY**

Second Supplemental Information Disclosure Statement

Mail Stop RCE

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Commissioner:

Listed on accompanying IDS Forms PTO/SB/08a; PTO/SB/08b equivalent are documents that are submitted under 37 C.F.R. §1.56 and in compliance with the duty of disclosure requirements of 37 C.F.R. §§ 1.97 and 1.98. The numbering on this Second Supplemental Information Disclosure Statement is a continuation of the numbering in Applicant's First Supplemental Information Disclosure Statement filed on **May 29, 2018** in connection with the above-captioned application.

Applicant has listed publication dates on the attached IDS Forms based on information presently available to the undersigned. However, the listed publication dates should not be construed as an admission that the information was actually published on the date indicated.

Applicant reserves the right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered.

This statement should not be construed as a representation that a search has been made, or that information more material to the examination of the present patent application does not exist. The Examiner is specifically requested not to rely solely on the material submitted herewith.

This is a filing under 37 C.F.R. § 1.97(b). This Information Disclosure Statement is being filed before the mailing of a first Office Action after the filing of a request for continued examination under 37 C.F.R. § 1.114. No statement or fee is required.

Copies of documents **NPL38-NPL46** are submitted. However, in accordance with 37 C.F.R. § 1.98(a)(2)(ii), no copies of U.S. patent application publications cited as documents **US47-US49** on the attached IDS Forms are submitted.

In accordance with the Federal Circuit decision in *Dayco Prods., Inc. v. Total Containment, Inc.* 329 F.3d 1358 (Fed. Cir. 2003), Applicants submit herewith an Office Action from the co-pending U.S. Patent Application No. 15/311,409, filed November 15, 2016, as document **NPL40**; Office Action from the co-pending U.S. Patent Application No. 15/210,612, filed July 14, 2016, as document **NPL41**; and an Office Action from the co-pending U.S Patent Application No. 15/141,772, filed April 28, 2016, as document **NPL46**. The identification of these Office Actions is not to be construed as a waiver of secrecy as to those applications now or upon issuance of the present application as a patent. The Examiner is respectfully requested to consider the cited applications and the art cited therein during examination.

It is respectfully requested that the Examiner initial and return a copy of the enclosed IDS Forms, and indicate in the official file wrapper of this patent application that the documents have been considered.

The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 19-0036.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

/Ji-Eun Kim/

Ji-Eun Kim
Attorney for Applicant
Registration No. 68,504

Date: September 20, 2018

1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

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Substitute for form 1449/PTO

SECOND SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 2

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG; Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	US47	US-20160022814-A1	01-28-2016	PETIT; R et al.	
	US48	US-20150290316-A1	10-15-2015	GRAZIANO; R.F. et al.	
	US49	US-20090217401-A1	08-27-2009	KORMAN; A.J. et al.	

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author(in CAPITAL LETTERS),title of the article(when appropriate), title of the item (book,magazine,journal,serial,symposium,catalog,etc.),date,page(s),volume-issue number(s),publisher, city and/or country where published.	T[2]
	NPL38	Kuenen, B., *et al*. "A Phase I Pharmacologic Study of Necitumumab (IMC-11 F8), a Fully Human IgG1 Monoclonal Antibody Directed Against EGFR in Patients with Advanced Solid Malignancies," *Clin Cancer Res*., 16(6):1915-23, United States (2010).	☐
	NPL39	Wang, D.D., *et al.* "Fixed Dosing Versus Body Size-Based Dosing of Monoclonal Antibodies in Adult Clinical Trials," J Clin Pharmacol., 49(9):1012-24, United States (2009)	☐
	NPL40	Office Action mailed May 2, 2018, in United States Patent Application No. 15/311,409, Feltquate, D. *et al*., filed November 15, 2016, 13 pages.	☐
	NPL41	Office Action mailed June 13, 2018, in United States Patent Application No. 15/210,612, Manekas, D. *et al*., filed July 14, 2016, 16 pages.	☐
	NPL42	BAI, S., et al., "A Guide to Rational Dosing of Monoclonal Antibodies," Clin Pharmacokinetics 51(2):119-135, Springer-Business, United States (February 2012).	☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

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Substitute for form 1449/PTO

SECOND SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 2 of 2

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG; Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author(in CAPITAL LETTERS),title of the article(when appropriate), title of the item (book,magazine,journal,serial,symposium,catalog,etc.),date,page(s),volume-issue number(s),publisher, city and/or country where published.	T[2]
	NPL43	GULER, E., et al., "A review of the fixed douse use of new oral anticoagulants in obese patients: Is it really enough?," Anatol J Cardiol 15:1020-1029, Turkish Society of Cardiology, Turkey (December 2015).	☐
	NPL44	PAN, S-D., et al., "Weight-based dosing in medication use: what should we know?," Patient Preference and Adherence 10:549-560, Dove Medical Press, United Kingdom (April 12, 2016).	☐
	NPL45	OPDIVO (nivolumab) [package insert], 32 pages; Bristol-Myers Squibb: Approved by U.S. Food and Drug Administration, United States; (August 2018).	☐
	NPL46	Advisory Action mailed August 3, 2018, in United States Patent Application No. 15/141,772, YANG, Arvin. *et al*., filed April 28, 2016, 3 pages.	☐

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Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. 1 Applicant's unique citation designation number (optional). 2 Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/141,769	Filing Date 04/28/2016	☐ To be Mailed

ENTITY: ☑ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $80 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $420 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	09/20/2018	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 20	Minus	** 20	= 0		x $100 =	0
	Independent (37 CFR 1.16(h))	* 2	Minus	*** 3	= 0		x $460 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	LIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	Moliki I May
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".	
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.	

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/141,769	04/28/2016	Arvin YANG	3338.0330001/ELE/C-K	6926

115018 7590 06/22/2018

Sterne, Kessler, Goldstein & Fox P.L.L.C.
1100 New York Avenue NW
Washington, DISTRICT OF COLUMBIA 20005
UNITED STATES OF AMERICA

EXAMINER	
OUSPENSKI, ILIA I	

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
06/22/2018	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

e-office@sternekessler.com

Office Action Summary	**Application No.** 15/141,769	**Applicant(s)** YANG, Arvin	
	Examiner ILIA I OUSPENSKI	**Art Unit** 1644	**AIA Status** Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 05/29/2018.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☑ This action is **FINAL.** 2b) ☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 1-2,8,14,16-18,20-21,25,27-28,32,34,36 and 46-50 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 1-2,8,14,16-18,20-21,25,27-28,32,34,36 and 46-50 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED ACTION

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

2. Applicant's amendment and remarks filed on 05/29/2018 are acknowledged. Claims 1, 2, 8, 14, 16-18, 20, 21, 25, 27, 28, 32, 34, 36 and 46-50 are pending.

3. The following is a quotation of **35 U.S.C. 112(a)**:

(a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

4. Claims 1, 2, 8, 14, 16-18, 20, 21, 25, 27, 28, 32, 34, 36 and 46-50 are rejected under **35 U.S.C. 112(a)** as failing to comply with the written description requirement. The claim(s) contain(s) subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention. ***This is a New Matter rejection.***

Applicant asserts that no New Matter has been added and points to the specification at paragraphs [0108]-[0110], [0112], [0116]-[0118] and [0122] for support for the amended and newly added claims.

However, neither the specification as filed nor the original claims appear to provide adequate written description of the following two newly added limitations:

(1) In claims 1 and 2 – a regimen wherein combined administration of anti-PD-1 and anti-CTLA4antibodies is "followed by a flat dose" of PD-1 antibody.

(2) In claims 1, 2 and 48 – a flat dose of the anti-PD-1 antibody of about "240 mg every two weeks."

Regarding limitation (1), the closest support is found at paragraphs [0110] and [0112] of the specification (emphasis added):

[0110] In some embodiments, the dosage of each of the anti-PD-1 and anti-CTLA-4 antibodies is kept constant during the induction dosing schedule and the maintenance dosing schedule. In certain embodiments, a regimen comprises: (i) an induction dosing schedule comprising **combined administration of the anti-PD-1 and anti-CTLA-4 antibodies** at a dosing frequency of at least once about every 2 weeks, at least **once about every 3 weeks**, or at least once about every 4 weeks, or at least about once a month, **for at least** 2, **4**, 6, 8 or 10 **doses**, **followed by** administration of the **anti-PD-1 antibody alone** at a dosing frequency of at least once **every 2**, 3 or 4 **weeks**, or at least once a month, for at least 2, 4, 6, 8 or 12 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of at least about once every 8 weeks, once about every 12 weeks, or once about every 16 weeks, or at least once a quarter, for at least 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In another embodiment, the dosing schedule comprises administering about 3 mg/kg of an anti-PD-1 antibody every 2 weeks, or about **1 mg/kg of an anti-PD-1 antibody every 3 weeks plus about 3 mg/kg of an anti-CTLA-4 antibody every 3 weeks for 4 doses**, **followed by** about **3 mg/kg of an anti-PD-1 antibody every 2 weeks** for cycle 3 and beyond, or 3 mg/kg of an anti-CTLA-4 antibody every 3 weeks for 4 doses. In one particular embodiment, the dosing schedule comprises administering about **1 mg/kg of an anti-PD-1 antibody** followed by an anti-CTLA-4 antibody (e.g., about **3 mg/kg anti-CTLA-4 antibody**) on the same day **every 3 weeks for 4 doses**, **followed by** about **3 mg/kg of an anti-PD-1 antibody every 2 weeks.**

[0112] In a particular embodiment, the anti-PD-1 and anti-CTLA-4 antibodies are administered at dosages of about 1 mg/kg anti-PD-1 antibody every three weeks and about 3 mg/kg of anti-CTLA-4 antibody every three weeks. In another embodiment, the patient is administered at a dose of about **1 mg/kg of an anti-PD-1 antibody**, followed by an **anti-CTLA-4 antibody on the same day**, **every 3 weeks for 4 doses**, **then** **3 mg/kg of an anti-PD-1 antibody every 2 weeks.**

In the above excerpts, there are four instances of disclosure of combined administration of anti-PD-1 and anti-CTLA4 antibodies followed by administration of anti-PD-1 antibody alone, wherein the dosage of the anti-PD-1 antibody administered alone is either unspecified (one instance) or specified as 3 mg/kg (three instances).

There does not appear to be any disclosure of a regiment wherein the combined administration of anti-PD-1 and anti-CTLA4 antibodies is followed by administration of a flat dose of an anti-PD-1 antibody.

Regarding limitation (2), a flat dose of the anti-PD-1 antibody of about "240 mg every two weeks," the closest support is found at paragraphs [0116] and [0117] of the specification (emphasis added):

[0116] In certain embodiments of the present methods, the therapeutically effective dosage of the **anti-PD-1 antibody** or antigen-binding portion thereof is a **flat dosing** (not a bodyweight based dosing) and comprises **about 60 mg, about 80 mg, about 100 mg, about 120 mg, about 140 mg, about 160 mg, about 180 mg, about 200 mg, about 220 mg, about 240 mg, about 260 mg, about 280 mg, or about 300 mg**. In other embodiments, the therapeutically effective dosage of the **anti-PD-1 antibody** or antigen-binding portion thereof comprises about **320 mg, 360 mg, 400 mg, 420 mg, 480 mg, 500 mg, 540 mg, 550 mg, 600 mg, 620 mg, 650 mg, 680 mg, 700 mg, 720 mg, 780 mg, 800 mg, 840 mg, or 900 mg**. In some embodiments, the dose of the anti-PD-1 antibody in the composition is between about 60 mg and about 300 mg, between about 60 mg and about 100 mg, between about 100 mg and about 200 mg, or between about 200 mg and about 300 mg. In some embodiments, the amount of the anti-PD-1 antibody in the composition is at least about 80 mg, about 160 mg, or about 240 mg. In some embodiments, the dose of the anti-PD-1 antibody in the composition is at least about 240 mg or at least about 80 mg.

[0117] In other embodiments, the dosages are given **every week, every two weeks, every three weeks, every four weeks, every five weeks, every six weeks, every seven weeks, every eight weeks, every nine weeks, or every 10 weeks**.

Paragraph [0116] discloses a flat dose of anti-PD-1 antibody of about 240 mg as one of a list of 31 different flat doses of anti-PD-1 antibody. Paragraph [0117] discloses a regimen wherein unspecified dosages are given every two weeks as one of 10 different regimens of administration, ranging from once a week to once every 10 weeks. Thus there are 310 combinations of flat doses and regimens of their administration, with no indication as to which regimen is appropriate for a given dose, i.e. no disclosed reason for the skilled artisan to select the particular dosage of 240 mg out of the 31 disclosed doses, and to combine it with the particular regimen of administration of once every two weeks out of the 10 disclosed regimens. Accordingly, there is no specific support under 35 U.S.C. 112(a) in the specification for this limitation.

The amended claims now recite limitations which were not clearly disclosed in the specification or claims as filed, and change the scope of the instant disclosure as filed. These limitations introduce new concepts and violate the description requirement of the first paragraph of 35 U.S.C. 112.

Claims 8, 14, 16-18, 20, 21, 25, 27, 28, 32, 34, 36, 46, 47, 49 and 50 are included in the rejection, because they encompass the New Matter of the claims on which they depend.

Applicant is required to cancel the New Matter in the response to this Office Action. Alternatively, Applicant is invited to clearly point out the written support for the newly added limitations.

5. Conclusion: no claim is allowed.

6. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, THIS ACTION IS MADE FINAL. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

7. Any inquiry concerning this communication or earlier communications from the examiner should be directed to ILIA I OUSPENSKI whose telephone number is (571)272-2920. The examiner can normally be reached 8:30 AM – 5 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel Kolker can be reached at 571-272-3181. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ILIA I OUSPENSKI/
Primary Examiner, Art Unit 1644

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/141,769	YANG, Arvin
	Examiner	Art Unit
	ILIA I OUSPENSKI	1644

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
Priority application	02/27/2018	io
WEST: attached	02/27/2018	io
ProQuest: same as WEST	02/27/2018	io
Inventor search	02/27/2018	io
WEST and Inventor searches updated	06/18/2018	io

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

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FIRST SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 3

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG; Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/BMD

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	US34	US-8168179-B2	05-01-2012	HONJO; Tasuku et al.	
	US35	US-8728474-B2	05-20-2014	HONJO; Tasuku et al.	
	US36	US-9067999-B1	06-30-2015	HONJO; Tasuku et al.	
	US37	US-9073994-B2	07-07-2015	HONJO; Tasuku et al.	
	US38	US-9212224-B2	12-15-2015	COGSWELL; John et al.	
	US39	US-9393301-B2	07-19-2016	HONJO; Tasuku et al.	
	US40	US-9402899-B2	08-02-2016	HONJO; Tasuku et al.	
	US41	US-9439962-B2	09-13-2016	HONJO; Tasuku et al.	
	US42	US-20150125463-A1	05-07-2015	COGSWELL; John P. et al.	
	US43	US-20160090417-A1	03-31-2016	COGSWELL; John P. et al.	
	US44	US-20160362489-A1	12-15-2016	YANG; Arvin et al.	
	US45	US-20170051060-A1	02-23-2017	HONJO; Tasuku et al.	
	US46	US-20170088615-A1	03-30-2017	ALAN; J. Korman	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	FP8	WO-2004004771-A1	01-15-2004	ONO PHARMACEUTICAL CO [JP], et al.		☒
	FP9	WO-2015042246-A1	03-26-2015	SQUIBB BRISTOL MYERS CO [US]		☐

Examiner Signature		Date Considered	

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Sheet 2 of 3

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG; Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/BMD

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	FP10	WO-2016176503-A1	11-03-2016	BRISTOL-MYERS SQUIBB COMPANY [US]		☐
	FP11	WO-2016176504-A1	11-03-2016	BRISTOL-MYERS SQUIBB COMPANY [US]		☐

Examiner Signature		Date Considered	

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Sheet 3 of 3

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG; Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/BMD

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author(in CAPITAL LETTERS),title of the article(when appropriate), title of the item (book,magazine,journal,serial,symposium,catalog,etc.),date,page(s),volume-issue number(s),publisher, city and/or country where published.	T[2]
	NPL32	BOWYER, S., et al., "Efficacy and Toxicity of Treatment with the Anti-CTLA-4 Antibody Ipilimumab in Patients with Metastatic Melanoma after Prior Anti-PD-1 Therapy," British Journal of Cancer 114(10):1084-1089, Nature Publishing Group, England (May 2016).	☐
	NPL33	NCCN GUIDELINES (2014), available at: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp#site, last accessed May 30, 2014.	☐
	NPL34	Office Action mailed December 6, 2017, in United States Patent Application No. 15/141,772, Yang, A. et al., filed April 28, 2016, 17 pages.	☐
	NPL35	Office Action mailed April 12, 2018, in United States Patent Application No. 15/141,772, Yang, A. et al., filed April 28, 2016, 13 pages.	☐
	NPL36	TZARTOS, S.J., et al., "Epitope Mapping by Antibody Competition. Methodology and Evaluation of the Validity of the Technique," Methods in Molecular Biology 66:55-66, Humana Press, United States (1996).	☐
	NPL37	International Search Report and Written Opinion for International Application No. PCT/US2016/029878, European Patent Office, Rijswijk, mailed on July 13, 2016, 15 pages.	☐

9396647_1.docx

Examiner Signature	/ILIA I OUSPENSKI/	Date Considered	06/18/2018

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☐	L6	L2 and yang-a $.in.	2
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☐	L4	L2 and @ pd > 20180225	54
☐	L3	L2 and (yang-a $.in. or Bristol $.as. or Bristol $.aanm.)	34
☐	L2	L1 and melanoma.clm.	451
☐	L1	((((pd-1 $ or pd1 $ or pdhd1 $ or cd279 $ or (programmed cell death protein 1) or (programmed cell death protein-1) or (programmed death protein 1) or (programmed death protein-1) or (programmed death 1) or (programmed death-1) or anti-pd-1 $ or anti-pd1 $ or anti-pdhd1 $ or anti-cd279 $ or ".alpha.pd-1$" or ".alpha.pd1$" or ".alpha.pdhd1$" or ".alpha.cd279$" or pd-1-binding or pd1-binding or pdhd1-binding or cd279-binding or (pd-1 binding) or (pd1 binding) or (pdhd1 binding) or (cd279 binding)) or (nivolumab $ or 5C4 or BMS-936558 or bms936558 or MDX-1106 or mdx1106 or GTPL7335 or gtpl-7335 or ONO-4538 or ono4538 or Opdivo $ or pembrolizumab or Keytruda $ or pidilizumab or CT-011 or CT011 or lambrolizumab or MK-3475 or MK3475 or (SCH 900475) or SCH-900475 or SCH900475 or REGN2810 or REGN-2810 or MEDI0680 or MEDI-0680 or AMP514 or AMP-514 or PDR001 or PDR-001 or SHR-1210 or SHR1210 or TSR-042 or TSR042) with (anti-ctla4 or anti-ctla-4 or anti-cd152 or ".alpha.ctla4" or ".alpha.ctla-4" or ".alpha.cd152" or ctla4 $ or ctla-4 $ or cd152 $ or ipilimumab or mdx-010 or 10D1 or tremelimumab or ticilimumab or "CP-675,206" or "CP-675206" or "CP675,206" or CP675206 or "11.2.1")) with (cancer $ or tumor $ or tumour $ or malignan $ or neoplas $ or metasta $ or onco $) and ((("PD-L1$" or pdL1 $ or pdL-1 $ or b7-h1 $ or b7h1 $ or b7-4 $ or cd274 $) with (negativ $ or positiv $ or express $ or level $ or status $))) with ((cancer $ or tumor $ or tumour $ or malignan $ or neoplas $ or metasta $ or onco $) or (sample $ or biopsy $ or patient $ or subject $ or human $))))	1200

END OF SEARCH HISTORY

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor: Arvin YANG	Confirmation No.: 6926
Applicant: Bristol-Myers Squibb Company	Art Unit: 1644
Application No.: 15/141,769	Examiner: OUSPENSKI, Ilia I.
Filing Date: April 28, 2016	Atty. Docket: 3338.0330001/ELE/C-K

Title: **TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY**

Amendment and Reply Under 37 C.F.R. § 1.111

Mail Stop Amendment

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Commissioner:

In reply to the Office Action dated February 28, 2018, Applicant submits the following Amendment and Remarks.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks and Arguments begin on page 6 of this paper.

It is not believed that extensions of time are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any additional fees required to continue prosecution or appeal of this application (including issue fee, fees for net addition of claims or forwarding to appeal) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Currently Amended) A method for treating a melanoma tumor in a human patient ~~subject~~ in need thereof comprising:

(i) identifying a patient having a PD-L1-negative melanoma tumor; and

(ii) administering to the patient:

(a) about 1 mg/kg of an ~~anti-PD-1~~ antibody or an antigen-binding portion thereof that binds specifically to a human PD-1 ("an anti-PD-1 antibody")[[;]] and

~~(b)~~ a dose of an ~~anti-CTLA-4~~ antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4 ("an anti-CTLA-4 antibody") every three weeks for about 4 doses; followed by

(b) a flat dose of the anti-PD-1 antibody of about 240 mg every two weeks or about 480 mg every four weeks.

2. (Currently Amended) A method for treating a melanoma tumor in a human patient in need thereof comprising administering to the patient:

(a) about 1 mg/kg of an ~~anti-PD-1~~ antibody or an antigen-binding portion thereof that binds specifically to a human PD-1 ("an anti-PD-1 antibody")[[;]] and

~~(b)~~ a dose of an ~~anti-CTLA-4~~ antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4 ("an anti-CTLA-4 antibody") every three weeks for about 4 doses; followed by

(b) a flat dose of the anti-PD-1 antibody of about 240 mg every two weeks or about 480 mg every four weeks

~~wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration~~.

3-7. (Cancelled)

8. (Previously Presented) The method of claim 2, wherein the patient is characterized by (i) extended progression-free survival for over 8 months following the administration, (ii)

tumor size reduction at least about 10% compared to the tumor size prior to the administration, or (iii) both (i) and (ii).

9-13. (Cancelled)

14. (Currently Amended) The method of claim 2, further comprising measuring a PD-L1 expression on the melanoma tumor prior to the administration.

15. (Cancelled)

16. (Previously Presented) The method of claim 14, wherein the measuring comprises providing a test tissue sample obtained from the patient, the test tissue sample comprising tumor cells and/or tumor-infiltrating inflammatory cells.

17. (Original) The method of claim 16, wherein the measuring further comprises assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface.

18. (Previously Presented) The method of claim 17, wherein the test tissue sample is a formalin-fixed paraffin-embedded (FFPE) tissue sample, and wherein the presence of PD-L1 is determined using an automated IHC assay.

19. (Cancelled)

20. (Currently Amended) The method of claim 18, wherein the IHC assay is performed using an anti-PD-L1 monoclonal antibody that specifically binds to the PD-L1 and wherein the anti-PD-L1 monoclonal antibody comprises a variable heavy region comprising the amino acid sequence set forth in SEQ ID NO: 1 and a variable light region comprising the amino acid sequence set forth in SEQ ID NO: 2 ~~is selected from the group consisting of 28-8, 28-1, 28-12, 29-8, 5H1, and any combination thereof~~.

21. (Currently Amended) The method of claim 2, wherein ~~the PD-L1-negative melanoma tumor is characterized by having~~ less than about 5% of tumor cells show ~~showing~~ binding to an[[the]] anti-PD-L1 antibody or an antigen-binding portion thereof.

22-24. (Cancelled)

25. (Previously Presented) The method of claim 2, wherein the anti-PD-1 antibody is nivolumab.

26. (Cancelled)

27. (Currently Amended) The method of claim 2, wherein the flat dose of the anti-PD-1 antibody ~~or antigen-binding portion thereof~~ is about 240 mg administered ~~at a dose ranging from 0.1 to 10.0 mg/kg body weight~~ once every two ~~2, 3 or 4~~ weeks.

28. (Currently Amended) The method of claim 2[[27]], wherein the flat dose of the anti-PD-1 antibody ~~or antigen-binding portion thereof~~ is about 480 mg administered ~~at a dose of 1 or 3 mg/kg body weight~~ once every four[[3]] weeks.

29-31. (Cancelled)

32. (Previously Presented) The method of claim 2, wherein the anti-CTLA-4 antibody is ipilimumab.

33. (Cancelled)

34. (Currently Amended) The method of claim 2, wherein the dose of the anti-CTLA-4 antibody ~~or antigen-binding portion thereof~~ is ~~administered at a dose ranging~~ from 0.1 mg/kg to 10.0 mg/kg ~~body weight once every 2, 3 or 4 weeks~~.

35. (Cancelled)

36. (Currently Amended) The method of claim 34, wherein ~~the anti-PD-1 antibody or an antigen-binding portion thereof is administered at a dose of 1 mg/kg body weight once every 3 weeks and~~ the dose of the anti-CTLA-4 antibody ~~or an antigen-binding portion thereof~~ is ~~administered at a dose of~~ 3 mg/kg ~~body weight~~ ~~once every 3 weeks~~.

37-45. (Cancelled)

46. (Previously Presented) The method of claim 2, further comprising administering an anti-cancer agent.

47. (Currently Amended) A kit for treating a patient afflicted with a melanoma tumor, the kit comprising:

(a) a dosage of about 1 mg/kg ~~ranging from 0.1 to 10 mg/kg body weight~~ of an anti-PD-1 antibody ~~or an antigen-binding portion thereof~~;

(b) a dosage ranging from 0.1 mg/kg to 10 mg/kg ~~body weight~~ of an anti-CTLA-4 antibody ~~or an antigen-binding portion thereof~~; and

(c) instructions for using the anti-PD-1 antibody ~~or the antigen-binding portion thereof~~ and the anti-CTLA-4 antibody ~~or the antigen-binding portion thereof~~ in the method of claim [[1]]2.

48. (New) The method of claim 1, wherein the flat dose of the anti-PD-1 antibody is about 240 mg administered once every two weeks.

49. (New) The method of claim 1, wherein the flat dose of the anti-PD-1 antibody is about 480 mg administered once every four weeks.

50. (New) The method of claim 1, wherein the dose of the anti-CTLA-4 antibody is 3 mg/kg.

Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 1, 2, 8, 14, 16-18, 20, 21, 25, 27, 28, 32, 34, 36, and 46-50 are pending in the application, with claims 1 and 2 being the independent claims. Claims 1, 2, 14, 20, 21, 27, 28, 34, 36, and 47 are sought to be amended. Claims 3-7, 9-12, 15, 19, 23, 24, 26, 30, 31, 33, 35, and 37-45 were previously cancelled, and claims 13, 22, and 29 are sought to be canceled without prejudice to or disclaimer of the subject matter therein. New claims 48-50 are sought to be added.

Support for the amendments to the claims can be found throughout the specification as filed. In particular, support for the amendments to claims 1, 2, 27, 28, and 47 can be found, *inter alia*, at paragraphs [0108]-[0110], [0112], [0118], and [0122].

Support for new claims 48-50 can be found throughout the specification as filed, including, *inter alia*, at paragraphs [0108], [0109], and [0116]-[0118].

These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicant respectfully requests that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Rejections under 35 U.S.C. § 101

The Examiner rejected claim 13 under 35 U.S.C. § 101 for allegedly being directed to unpatentable subject matter. Office Action, pp. 2-4. Applicant respectfully disagrees. However, solely to advance prosecution, and without acquiescing to the Examiner's arguments, claim 13 is cancelled rendering the Examiner's rejection of claim 13 moot. Applicant reserves the right to

establish the patentability of the subject matter of claim 13 at a later time in a continuing application. Applicant requests that the Examiner withdraw this rejection of claim 13 under 35 U.S.C. § 101.

Rejections under 35 U.S.C. § 112(b)

The Examiner rejected claims 1, 2, 8, 13, 14, 16-18, 20-22, 25, 27-29, 32, 34, 36, 46, and 47 under 35 U.S.C. § 112(b), for allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor regards as the invention. Office Action, pp. 4-6. Applicant respectfully requests that this rejection be withdraw for the following reasons.

Rejection of Claim 1

The Examiner rejected claim 1 for allegedly being indefinite for reciting a "subject" in the preamble and a "patient" in the body of the claim, rendering the claim unclear. Office Action, pp. 4-5. Solely to advance examination, claim 1 is amended to recite a method of treating a melanoma tumor in a human patient in need thereof. A person of ordinary skill in the art would have been able to readily identify the metes and bounds of amended claim 1, and therefore amended claim 1 is clear and definite. Accordingly, Applicant respectfully requests that the Examiner reconsider and withdraw this rejection of claim 1 under 35 U.S.C. § 112(b).

Rejection of Claim 2

The Examiner rejected claim 2 for allegedly being indefinite for recitation of "PD-L1-negative melanoma tumor." Office Action, p. 5. In particular, the Examiner contends that the definition of the term "PD-L1-negative melanoma tumor" is unclear when viewed in light of dependent claim 21, which recites, *inter alia*, that the PD-L1-negative melanoma tumor is

characterized by having less than about 5% of tumor cells showing binding to the anti-PD-L1 antibody or an antigen-binding portion thereof. *Id.* Applicant respectfully disagrees.

However, solely to advance prosecution, claim 2 is amended to delete the phrase "PD-L1 negative melanoma tumor," rendering the Examiner's rejection moot. Therefore amended claim 2 is clear and definite. Accordingly, Applicant respectfully requests that the Examiner withdraw this rejection of claim 2 under 35 U.S.C. § 112(b).

Rejection of Claim 13

The Examiner rejected claim 13 for allegedly being indefinite. Office Action, p. 4. Applicant respectfully disagrees. However, solely to advance prosecution, and without acquiescing to the Examiner's arguments, claim 13 is cancelled, rendering the Examiner's rejection moot. Accordingly, Applicant respectfully requests that the Examiner withdraw this rejection of claim 13 under 35 U.S.C. § 112(b).

Rejection of Claim 20

The Examiner rejected claim 20 for allegedly being indefinite for reciting "laboratory designations of antibodies," *e.g.*, "28-8" and "28-1." Office Action, p. 5. Applicant respectfully disagrees. A person of ordinary skill in the art would have been able to readily identify the metes and bounds of previously pending claim 20. Nonetheless, solely to advance prosecution, and without acquiescing to the Examiner's arguments, claim 20 is amended to remove recitation of the designations objected to by the Examiner and to recite, *inter alia*, that the anti-PD-L1 monoclonal antibody comprises a variable heavy region comprising the amino acid sequence set forth in SEQ ID NO: 1 and a variable light region comprising the amino acid sequence set forth in SEQ ID NO:

2. Therefore amended claim 20 is clear and definite. Accordingly, Applicant respectfully requests that the Examiner reconsider and withdraw this rejection of claim 20 under 35 U.S.C. § 112(b).

Rejection of Claims 8, 13, 14, 16-18, 20-22, 25, 27-29, 32, 34, 36, 46, and 47

The Examiner further rejected claims 8, 13, 14, 16-18, 20-22, 25, 27-29, 32, 34, 36, 46, and 47 for allegedly being indefinite insofar as they encompass an allegedly indefinite element of the claims on which they depend. Office Action, p. 6. Applicant respectfully disagrees.

Nonetheless, as discussed above, amended claims 1, 2, and 20 are definite under 35 U.S.C. § 112(b). Claims 13, 22, and 29 are cancelled, rendering the Examiner's rejection of these claims moot. Insofar as claims 8, 14, 16-18, 20, 21, 25, 27, 28, 32, 34, 36, 46, and 47 depend from claim 1, 2, or 20, claims 8, 14, 16-18, 20, 21, 25, 27, 28, 32, 34, 36, 46, and 47 are therefore also definite under 35 U.S.C. § 112(b). Accordingly, Applicant respectfully requests that the Examiner withdraw this rejection of claims 8, 13, 14, 16-18, 20-22, 25, 27-29, 32, 34, 36, 46, and 47 under 35 U.S.C. § 112(b).

Rejections under 35 U.S.C. § 112(a)

The Examiner rejected claim 20 under 35 U.S.C. § 112(a) for allegedly failing to comply with the enablement requirement. Office Action, pp. 6-7. In particular, the Examiner contends the specification does not enable the use of the recited 28-8, 28-1, 28-12, 29-8, and 5H1 anti-PD-L1 antibodies. *Id.* at p. 6. Applicant respectfully disagrees.

However, solely to advance prosecution, and without acquiescing to the Examiner's characterization of claim 20 or the specification, claim 20 is amended to recite, *inter alia*, that the anti-PD-L1 monoclonal antibody comprises a variable heavy region comprising the amino acid sequence set forth in SEQ ID NO: 1 and a variable light region comprising the amino acid sequence

set forth in SEQ ID NO: 2. A person of ordinary skill in the art at the time of the filing of the present application would not have required any additional information to practice the full scope of amended claim 20. Therefore, amended claim 20 is enabled. Accordingly, Applicant respectfully requests that the Examiner reconsider and withdraw this rejection to claim 20 under 35 U.S.C. § 112(a).

Rejections under 35 U.S.C. § 102

The Examiner rejected claims 1, 2, 8, 13, 14, 16-18, 20-22, 25, 27-29, 32, 34, 36, 46, and 47 under 35 U.S.C. § 102(a)(1) for allegedly being anticipated by Wolchok et al. (N Engl J Med 2013; 369:122-133) ("Wolchok"). Office Action, pp. 7-8. Applicant respectfully disagrees.

However, solely to advance prosecution, and without acquiescing to the Examiner's arguments, claims 13, 22, and 29 are cancelled, rendering the Examiner's rejection of these claims moot. In addition, claims 1 and 2 are amended to recite, *inter alia*, administering to the patient (a) about 1 mg/kg of an anti-PD-1 antibody and (b) a dose of an anti-CTLA-4 antibody every three weeks for about 4 doses; followed by (b) a flat dose of the anti-PD-1 antibody of about 240 mg every two weeks or about 480 mg every four weeks.

A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. M.P.E.P. 2131 (citing *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 361 (Fed. Cir. 1987)). There must be no difference between the claimed invention and the referenced disclosures, as viewed by a person of ordinary skill in the field of the invention. *Scripps Clinic v. Milgraum*, 52 U.S.P.Q. 2d 1303 (Fed. Cir. 1999).

Wolchok fails to disclose every element of amended claim 1 or 2. In particular, Wolchok does not disclose administering a flat dose of an anti-PD-1 antibody, let alone a flat dose of about 240 mg every two weeks or about 480 mg every four weeks, as recited in amended claims 1 and 2. Therefore, Wolchok does not disclose every element of amended claim 1 or 2 and thus does not anticipate amended claim 1 or 2.

Amended claims 8, 14, 16-18, 20, 21, 25, 27, 28, 32, 34, 36, 46, and 47 depend directly or indirectly from amended claim 1 or 2 and thus include every element of amended claim 1 or 2. Therefore, amended claims 8, 14, 16-18, 20, 21, 25, 27, 28, 32, 34, 36, 46, and 47 are also novel over Wolchok. Accordingly, Applicant respectfully request that the Examiner reconsider and withdraw this rejection of claims 1, 2, 8, 13, 14, 16-18, 20-22, 25, 27-29, 32, 34, 36, 46, and 47 under 35 U.S.C. § 102(a)(1).

Double Patenting Rejection

The Examiner rejected claims 2, 8, 21, 22, 25, 27-29, 32, 34, 36 and 46 as allegedly being unpatentable over claims 1-22 of commonly assigned U.S. Patent No. 9,856,320 ("the '320 patent"), as evidenced by Wolchok. Office Action, pp. 8-9. Though the Examiner concedes that "the claims at issue are not identical," the Examiner contends that "they are not patentably distinct from each other because the instant claims are anticipated by the claims of" the '320 patent. *Id.* at p. 10. Applicant respectfully disagrees.

However, solely to advance prosecution, and without acquiescing to the Examiner's characterization the present claims or the claims of the '320 patent, claims 22 and 29 are cancelled, rendering the Examiner's rejection of these claims moot. In addition, claim 2 is amended to recite, *inter alia*, administering to the patient (a) about 1 mg/kg of an anti-PD-1 antibody and (b) a dose of

an anti-CTLA-4 antibody every three weeks for about 4 doses; followed by (b) a flat dose of the anti-PD-1 antibody of about 240 mg every two weeks or about 480 mg every four weeks. The Examiner points to no claim in the '320 patent that recites administering a flat dose of about 240 mg or about 480 mg of an anti-PD-1 antibody. Further, as noted above, Wolchok does not disclose administering a flat dose of about 240 mg or about 480 mg of an anti-PD-1 antibody. Therefore, amended claim 2 is novel and patentably distinct from the claims of the '320 patent, as cited by the Examiner.

Amended claims 8, 21, 25, 27, 28, 32, 34, 36, and 46 depend directly or indirectly from amended claim 2 and thus include every element of amended claim 2. Therefore, amended claims 8, 21, 25, 27, 28, 32, 34, 36, and 46 are also novel and patentably distinct from the claims of the '320 patent, as cited by the Examiner.

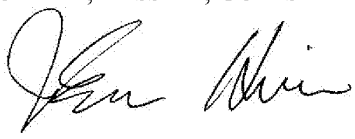
Conclusion

All of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding rejections and that they be withdrawn. Applicant believes that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



Ji-Eun Kim
Attorney for Applicant
Registration No. 68,504

Date: May 29, 2018

1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor: Arvin YANG	Confirmation No.: 6926
Applicant: Bristol-Myers Squibb Company	Art Unit: 1644
Application No.: 15/141,769	Examiner: OUSPENSKI, Ilia I.
Filing Date: April 28, 2016	Atty. Dkt. No.: 3338.0330001/ELE/C-K

Title: **TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY**

First Supplemental Information Disclosure Statement

Mail Stop Amendment

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Commissioner:

Listed on accompanying IDS Forms PTO/SB/08a equivalent and PTO/SB/08b equivalent are documents for consideration in view of 37 C.F.R. §1.56 and in compliance with the duty of disclosure requirements of 37 C.F.R. §§ 1.97 and 1.98. The numbering on this First Supplemental Information Disclosure Statement is a continuation of the numbering in Applicant's Information Disclosure Statement filed on **February 22, 2017** in connection with the above-captioned application.

Where the publication date of a listed document does not provide a month of publication, the year of publication of the listed document is sufficiently earlier than the effective U.S. filing date and any foreign priority date so that the month of publication is not in issue. Applicant has listed publication dates on the attached IDS Forms based on information presently available to the undersigned. However, the listed publication dates should not be construed as an admission that the information was actually published on the date indicated.

Applicant reserves the right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered.

This statement should not be construed as a representation that a search has been made, or that information more material to the examination of the present patent application does not exist. The Examiner is specifically requested not to rely solely on the material submitted herewith.

This is a filing under 37 C.F.R. § 1.97(c). This Information Disclosure Statement is being filed more than three months after the U.S. filing date AND after the mailing date of the first Office Action on the merits, but before the mailing date of a Final Rejection, or Notice of Allowance, or an action that otherwise closes prosecution in the application.

The required fee is provided through online credit card payment authorization in the amount of $240.00 in payment of the fee under 37 C.F.R. § 1.17(p).

A concise explanation of the relevance of the non-English language document appears below in accordance with 37 C.F.R. § 1.98(a)(3).

Document **FP8**, WO-2004004771-A1, is in a foreign language. An English language abstract is appended to the face page of Document **FP8**. Additionally, Document **US45**, US-20170051060-A1, is believed to be an English language equivalent of Document **FP8**.

Copies of documents **FP8-FP11** and **NPL32-NPL37** are submitted. However, in accordance with 37 C.F.R. § 1.98(a)(2)(ii), no copies of U.S. patents and patent application publications cited as documents **US34-US46** on the attached IDS Forms are submitted.

In accordance with the Federal Circuit decision in *Dayco Prods., Inc. v. Total Containment, Inc. 329* F.3d 1358 (Fed. Cir. 2003), Applicant submits herewith Office Actions from the co-pending U.S. Patent Application No. 15/141,772, filed April 28, 2016, as documents **NPL34** and **NPL35**.

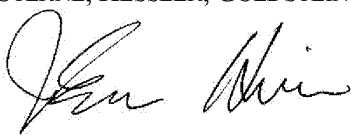
It is respectfully requested that the Examiner initial and return a copy of the enclosed IDS Forms, and indicate in the official file wrapper of this patent application that the documents have been considered.

The identification of this Office Action is not to be construed as a waiver of secrecy as to that application now or upon issuance of the present application as a patent. The Examiner is respectfully requested to consider the cited application and the art cited therein during examination.

The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 19-0036.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



Ji-Eun Kim
Attorney for Applicant
Registration No. 68,504

Date: May 29, 2018

1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

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Atty. Dkt. No. 3338.0330001/ELE/C-K

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FIRST SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 3

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG; Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/BMD

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	US34	US-8168179-B2	05-01-2012	HONJO; Tasuku et al.	
	US35	US-8728474-B2	05-20-2014	HONJO; Tasuku et al.	
	US36	US-9067999-B1	06-30-2015	HONJO; Tasuku et al.	
	US37	US-9073994-B2	07-07-2015	HONJO; Tasuku et al.	
	US38	US-9212224-B2	12-15-2015	COGSWELL; John et al.	
	US39	US-9393301-B2	07-19-2016	HONJO; Tasuku et al.	
	US40	US-9402899-B2	08-02-2016	HONJO; Tasuku et al.	
	US41	US-9439962-B2	09-13-2016	HONJO; Tasuku et al.	
	US42	US-20150125463-A1	05-07-2015	COGSWELL; John P. et al.	
	US43	US-20160090417-A1	03-31-2016	COGSWELL; John P. et al.	
	US44	US-20160362489-A1	12-15-2016	YANG; Arvin et al.	
	US45	US-20170051060-A1	02-23-2017	HONJO; Tasuku et al.	
	US46	US-20170088615-A1	03-30-2017	ALAN; J. Korman	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	FP8	WO-2004004771-A1	01-15-2004	ONO PHARMACEUTICAL CO [JP], et al.		☒
	FP9	WO-2015042246-A1	03-26-2015	SQUIBB BRISTOL MYERS CO [US]		☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Sheet 2 of 3

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG; Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/BMD

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	FP10	WO-2016176503-A1	11-03-2016	BRISTOL-MYERS SQUIBB COMPANY [US]		☐
	FP11	WO-2016176504-A1	11-03-2016	BRISTOL-MYERS SQUIBB COMPANY [US]		☐

Examiner Signature		Date Considered	

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FIRST SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 3 of 3

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG; Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/BMD

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author(in CAPITAL LETTERS),title of the article(when appropriate), title of the item (book,magazine,journal,serial,symposium,catalog,etc.),date,page(s),volume-issue number(s),publisher, city and/or country where published.	T[2]
	NPL32	BOWYER, S., et al., "Efficacy and Toxicity of Treatment with the Anti-CTLA-4 Antibody Ipilimumab in Patients with Metastatic Melanoma after Prior Anti-PD-1 Therapy," British Journal of Cancer 114(10):1084-1089, Nature Publishing Group, England (May 2016).	☐
	NPL33	NCCN GUIDELINES (2014), available at: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp#site, last accessed May 30, 2014.	☐
	NPL34	Office Action mailed December 6, 2017, in United States Patent Application No. 15/141,772, Yang, A. et al., filed April 28, 2016, 17 pages.	☐
	NPL35	Office Action mailed April 12, 2018, in United States Patent Application No. 15/141,772, Yang, A. et al., filed April 28, 2016, 13 pages.	☐
	NPL36	TZARTOS, S.J., et al., "Epitope Mapping by Antibody Competition. Methodology and Evaluation of the Validity of the Technique," Methods in Molecular Biology 66:55-66, Humana Press, United States (1996).	☐
	NPL37	International Search Report and Written Opinion for International Application No. PCT/US2016/029878, European Patent Office, Rijswijk, mailed on July 13, 2016, 15 pages.	☐

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Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. 1 Applicant's unique citation designation number (optional). 2 Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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(12)特許協力条約に基づいて公開された国際出願

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特願2002-194491 2002 年7 月3 日 (03.07.2002) JP
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(71) 出願人 (米国を除く全ての指定国について): 小野薬品工業株式会社 (ONO PHARMACEUTICAL CO., LTD.) [JP/JP]; 〒541-8526 大阪府 大阪市 中央区道修町２丁目１番５号 Osaka (JP).

(71) 出願人 および
(72) 発明者: 本庶 佑 (HONJO,Tasuku) [JP/JP]; 〒606-0001 京都府 京都市 左京区岩倉大鷺町１９－４ Kyoto (JP).

(72) 発明者; および
(75) 発明者/出願人 (米国についてのみ): 湊 長博 (MINATO,Nagahiro) [JP/JP]; 〒606-8105 京都府 京都市 左京区高野上竹屋町１０－３６ Kyoto (JP). 岩井 佳子 (IWAI,Yoshiko) [JP/JP]; 〒602-0855 京都府 京都市 上京区上生洲町２０１－４０３ Kyoto (JP). 柴山 史朗 (SHIBAYAMA,Shiro) [JP/JP]; 〒300-4247 茨城県 つくば市 和台１７番地２ 小野薬品工業株式会社内 Ibaraki (JP).

(74) 代理人: 大家 邦久 (OHIE,Kunihisa); 〒103-0013 東京都 中央区 日本橋人形町２丁目２番６号 堀口第２ビル７階 大家特許事務所 Tokyo (JP).

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(54) Title: IMMUNOPOTENTIATING COMPOSITIONS

(54) 発明の名称: 免疫賦活組成物

(57) Abstract: Compositions for treating cancer or infectious diseases mediated by immunopotentiation which is caused by inhibiting a immunosuppressive signal induced by PD-1, PD-L1 or PD-L2; a therapeutic method using the same; methods of screening an immunopotentiator, an anticancer substance or a substance against infectious diseases contained in these compositions as the active ingredient; a cell line to be used in these screening methods; a method of evaluating an anticancer agent; and a mammal having cancer cells transplanted therein which is to be used in the evaluation. The above compositions inhibiting the function of PD-1, PD-L1 or PD-L2 are useful in treating cancer or infectious diseases.

(57) 要約: PD-1、PD-L1またはPD-L2によって誘導される免疫抑制シグナルを阻害した結果惹起される免疫賦活を介した癌の治療若しくは感染症の治療のための組成物およびそれらを用いた治療方法、その有効成分として含まれる免疫賦活物質、癌治療物質若しくは感染症治療物質のスクリーニング方法、それらスクリーニング方法に使用される細胞株、癌治療物質を選別する評価法、およびその評価に用いられる癌細胞移植哺乳動物に関する。PD-1、PD-L1、またはPD-L2の機能を阻害する本発明の組成物は、癌または感染症に対する治療に有用である。

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明　細　書

免疫賦活組成物

技術分野

本発明は、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２によって誘導される免疫抑制シグナルを阻害することを特徴とする免疫賦活、癌の治療若しくは感染症の治療のための組成物、およびそれらを用いる治療方法に関する。

さらに詳しく言えば、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２によって誘導される免疫抑制シグナルを阻害した結果惹起される免疫賦活を介した癌の治療若しくは感染症の治療ための組成物、それらを用いる治療方法、その組成物に有効成分として含まれる免疫賦活物質、癌治療物質若しくは感染症治療物質のスクリーニング方法、それらスクリーニング方法に使用される細胞株、癌治療物質を選別する評価法、およびその評価に用いられる癌細胞移植哺乳動物に関する。

背景技術

免疫療法は、ほとんどの薬物療法において避け難い副作用が軽減され、極めて特異性の高い治療方法として期待されている。特に、癌治療や感染症治療における薬物療法が、患者に対して大きな負担を課す治療方法であるために、患者のＱＯＬ（クオリティ・オブ・ライフ）の回復が重要視されているが、免疫療法は、ヒトがもともと備え持っている免疫反応を外因的な方法によって賦活化させ、薬物投与による負担の一部を肩代わりさせることによって、患者のＱＯＬを回復させる目的のもとに行なうことができる。

免疫の賦活化は、Ｔリンパ球細胞の免疫反応を活性化させる方法で行なうことができる。Ｔ細胞の活性化には、抗原レセプター（ＴＣＲ）を介した刺

激だけでなく、共役刺激分子群（例えば、ＣＤ２８）を介した付加的な刺激誘導が必要であるといわれている。一方、最近、共役刺激分子群と相同的な構造を有する分子群、ＣＴＬＡ－４とＰＤ－１が発見され、抗原レセプター（ＴＣＲ）シグナルを抑制するシグナルを発していることが報告されている。Ｔ細胞の活性化の方法として、この共役抑制分子の機能を抑制することも有効な１つの手段であると考えられている。

ＰＤ－１は免疫グロブリンファミリーに属する５５ｋＤのＩ型膜タンパクとしてクローニングされた（The EMBO Journal，1992年，第11巻，第11号，p.3887～3895、特開平5-336973号公報、特開平7-291996号公報）。ヒトＰＤ－１ｃＤＮＡは、EMBL / GenBank Acc. No. NM_005018に示される塩基配列で構成され、マウスＰＤ－１ｃＤＮＡは、Acc. No. X67914に示される塩基配列で構成され、それら発現は、胸腺細胞においてはＣＤ４－ＣＤ８－からＣＤ４＋ＣＤ８＋細胞に分化する際に認められる（International Immunology，1996年，第18巻，第5号，p.773～780、Journal of Experimental Medicine，2000年，第191巻，第5号，p.891～898）。また、末梢におけるＰＤ－１の発現は、抗原レセプターからの刺激により活性化したＴ細胞、Ｂ細胞（International Immunology，1996年，第18巻，第5号，p.765～772参照）または活性化マクロファージを含む骨髄細胞に認められることが報告されている。

ＰＤ－１の細胞内領域には、ＩＴＩＭモチーフ（Immunoreceptor Tyrosine - based Inhibitory Motif）があり、免疫反応に対する抑制ドメインと考えられている。さらに、ＰＤ－１欠損マウスが、糸球体腎炎、関節炎といったループス様自己免疫病（Ｃ５７ＢＬ／６遺伝子背景の場合）（International Immunology，1998年，第10巻，第10号，p.1563～1572、Immunity，1999年，第11巻，第２号，p.141～151）や拡張性心筋症様疾患（ＢＡＬＢ／ｃ遺伝子背景の場合）（Science，2001年，第291巻，第5502号，p.319～332）を発症することから、ＰＤ－１が自己免疫疾患発症、特に、末梢自己免疫寛容の制

御因子であることも示唆されている。

ＰＤ－１のリガンドであるＰＤ－Ｌ１（ヒトＰＤ－Ｌ１ｃＤＮＡはEMBL/GenBank Acc. No. AF233516、マウスＰＤ－Ｌ１ｃＤＮＡはNM_021893で示される塩基配列で構成される。）は、活性化した単球や樹状細胞などのいわゆる抗原提示細胞に発現している（Journal of Experimental Medicine，2000年，第19巻，第7号，p.1027～1034）。これら細胞は、Ｔリンパ球細胞に対して、さまざまな免疫誘導シグナルを誘導する相互作用分子を提示しており、ＰＤ－Ｌ１は、ＰＤ－１による抑制シグナルを誘導する分子の１つである。ＰＤ－Ｌ１リガンド刺激は、ＰＤ－１を発現しているＴリンパ球細胞の活性化（細胞増殖、各種サイトカイン産生誘導）を抑制することが示されている。さらに、ＰＤ－Ｌ１の発現は、免疫担当細胞のみでなく、ある種の腫瘍細胞株（単球性白血病由来細胞株、肥満細胞種由来細胞株、肝癌由来細胞株、神経芽細胞種由来細胞株、乳癌由来各種細胞株）でも確認されている（Nature Immunology，2001年，第2巻，第3号，p.261～267参照）。

ＰＤ－Ｌ２（ヒトＰＤ－Ｌ２ｃＤＮＡはEMBL / GenBank Acc. No. NM_025239、マウスＰＤ－Ｌ２ｃＤＮＡはNM_021896で示される塩基配列で構成される（Nature Immunology，2001年，第2巻，第3号，p.261～267）。）は、ＰＤ－１の第２番目リガンドとして同定されたが、その発現と機能はＰＤ－Ｌ１とほぼ同じであることが報告されている。

ＰＤ－１に代表される共役抑制分子からの抑制シグナルは、抗原レセプター（ＴＣＲ）および共役刺激分子によるポジティブなシグナルを適性に制御するメカニズムによって、リンパ球発生または成熟過程での免疫寛容や自己抗原に対する異常な免疫反応を制御していると考えられている。また、ある種の腫瘍やウイルスは、直接的もしくは間接的なメカニズムによって、Ｔ細胞の活性化および増殖を遮断し、これら共役抑制分子を自らに対する宿主免疫反応を衰弱させるのに利用していると考えられている（Cell，1992年，第

71巻，第7号，p.1093～1102、Science，1993年，第259巻，第5093号，p.368～370参照）。さらに、T細胞の機能障害に起因すると考えられている疾患の一部では、これら共役抑制分子の異常がT細胞の機能障害を起していると考えられている。

発明の開示

本発明の課題は、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２による抑制シグナルを阻害して、免疫賦活させる組成物およびこの機構を介した癌治療または感染症治療のための組成物の提供にある。

本発明者らは、癌治療または感染症治療における新たな標的として、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２に注目し、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２による抑制シグナルを阻害する物質が、免疫機能の回復、さらには賦活機構を介して癌の増殖を阻害することを見出した。さらに、感染したウイルスの排除にＰＤ－１シグナル、具体的にはＰＤ－１とＰＤ－Ｌ１またはＰＤ－１とＰＤ－Ｌ２の相互作用が関与していることを見出した。これら事実に基いて、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２による抑制シグナルを阻害する物質が、癌または感染症に対して治療効果を有することを見出し、本発明を完成した。

すなわち、本発明は、

１．ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を含有してなる免疫賦活組成物、

２．ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を含有してなる癌治療組成物、

３．癌転移を抑制する組成物である前項２記載の癌治療組成物、

４．ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を含有してなる感染症治療組成物、

５．免疫賦活を介して作用することを特徴とする前項２または３記載の癌治療組成物、

６．免疫賦活を介して作用することを特徴とする前項４記載の感染症治療組成物、

７．ＰＤ－１とＰＤ－Ｌ１若しくはＰＤ－１とＰＤ－Ｌ２の相互作用阻害物質、ＰＤ－１の細胞内シグナル阻害物質、およびＰＤ－１、ＰＤ－Ｌ１若しくはＰＤ－Ｌ２の産生阻害物質から選択される一以上の免疫抑制シグナル阻害物質である前項１乃至６のいずれかに記載の組成物、

８．ＰＤ－１抗体、ＰＤ－Ｌ１抗体、可溶化ＰＤ－１、および可溶化ＰＤ－Ｌ１から選択される一以上のＰＤ－１とＰＤ－Ｌ１の相互作用阻害物質である前項７記載の組成物、

９．国際受託番号 FERM BP-8392 で識別されるハイブリドーマが産生する抗ヒトＰＤ－１抗体、非ヒト抗体をヒト化させた抗ＰＤ－１抗体、および完全ヒト型抗ヒトＰＤ－１抗体から選択されるＰＤ－１抗体である前項８記載の組成物、

１０．遺伝子改変によりＰＤ－１発現が阻害されたリンパ球細胞が、免疫抑制シグナル阻害物質である前項１乃至６のいずれかに記載の組成物、

１１．ＰＤ－１とＰＤ－Ｌ１若しくはＰＤ－１とＰＤ－Ｌ２の相互作用阻害物質、ＰＤ－１の細胞内シグナル阻害物質、またはＰＤ－１、ＰＤ－Ｌ１若しくはＰＤ－Ｌ２の産生阻害物質が、タンパク質、ポリペプチド若しくはペプチド、ポリヌクレオチド若しくはポリヌクレオシド、抗体若しくはそれらの誘導体、有機合成化合物、無機化合物、および天然物から選択される一以上の物質である前項７記載の組成物、

１２．ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を投与することからなる免疫賦活方法、

１３．ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物

質を投与することからなる癌治療方法、

１４．癌転移を抑制する前項１３記載の癌治療方法、

１５．ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を投与することからなる感染症治療方法、

１６．免疫賦活を介して作用することを特徴とする前項１３または１４記載の癌治療方法、

１７．免疫賦活を介して作用することを特徴とする前項１５記載の感染症治療方法、

１８．ＰＤ－１とＰＤ－Ｌ１若しくはＰＤ－１とＰＤ－Ｌ２の相互作用阻害物質、ＰＤ－１の細胞内シグナル阻害物質、およびＰＤ－１、ＰＤ－Ｌ１若しくはＰＤ－Ｌ２の産生阻害物質から選択される一以上の免疫抑制シグナル阻害物質である前項１２乃至１７のいずれかに記載の方法、

１９．ＰＤ－１抗体、ＰＤ－Ｌ１抗体、可溶化ＰＤ－１、および可溶化ＰＤ－Ｌ１から選択される一以上のＰＤ－１とＰＤ－Ｌ１の相互作用阻害物質である前項１８記載の方法、

２０．国際受託番号 FERM BP-8392 で識別されるハイブリドーマが産生する抗ヒトＰＤ－１抗体、非ヒト抗体をヒト化させたＰＤ－１抗体、および完全ヒト型抗ヒトＰＤ－１抗体から選択されるＰＤ－１抗体である前項１９記載の方法、

２１．遺伝子改変によりＰＤ－１発現が阻害されたリンパ球細胞が、免疫抑制シグナル阻害物質である前項１２乃至１７のいずれかに記載の方法、

２２．ＰＤ－１とＰＤ－Ｌ１若しくはＰＤ－１とＰＤ－Ｌ２の相互作用阻害物質、ＰＤ－１の細胞内シグナル阻害物質、またはＰＤ－１、ＰＤ－Ｌ１若しくはＰＤ－Ｌ２の産生阻害物質が、タンパク質、ポリペプチド若しくはペプチド、ポリヌクレオチド若しくはポリヌクレオシド、抗体若しくはそれらの誘導体、有機合成化合物、無機化合物、および天然物から選択される１つ

以上の物質である前項１８記載の方法、

２３．免疫賦活組成物を製造するためのＰＤ－1、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質の使用、

２４．癌治療組成物を製造するためのＰＤ－1、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質の使用、

２５．癌治療組成物が、癌転移抑制組成物である前項２４記載の物質の使用、

２６．感染症治療組成物を製造するためのＰＤ－1、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質の使用、

２７．ＰＤ－Ｌ１またはＰＤ－Ｌ２を発現するように形質転換されたスクリーニング用癌細胞株、

２８．前項２７記載の細胞、リンパ球細胞および被験物質を接触させて、前項２７記載の細胞へのリンパ球細胞の免疫反応に対する被験物質の増強作用を評価することを特徴とする免疫賦活物質のスクリーニング方法、

２９．癌細胞である前項２７記載の細胞、リンパ球細胞および被験物質を接触させて、その癌細胞へのリンパ球細胞の免疫反応に対する被験物質の増強作用またはその腫瘍細胞の増殖に対する阻害作用を評価することを特徴とする癌治療物質のスクリーニング方法、

３０．病原体を感染させた前項２７記載の細胞またはＰＤ－Ｌ１若しくはＰＤ－Ｌ２を発現する細胞、リンパ球細胞および被験物質を接触させて、その感染細胞へのリンパ球細胞の免疫反応に対する被験物質の増強作用または病原体増殖に対する阻害作用を評価することを特徴とする感染症治療物質のスクリーニング方法、

３１．前項２７記載の癌細胞株を移植して作出した哺乳動物、

３２．前項３１記載の哺乳動物に被験物質を投与し、被験物質による移植癌細胞の増殖に対する抑制率またはその被移植哺乳動物の生存率を評価することを特徴とする癌治療物質の選別方法に関する。

本発明中のＰＤ－１、ＰＤ－Ｌ１またはＰＤ－Ｌ２は、それぞれマウス、ラット、ハムスター、モルモット、イヌ、ブタ、サルまたはヒトを含む霊長類の哺乳動物を由来とするものを含む。好ましくは、ヒトＰＤ－１、ヒトＰＤ－Ｌ１およびヒトＰＤ－Ｌ２である。

本発明中のＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２による免疫抑制シグナルは、少なくともＰＤ－１とＰＤ－Ｌ１またはＰＤ－１とＰＤ－Ｌ２との相互作用、ＰＤ－１の細胞内シグナルから構成される。さらに、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２分子自身の産生もこれに含まれる。

本発明中のＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２による免疫抑制シグナルは、ＰＤ－１とＰＤ－Ｌ１またはＰＤ－１とＰＤ－Ｌ２との相互作用またはＰＤ－１の細胞内シグナルの直接的あるいは間接的な阻害によって阻害される。これらの阻害活性を有する物質として、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２にそれぞれ選択的に結合する物質が挙げられる。好ましくは、例えば、タンパク質、ポリペプチド若しくはペプチド、ポリヌクレオチド若しくはポリヌクレオシド、抗体若しくはそれら誘導体、有機合成化合物、無機化合物、または天然物が挙げられる。特に、特異性に優れた物質として、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２に対する抗体が挙げられる。

また、当該免疫抑制シグナルは、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２分子自身の産生の阻害によっても阻害される。

ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２に対する抗体は、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２による免疫抑制シグナルを阻害するものであれば、ヒト由来抗体、マウス由来抗体、ラット由来抗体、ウサギ由来抗体またはヤギ由来抗体のいずれの抗体でもよく、さらにそれらのポリクローナル若しくはモノクローナル抗体、完全型若しくは短縮型（例えば、Ｆ（ａｂ'）$_2$、Ｆａｂ'、ＦａｂまたはＦｖ断片）抗体、キメラ化抗体、ヒト化抗体または完全ヒト型抗体のいずれのものでもよい。

そのような抗体は、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の細胞外領域の部分タンパク質を抗原として、公知の抗体または抗血清の製造法に従って製造することができる。細胞外領域の部分タンパク質は、公知のタンパク質発現ならびに精製法によって調製することができる。

ポリクローナル抗体は、公知の方法によって製造することができる。例えば、抗原タンパク質あるいはそれとキャリアー蛋白質との混合物で、適当な動物に免疫を行ない、その免疫動物から抗原タンパク質に対する抗体含有物を採取して、抗体の分離精製を行なうことにより製造することができる。用いられる動物としては、マウス、ラット、ヒツジ、ヤギ、ウサギ、モルモットが一般的に挙げられる。抗体産生能を高めるため、完全フロイントアジュバントや不完全フロイントアジュバントを抗原タンパク質と共に投与することができる。投与は、通常約２週毎に１回ずつ、計約３～１０回程度行なうのが一般的である。ポリクローナル抗体は、上記の方法で免疫された動物の血液、腹水などから採取することができる。抗血清中のポリクローナル抗体価の測定は、ＥＬＩＳＡ法によって測定することができる。ポリクローナル抗体の分離精製は、例えば、抗原結合固相あるいはプロテインＡあるいはプロテインＧなどの活性吸着剤を用いた精製法、塩析法、アルコール沈殿法、等電点沈殿法、電気泳動法、イオン交換体による吸脱着法、超遠心法、ゲルろ過法などの免疫グロブリンの分離精製法に従って行なうことができる。

抗体製剤としては、モノクローナル抗体あるいはその修飾体がより好ましい。

モノクローナル抗体産生細胞の作製は、抗原で免疫された動物から抗体価の認められた個体を選択し、最終免疫の２～５日後に脾臓またはリンパ節を採取し、それらに含まれる抗体産生細胞を同種または異種動物の骨髄腫細胞と融合させることにより、継代培養可能なモノクローナル抗体産生ハイブリドーマを作製することにより行なうことができる。抗原タンパク質の投与は、

抗体産生が可能な部位にそれ自体あるいは担体、希釈剤と共に行なう。投与には、抗体産生能を高めるため、完全フロイントアジュバントや不完全フロイントアジュバントを投与するのが一般的である。また、“ＤＮＡ免疫”と呼ばれる方法によっても、動物を免疫することができる。この方法は、免疫動物の後足前脛骨筋にカルジオトキシン（Cardiotoxin）を処置し、さらに抗原タンパク質を発現するベクターを導入した後、組織修復の過程でベクターが筋細胞に取りこまれ、タンパク質を発現する現象を利用した方法である（Nature Immunology，2001年，第2巻，第3号，p.261～267）。

免疫される動物としては、マウス、ラット、ヒツジ、ヤギ、ウサギまたはモルモットが可能であるが、好ましくはマウスまたはラットが用いられる。融合操作は、コーラー（Kohler）とミルシュタイン（Milstein）の方法（Nature，1975年，第256巻，第5517号，p.495～497）で実施することができ、融合促進剤としては、ポリエチレングリコール（ＰＥＧ）やセンダイウィルスなどが用いられる。骨髄腫細胞としては、Ｐ３Ｕ１、ＮＳ１、ＳＰ２／０、ＡＰ１などの骨髄腫細胞が挙げられるが、通常Ｐ３Ｕ１がよく利用される。モノクローナル抗体産生細胞の選別は、例えば、抗原タンパク質を直接あるいは担体と共に吸着させた固相にハイブリドーマ培養上清を添加することによるＥＬＩＳＡ法などにより検出して行なうことができる。さらに、ハイブリドーマ培養上清の抗体価は、ＥＬＩＳＡ法によって測定できる。モノクローナル抗体の分離精製は、上記のポリクローナル抗体の分離精製と同様の免疫グロブリンの分離精製法に従って行なうことができる。具体的には、国際受託番号FERM BP-8392で識別されるハイブリドーマが産生する抗ヒトＰＤ－１抗体あるいは国際受託番号FERM BP-8396で識別されるハイブリドーマが産生する抗マウスＰＤ－Ｌ１抗体である。

国際受託番号FERM BP-8392で識別されるハイブリドーマは、2002年12月19日付で日本国茨城県つくば市東１丁目１番地１　中央第６（郵便番号

305-8566）、独立行政法人産業技術総合研究所特許生物寄託センターに受託番号 FERM P-19162 で寄託され、2003 年 6 月 5 日付で国際寄託に移管されている。また、国際受託番号 FERM BP-8396 で識別されるハイブリドーマは、2002 年 6 月 25 日付で同センターに受託番号 FERM P-18908 で寄託され、2003 年 6 月 11 日付で国際寄託に移管されている。

抗体断片とは、Ｆ（ａｂ'）$_2$、Ｆａｂ'、Ｆａｂまたはｓｃｆｖ抗体フラグメントであり、プロテアーゼ酵素により処理し、場合により還元して得ることができる。

Ｆ（ａｂ'）$_2$抗体フラグメントは、精製されたモノクローナル抗体をペプシンで完全に消化し、イオン交換クロマトグラフィー、ゲルろ過、プロテインＡあるいはプロテインＧカラムなどのアフィニティークロマトグラフィーのいずれかの方法により精製することができる。ペプシンの消化時間は、Ｉｇサブタイプにより異なるため、適当に調製することが必要である。Ｆａｂ'抗体フラグメントは、調製したＦ（ａｂ'）$_2$を２－メルカプトエチルアミンで部分還元することによって作製することができる。また、Ｆａｂ抗体フラグメントは、システイン存在下で消化酵素パパインで直接消化し、精製して作製することができる。

また、モノクローナル抗体は、抗体のアミノ酸配列を決定したり、ハイブリドーマから単離できる抗体をコードするＤＮＡ配列を利用して、遺伝子組換え技術により改変し、改変抗体やハイブリッド抗体を産生させることも可能である。例えば、通常の完全型抗体ではなく単鎖抗体として作製することもできる。ｓｃＦｖ抗体（Single Chain Fv）は、ジョストらの方法（Journal of Biological Chemistry, 1994 年, 第 269 巻, 第 42 号, p.26267～26273）により行なうことができる。重鎖および軽鎖の可変領域をそれぞれコードするＤＮＡ断片を中性アミノ酸（グリシンあるいはセリン）をコードするスペーサーで連結し、この融合ＤＮＡを含む発現ベクターを適当な宿主細胞で発現させ

ることによって、本来の抗体の特性と親和性を保持する単鎖の抗体を作製することができる。

非ヒト抗体をヒトの治療に用いる場合、その抗体の抗原性を減少させることが不可欠である。患者の抗体に対する免疫反応は、有効な治療期間を短縮させる場合が多く、抗体をヒト化あるいは完全ヒト型化させ、抗体の抗原性を減らす工程が必要である。ヒトへの投与が可能となるよう改変されたヒト化抗体とは、同抗体をヒトに投与したときに薬理学的に許容し得る程度に、抗原性が減少あるいは血中動態が改善するよう改変された抗体である。

本発明の明細書中のヒトＰＤ－１抗体またはヒトＰＤ－Ｌ１抗体は、これをヒト化あるいは完全ヒト型化した抗体をも含む。

ヒト化抗体は、ヒト以外の哺乳動物に免疫して作製された非ヒト抗体の一部をヒト抗体の一部に置換することによって作製できる。具体的には、ヒト抗体の定常領域をコードする遺伝子とのキメラを構築することによって作製できることが知られている（Proc. Natl. Acad. Sci. （USA），1987 年，第 84 巻，p.3439～3443、Journal of Immunology, 1987 年，第 139 巻，第 1 号，p.3521）。ヒトの定常領域のＤＮＡ配列は文献に記載されており、その定常領域遺伝子は既知のクローンから容易に入手できる。続いて、抗体の可変領域をコードするＤＮＡ配列をヒトの定常領域の配列に融合させる。ヒトの定常領域のアイソタイプは所望のエフェクター機能または抗体依存細胞性細胞毒性における活性によって選択できる。好ましいアイソタイプはＩｇＧ１、ＩｇＧ３およびＩｇＧ４である。また、ヒト軽鎖定常領域、κ鎖またはλ鎖のいずれも用いることができる。このヒト化キメラ抗体は通常の方法によって発現させることができる。

完全ヒト型抗体は、ヒト免疫グロブリンの定常領域遺伝子を導入されたマウス（ゼノマウス（Chemical Biology，2000 年，第 7 巻，第 8 号，p.R185-6）、ヒューマブマウス（infection and immunity, 2002 年，第 70 巻，第 2 号，p.612-9）、

ＴＣマウス（Biotechnology and Genetics Enginnering Revew, 2002年, 第19巻, p.73～82）、ＫＭマウス（Cloning Stem Cells, 2002年, 第4巻, 第1号, p.91-102））を利用して作製することができ、さらにそれらマウスから単離した抗体産生リンパ球をハイブリドーマにして、目的の抗体を量産させることができる。また、ファージ・ディスプレー法（FEBS Letter, 1998年, 第441巻, p.20-24）によっても作製することができる。この方法は、環状一本鎖ＤＮＡにヒト抗体遺伝子を組み込んだファージを利用して、ファージを構成する外殻タンパク質と融合した形で、ヒト型抗体をファージの表面に発現されることができる。

ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２に結合するポリペプチドまたはその誘導体は、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２のそれぞれの部分タンパク質であって、免疫抑制シグナルを誘導しないものが挙げられる。ＰＤ－１の免疫抑制シグナルの誘導には、ＰＤ－１が抗原レセプターの近傍に存在することが不可欠であり、そのためには抗原提示細胞、腫瘍あるいは癌細胞に存在するＰＤ－Ｌ１またはＰＤ－Ｌ２との相互作用による拘束が必要である。したがって、細胞外ドメインのみであってＰＤ－１と相互作用する部分を有する可溶化ＰＤ－Ｌ１または可溶化ＰＤ－Ｌ２は、ＰＤ－１の免疫抑制シグナルを阻害することができる。逆に、同様の構造を有し、ＰＤ－Ｌ１またはＰＤ－Ｌ２に結合できる可溶化ＰＤ－１も、免疫抑制シグナルを阻害することができる。これら可溶化タンパク質は、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２に結合するのに必要十分な細胞外領域を含むものであればよく、公知のタンパク質発現法および精製法によって調製することができる。

ＰＤ－１とＰＤ－Ｌ１またはＰＤ－１とＰＤ－Ｌ２の相互作用阻害物質がタンパク質またはポリペプチドであり、それらの相互作用に必須の領域が、連続するポリペプチドのみによって構成されている場合、そのようなポリペプチド断片は、互いの拮抗物質になり得る。さらに、このポリペプチド断片

を化学的に修飾されたあるいはポリペプチド断片の立体構造をもとにコンピュータによって設計された分子群から、より強力な活性を有する拮抗物質を同定することが可能となる。また、相互作用領域のタンパク質立体構造解析データをもとにコンピュータによって設計された分子群の中から、最適の拮抗分子をより効率的に選択することもできる。

また、ＰＤ－１とＰＤ－Ｌ１またはＰＤ－１とＰＤ－Ｌ２のの相互作用を阻害する物質を直接スクリーニングすることができる。そのような物質は、タンパク質、ポリペプチド若しくはペプチド、ポリヌクレオチド若しくはポリヌクレオシド、非ペプチド性化合物、有機合成化合物、または天然物（例えば、発酵生産物、細胞抽出物、植物抽出物、動物組織抽出物）のライブラリーから同定することが可能である。

ＰＤ－１細胞内ドメインの抑制シグナルは、ＰＤ－１細胞内ドメインであるＩＴＩＭに結合した脱リン酸化酵素（例えば、ＳＨＰ－１、２（Sathish JG, Journal of Immunology），2001 年，第 166 巻，第 3 号，p.1763-70）が、抗原受容体複合体の細胞内複合体に接触することによって生じることから、総じて抗原受容体複合体とＰＤ－１の接触の阻害によって阻害される。抑制シグナルを阻害する物質として、ＩＴＩＭのチロシン残基のリン酸化を阻害する物質、ＩＴＩＭへの脱リン酸化酵素の結合を阻害する物質、その脱リン酸化酵素の活性を直接阻害する物質などが挙げられる。抗原受容体複合体としては、Ｔ細胞受容体複合体、Ｂ細胞受容体複合体が挙げられる。

ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の産生は、特定のポリヌクレオチド若しくはポリヌクレオシド、有機合成化合物、無機化合物、または天然物などによって阻害できる。特に、好適なポリヌクレオチドまたはポリヌクレオシドとして、リボザイムと呼ばれるアンチセンスヌクレオチド誘導体が挙げられる。これは、ＰＤ－１、ＰＤ－Ｌ１または、ＰＤ－Ｌ２のｍＲＮＡに相補的なポリヌクレオチド誘導体を、対象の発現細胞に導入することによ

って、発現するｍＲＮＡが破壊される機構を利用したものである。さらに、ベクターは、患者から採取したリンパ球前駆細胞に対して、ＰＤ－１の発現を阻害するような遺伝子操作に使用でき、その操作された細胞は、増殖、分化、そして、活性化させて、再び患者に投与する細胞医療に利用することができる。特に、癌に対する免疫療法においては、リンパ球前駆細胞の成熟化および活性化の際に、標的細胞の特異抗原を添加することによって、標的細胞に特異的で、よりクローナルなリンパ球細胞を調製することができる。

本発明のスクリーニング法は、細胞機能を測定する方法で行なうことができる。同方法で使用されるＰＤ－Ｌ１またはＰＤ－Ｌ２を発現するように形質転換したスクリーニング用癌細胞株とは、ＰＤ－Ｌ１またはＰＤ－Ｌ２を発現するように構築された発現ベクターを公知の方法によって細胞に導入し、一過性あるいは安定的に形質転換された癌細胞株を含む。使用される癌細胞株としては、サルＣＯＳ－１細胞、ＣＯＳ－７細胞、Ｖｅｒｏ、チャイニーズハムスター細胞ＣＨＯ（以下、ＣＨＯ細胞と略記する。）、ｄｈｆｒ遺伝子欠損チャイニーズハムスター細胞ＣＨＯ（以下、ＣＨＯ（ｄｈｆｒ－）細胞と略記する。）、マウスＬ細胞、マウスＡｔＴ－２０、マウスミエローマ細胞、ラットＧＨ３、ＨＥＫ２９３Ｔ細胞、ヒトＦＬ細胞などが用いられる。特に、動物細胞を形質転換する場合には、例えば、「細胞工学別冊８新細胞工学実験プロトコール」（秀潤社、1995年、第263）や「Virology」（1973年、第52巻、第456号）に記載の方法に従って行なうことができる。

また、自然にＰＤ－Ｌ１またはＰＤ－Ｌ２を発現する細胞を用いることもできる。そのような細胞として、白血球細胞、好ましくは単球、マクロファージ若しくは抗原提示細胞、上皮系細胞、腫瘍細胞、癌細胞またはそれらの細胞株などである。腫瘍細胞または癌細胞として使用できる細胞として、例えば、Ｐ３８Ｄ１細胞、Ｐ８１５細胞、ＮＢ４１Ａ３細胞、ＭＤＡ－２３１細胞、ＳＫＢＲ－３細胞、ＭＣＦ－７細胞、ＢＴ４７４細胞、Ｊ５５８Ｌ細

胞、Ｐ３Ｕ１細胞、ＰＡＩ細胞、Ｘ６３細胞またはＳＰ２／０細胞を用いることができる。

ＰＤ－Ｌ１若しくはＰＤ－Ｌ２を自然に発現する細胞または強制的に発現させた細胞であって、病原体を感染させた細胞を用いることもできる。感染させる病原体としては、ヒト肝炎ウイルス（Ｂ型肝炎、Ｃ型肝炎、Ａ型肝炎）またはＥ型肝炎）、ヒトレトロウイルス、ヒト免疫不全ウイルス（ＨＩＶ１、ＨＩＶ２）、ヒトＴ細胞白血病ウイルス、ヒトＴリンパ向性ウイルス（ＨＴＬＶ１、ＨＴＬＶ２）、単純ヘルペスウイルス１型若しくは２型、エプスタイン・バーウイルス、サイトメガロウイルス、水痘－帯状疱疹ウイルス、ヒトヘルペスウイルス６を含むヒトヘルペスウイルス、ポリオウイルス、麻疹ウイルス、風疹ウイルス、日本脳炎ウイルス、おたふくウイルス、インフルエンザウイルス、アデノウイルス、エンテロウイルス、ライノウイルス、重症急性呼吸器症候群（ＳＡＲＳ）を発症するウイルス、エボラウイルス、西ナイルウイルス、またはそれらを人為的に改変されたウイルスが挙げられる。その他の病原体として、例えば、病原性原生動物（例えば、トリパノソーマ、マラリアおよびトキソプラズマ）、細菌（例えば、マイコバクテリウム、サルモネラおよびリステリア）、または真菌（例えば、カンジダ）が挙げられる。

本発明のスクリーニング法で使用されるリンパ球細胞としては、Ｔ細胞またはＢ細胞であり、好ましくは、細胞傷害性Ｔリンパ球細胞（ＣＴＬ）である。また、本発明のスクリーニング法におけるリンパ球細胞の免疫反応は、細胞傷害反応（例えば、腫瘍免疫反応）、混合リンパ球反応、サイトカイン、抗体、補体若しくはその他細胞表面抗原の産生、または細胞増殖が挙げられる。

本発明の免疫賦活または癌治療組成物の有効成分のスクリーニング法は、具体的には、細胞傷害性Ｔリンパ球の対象細胞に対する細胞傷害活性を測定

し、その活性に対する被験物質の効果を定量することによって行なうことができる。この方法は、ＰＤ－１を自然に発現する細胞傷害性Ｔリンパ球細胞（ＣＴＬ）あるいは細胞株（例えば、２Ｃ細胞）および同系マウス由来であってＰＤ－Ｌ１またはＰＤ－Ｌ２を自然に発現するもしくは強制的に発現させた細胞の混合培養に対して、被験物質を添加することによる細胞傷害活性の回復あるいは増強を定量するものである。本法の特徴は、ＰＤ－Ｌ１またはＰＤ－Ｌ２を発現していない細胞に対する細胞傷害活性に比べ、ＰＤ－Ｌ１またはＰＤ－Ｌ２を発現している細胞に対する細胞傷害活性が低く、被験物質による細胞傷害活性の回復（上昇幅）をより明確に測定できるところにある。被験物質による細胞傷害性の回復は、本発明の特徴とするＰＤ－Ｌ１またはＰＤ－Ｌ２による細胞傷害性の抑制の阻害に相当するものとして評価することができる。さらに、被験物質による細胞毒性を任意に測定することがより望ましい。これに使用される細胞としては、自然にＰＤ－Ｌ１またはＰＤ－Ｌ２を発現する腫瘍細胞株または癌細胞株（Nature Immunology, 2001年, 第2巻, 第3号, p.261～267）、例えば、Ｐ３８Ｄ１細胞、Ｐ８１５細胞、ＮＢ４１Ａ３細胞、ＭＤＡ－２３１細胞、ＳＫＢＲ－３細胞、ＭＣＦ－７細胞、ＢＴ４７４細胞、Ｊ５５８Ｌ細胞、Ｐ３Ｕ１細胞、ＰＡＩ細胞、Ｘ６３細胞、またはＳＰ２／０細胞を用いることができるが、ＰＤ－Ｌ１またはＰＤ－Ｌ２を安定的にあるいは一過的に発現するように形質転換させた腫瘍細胞株または癌細胞株も使用することができる。

一方、使用できる細胞傷害性リンパ球は、ＰＤ－１を発現しているものであって、対象とする細胞に対して同系の動物由来の細胞であることが好ましい。

本発明の感染症治療組成物の有効成分のスクリーニングは、ＰＤ－１を自然に発現する細胞傷害性Ｔリンパ球細胞（ＣＴＬ）あるいは細胞株（例えば、２Ｃ細胞）、病原菌またはウイルスを感染させた同系由来のＰＤ－Ｌ１また

はＰＤ－Ｌ２を自然に発現するもしくは強制的に発現させた細胞の混合培養に対して、被験物質を添加することによるその感染細胞へのリンパ球細胞の免疫反応の増強作用または病原菌若しくはウイルスの増殖活性に対する阻害作用を定量するものである。

さらに、同様の原理を利用した評価法では、上記のＰＤ－Ｌ１またはＰＤ－Ｌ２を発現するように形質転換させたスクリーニング用癌細胞株または自然にＰＤ－Ｌ１またはＰＤ－Ｌ２を発現する細胞を、同系哺乳動物に移植して作出した哺乳動物を使用することができる。作出する工程としては、細胞を移植する工程、評価対象が適切となるまで同哺乳動物を飼育する工程が不可欠である。この評価法は、移植細胞の増殖、各種サイトカイン若しくは細胞表面抗原の産生量、特に、細胞が癌細胞の場合は、同細胞の増殖、浸潤若しくは転移の組織学的解析または被移植哺乳動物の生存率を評価することを特徴とする。細胞増殖は、腹水癌または血液癌の場合はその単位容量当りの癌細胞数、固形癌の場合はその大きさまたは摘出後の重量によって評価することができる。本法における被験物質による癌治療効果は、ＰＤ－Ｌ１またはＰＤ－Ｌ２による細胞傷害性抑制の阻害に由来する効果に相当するものとして評価することができる。移植する癌細胞株は、インビトロでのスクリーニング法で使用できる癌細胞のいずれも使用できるが、移植哺乳動物と同系由来のもので、増殖性が良いものがより好ましい。哺乳動物としては、ヒトを除く霊長類、マウス、ラット、ハムスター、モルモット、イヌ、ブタ、サルが挙げられる。

産業上の利用可能性

本発明者らは、癌細胞移植動物モデルへの投与によって、癌の増殖を顕著に抑制し個体の延命効果を発揮する物質として、ＰＤ－１、ＰＤ－Ｌ１の機能を阻害するそれぞれの特異的抗体（抗ＰＤ－１抗体、抗ＰＤ－Ｌ１抗体）

を発明した。これら抗体は、ＰＤ－１を発現しているＣＴＬ（細胞傷害性Ｔリンパ球細胞）へのＰＤ－Ｌ１リガンドの提示によって相対的に低下する細胞傷害活性を回復あるいは増強させる作用を示した（実施例１，第１図参照）。これは、ＣＴＬによる癌細胞に対する細胞傷害活性が、これら抗体の投与によって増強しうることを示唆するものである。さらに、抗ＰＤ－Ｌ１抗体の投与は、ＰＤ－Ｌ１を人為的に発現させた肥満細胞腫由来細胞株を移入した同系マウスを用いた癌細胞移植動物モデル（蛋白質・核酸・酵素、1981 年、26 巻、3 号、p.208～224）において、癌細胞の増殖、浸潤および転移を抑制し、個体の延命効果を示した（第２図、第３図参照）。さらに、この抗体によるＰＤ－Ｌ１機能の阻害による効果と同様の効果が、ＰＤ－１の機能あるいは産生を阻害することによって得られることが示唆された。これは、ＰＤ－１欠損マウスを用いた癌移入モデルでは、移入癌細胞の増殖が全く認められなかったからであり、ＰＤ－１の機能阻害あるいは産生阻害も有効な癌治療方法になることを示すものである（実施例５，第５図参照）。

実際に、癌細胞移植動物モデルにおいて、抗ＰＤ－１抗体の投与が移入癌細胞の肝臓への転移を有意に抑制することが証明されていた（実施例１３参照）。

さらに、発明者らは、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２によって誘導される免疫抑制シグナルを阻害する物質が、感染症治療に有用であることを示した（実施例１１，第１５図，第１６図参照）。

本発明者らが実験的に示したこれら結果は、ＰＤ－１抗体またはＰＤ－Ｌ１抗体のみが既述の効果を呈することを示すものではなく、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２からの免疫抑制シグナルを阻害し得るいずれの物質もほぼ同様の効果を呈することを証明するものである。そのような効果を有する物質として、例えば、抗ＰＤ－Ｌ２抗体、可溶化ＰＤ－１、可溶化ＰＤ－Ｌ１、可溶化ＰＤ－Ｌ２、ＰＤ－１拮抗物質、ＰＤ－Ｌ１拮抗物質、Ｐ

Ｄ－Ｌ２拮抗物質、ＰＤ－１とＰＤ－Ｌ１若しくはＰＤ－１とＰＤ－Ｌ２の相互作用を阻害する物質、ＰＤ－１産生阻害物質、ＰＤ－Ｌ１産生阻害物質、ＰＤ－Ｌ２産生阻害物質、またはＰＤ－１による細胞内抑制シグナル阻害物質が挙げられる。

本発明の癌治療組成物の投与によって、その効果が期待される癌または腫瘍として、例えば、癌腫、扁平上皮癌（例えば、子宮頚管、瞼、結膜、膣肺、口腔、、皮膚、膀胱、舌、喉頭、食道）、腺癌（例えば、前立腺、小腸、子宮内膜、子宮頚管、大腸、肺、膵、食道、直腸、子宮、胃、乳房、卵巣）が挙げられる。さらに、肉腫（例えば、筋原性肉腫）、白血病、神経腫、メラノーマ、リンパ腫も含まれる。

これら癌または腫瘍のうち、特にＰＤ－Ｌ１またはＰＤ－Ｌ２を顕著に発現しているものに対してその効果が顕著である。ＰＤ－Ｌ１またはＰＤ－Ｌ２の発現を、外科的に切除した癌または腫瘍塊あるいは体外に採取した病変部をサンプルとする検査により同定でき、本発明の組成物の投与は、ＰＤ－Ｌ１またはＰＤ－Ｌ２を顕著に発現している腫瘍または癌を有する患者に対する外科的処置後の治療として、効率的で有効な方法となる。ＰＤ－Ｌ１またはＰＤ－Ｌ２の発現の同定は、例えば、ＰＤ－Ｌ１抗体若しくはＰＤ－Ｌ２抗体を用いた免疫化学的方法、ＲＴ－ＰＣＲ法またはＤＮＡアレイ法によって検査することができる。

癌に対する化学療法および放射線療法は、リンパ球の増殖を激しく減少させるという副作用が不可避である。本発明の組成物の投与は、減少したリンパ球細胞を刺激、増殖させる効果を示すと共に、通常の化学療法に付随する激烈な副作用を最小限に抑制することができる。また、放射線療法についても同様である。また、本発明の組成物との併用によって、化学療法剤の用量または照射放射線量を通常使用される用量あるいは照射量から大幅に減少させることができる。

本発明の癌治療組成物は、既存の化学療法剤と併用あるいは合剤化することができる。そのような化学療法剤として、例えば、アルキル化剤、ニトロソウレア剤、代謝拮抗剤、抗癌性抗生物質、植物由来アルカロイド、トポイソメラーゼ阻害剤、ホルモン療法剤、ホルモン拮抗剤、アロマターゼ阻害剤、Ｐ糖蛋白阻害剤、白金錯体誘導体、その他免疫療法剤またはその他の抗癌剤が挙げられる。さらに、患者のＱＯＬ回復のための癌治療補助剤である白血球（好中球）減少症治療薬、血小板減少症治療薬、制吐剤、癌性疼痛治療薬と併用あるいは合剤化ができる。

本発明の癌治療組成物は、その他の免疫賦活物質と併用あるいは合剤化することができる。そのような免疫賦活物質としては、例えば、各種サイトカインや腫瘍抗原などが挙げられる。免疫反応を刺激するサイトカインとしては、例えば、ＧＭ－ＣＳＦ、Ｍ－ＣＳＦ、Ｇ－ＣＳＦ、インターフェロン－α、β、γ、ＩＬ－１、ＩＬ－２、ＩＬ－３、ＩＬ－１２などが挙げられる。また、Ｂ７リガンド誘導体、抗ＣＤ３抗体および抗ＣＤ２８抗体、抗ＣＴＬＡ－４抗体も免疫反応を高めることができる。

癌抗原の投与も、癌細胞に対するＴリンパ球細胞の特異的免疫反応を高めることができ、本発明の癌治療組成物との併用によって、付加的あるいは相乗的な増強を与えることができる。癌抗原は、遺伝子が明らかなものについては精製タンパク質として、また不明なものについては、癌細胞自体の溶解物として調製することができる。

そのような癌抗原として、例えば、悪性黒色腫のＭＡＧＥ－１、ＭＡＧＥ－３由来のＨＬＡ－Ａ１およびＨＬＡ－Ａ２拘束ペプチドやＭＡＲＴ－１、ｇｐ１００が挙げられる。また、乳癌や卵巣癌のＨＥＲ２／ｎｅｕペプチドや腺癌のＭＵＣ－１ペプチド、さらに、転移性癌のＮＹ－ＥＳＯ－１も挙げることができる。

ウイルスは、感染宿主の免疫防御から逃れるための１つの方法として、Ｔ

リンパ球細胞の共役抑制因子を利用していると考えられている（Journal Experimental Medicine，2000年，第191巻，第11号，p.1987～1997）。ウイルス感染は、このようなウイルスのエスケープ機能に一部起因しており、本発明の組成物の投与によってＴリンパ球細胞のウイルスに対する免疫反応を高めることができると考えられる。

本発明の感染症治療組成物の投与は、例えば、ヒト肝炎ウイルス（Ｂ型肝炎、Ｃ型肝炎、Ａ型肝炎またはＥ型肝炎）、ヒトレトロウイルス、ヒト免疫不全ウイルス（ＨＩＶ１、ＨＩＶ２）、ヒトＴ細胞白血病ウイルスまたはヒトＴリンパ向性ウイルス（ＨＴＬＶ１、ＨＴＬＶ２）の感染治療に有効である。また、単純ヘルペスウイルス１型および２型、エプスタイン・バーウイルス、サイトメガロウイルス、水痘－帯状疱疹ウイルス、ヒトヘルペスウイルス６を含むヒトヘルペスウイルス、ポリオウイルス、麻疹ウイルス、風疹ウイルス、日本脳炎ウイルス、おたふくウイルス、インフルエンザウイルスまたは風邪ウイルスとして挙げられるアデノウイルス、エンテロウイルス若しくはライノウイルス、重症急性呼吸器症候群（ＳＡＲＳ）を発症するウイルス、エボラウイルス、または西ナイルウイルスの感染治療にも有効であると考えられる。

その他の病原体として、例えば、病原性原生動物（例えば、トリパノソーマ、マラリアおよびトキソプラズマ）、細菌（例えば、マイコバクテリウム、サルモネラおよびリステリア）または真菌（例えば、カンジダ）による感染に対しても有効であると考えられる。

本発明の感染症治療組成物は、既存の抗ＨＩＶ剤、抗ウイルス剤、抗生物質製剤、抗菌剤、内臓真菌症治療薬と併用あるいは合剤化することができる。

抗ＨＩＶ剤として、例えば、逆転写酵素阻害剤（例えば、ＡＺＴ，ｄｄＩ，３ＴＣ，ｄ４Ｔ）、プロテアーゼ阻害剤（例えば、メシル酸サキナビル、リトナビル、メシル酸ネルフィナビル、アンプレナビル、メシル酸デラビルジ

ン、サキナビル、ロピナビル／リトナビル）、またはＣＣＲ５受容体拮抗剤が挙げられる。抗ウイルス剤としては、例えば、抗ヘルペスウイルス剤、抗インフルエンザウイルス剤、インターフェロンーαおよびβ、または各種免疫グロブリンが挙げられる。

本発明の感染症治療組成物は、ウイルスもしくは病原体のワクチンと併用あるいは共に製剤化することができる。そのようなワクチンとしては、例えば、ポリオワクチン、麻疹ワクチン、日本脳炎ワクチン、ＢＣＧワクチン、３種混合ワクチン、おたふくワクチン、水痘ワクチン、インフルエンザワクチン、Ａ型肝炎ワクチン、Ｂ型肝炎ワクチン、またはコレラワクチンが挙げられる。

本発明の組成物は、通常、全身的または局所的に、経口または非経口の形で投与される。

投与量は、本発明に用いる薬物により異なると同時に、年齢、体重、症状、治療効果、投与方法、処理時間等により異なるが、経口投与の場合は、通常、成人一人あたり、１回につき、１μｇから１００ｍｇの範囲で、１日１回から数回投与される。非経口投与の場合は、成人一人あたり、１回につき、0.1ｎｇから１０ｍｇの範囲で、１日１回から数回投与され、その非経口投与形態は、好ましくは、静脈内投与であり、１日１時間から２４時間の範囲で静脈内に持続投与される。

もちろん前記したように、投与量は種々の条件により変動するので、上記投与量より少ない量で十分な場合もあるし、また範囲を越えて投与の必要な場合もある。

本発明の組成物と他の薬剤の併用剤を投与する際には、経口投与のための内服用固形剤、内服用液剤および、非経口投与のための注射剤、外用剤、坐剤、吸入剤、経鼻剤等として用いられる。

経口投与のための内服用固形剤には、錠剤、丸剤、カプセル剤、散剤、顆

粒剤等が含まれる。カプセル剤には、ハードカプセルおよびソフトカプセルが含まれる。また錠剤には舌下錠、口腔内貼付錠、口腔内速崩壊錠などが含まれる。

このような内服用固形剤においては、ひとつまたはそれ以上の活性物質はそのままか、または賦形剤（ラクトース、マンニトール、グルコース、微結晶セルロース、デンプン等）、結合剤（ヒドロキシプロピルセルロース、ポリビニルピロリドン、メタケイ酸アルミン酸マグネシウム等）、崩壊剤（繊維素グリコール酸カルシウム等）、滑沢剤（ステアリン酸マグネシウム等）、安定剤、溶解補助剤（グルタミン酸、アスパラギン酸等）等と混合され、常法に従って製剤化して用いられる。また、必要によりコーティング剤（白糖、ゼラチン、ヒドロキシプロピルセルロース、ヒドロキシプロピルメチルセルロースフタレート等）で被覆していてもよいし、また２以上の層で被覆していてもよい。さらにゼラチンのような吸収されうる物質のカプセルも包含される。

舌下錠は公知の方法に準じて製造される。例えば、ひとつまたはそれ以上の活性物質に賦形剤（ラクトース、マンニトール、グルコース、微結晶セルロース、コロイダルシリカ、デンプン等）、結合剤（ヒドロキシプロピルセルロース、ポリビニルピロリドン、メタケイ酸アルミン酸マグネシウム等）、崩壊剤（デンプン、Ｌ－ヒドロキシプロピルセルロース、カルボキシメチルセルロース、クロスカルメロースナトリウム、繊維素グリコール酸カルシウム等）、滑沢剤（ステアリン酸マグネシウム等）、膨潤剤（ヒドロキシプロピルセルロース、ヒドロキシプロピルメチルセルロース、カーボポール、カルボキシメチルセルロース、ポリビニルアルコール、キサンタンガム、グアーガム等）、膨潤補助剤（グルコース、フルクトース、マンニトール、キシリトール、エリスリトール、マルトース、トレハロース、リン酸塩、クエン酸塩、ケイ酸塩、グリシン、グルタミン酸、アルギニン等）安定剤、溶解補

助剤（ポリエチレングリコール、プロピレングリコール、グルタミン酸、アスパラギン酸等）、香味料（オレンジ、ストロベリー、ミント、レモン、バニラ等）等と混合され、常法に従って製剤化して用いられる。また、必要によりコーティング剤（白糖、ゼラチン、ヒドロキシプロピルセルロース、ヒドロキシプロピルメチルセルロースフタレート等）で被覆していてもよいし、また２以上の層で被覆していてもよい。また、必要に応じて常用される防腐剤、抗酸化剤、着色剤、甘味剤等の添加物を加えることもできる。口腔内貼付錠は公知の方法に準じて製造される。例えば、ひとつまたはそれ以上の活性物質に賦形剤（ラクトース、マンニトール、グルコース、微結晶セルロース、コロイダルシリカ、デンプン等）、結合剤（ヒドロキシプロピルセルロース、ポリビニルピロリドン、メタケイ酸アルミン酸マグネシウム等）、崩壊剤（デンプン、Ｌ－ヒドロキシプロピルセルロース、カルボキシメチルセルロース、クロスカルメロースナトリウム、繊維素グリコール酸カルシウム等）、滑沢剤（ステアリン酸マグネシウム等）、付着剤（ヒドロキシプロピルセルロース、ヒドロキシプロピルメチルセルロース、カーボポール、カルボキシメチルセルロース、ポリビニルアルコール、キサンタンガム、グアーガム等）、付着補助剤（グルコース、フルクトース、マンニトール、キシリトール、エリスリトール、マルトース、トレハロース、リン酸塩、クエン酸塩、ケイ酸塩、グリシン、グルタミン酸、アルギニン等）安定剤、溶解補助剤（ポリエチレングリコール、プロピレングリコール、グルタミン酸、アスパラギン酸等）、香味料（オレンジ、ストロベリー、ミント、レモン、バニラ等）等と混合され、常法に従って製剤化して用いられる。また、必要によりコーティング剤（白糖、ゼラチン、ヒドロキシプロピルセルロース、ヒドロキシプロピルメチルセルロースフタレート等）で被覆していてもよいし、また２以上の層で被覆していてもよい。また、必要に応じて常用される防腐剤、抗酸化剤、着色剤、甘味剤等の添加物を加えることもできる。

口腔内速崩壊錠は公知の方法に準じて製造される。例えば、ひとつまたはそれ以上の活性物質をそのまま、あるいは原末もしくは造粒原末粒子に適当なコーティング剤（エチルセルロース、ヒドキシプロピルセルロース、ヒドロキシプロピルメチルセルロース、アクリル酸メタクリル酸コポリマー等）、可塑剤（ポリエチレングリコール、クエン酸トリエチル等）を用いて被覆を施した活性物質に賦形剤（ラクトース、マンニトール、グルコース、微結晶セルロース、コロイダルシリカ、デンプン等）、結合剤（ヒドロキシプロピルセルロース、ポリビニルピロリドン、メタケイ酸アルミン酸マグネシウム等）、崩壊剤（デンプン、Ｌ－ヒドロキシプロピルセルロース、カルボキシメチルセルロース、クロスカルメロースナトリウム、繊維素グリコール酸カルシウム等）、滑沢剤（ステアリン酸マグネシウム等）、分散補助剤（グルコース、フルクトース、マンニトール、キシリトール、エリスリトール、マルトース、トレハロース、リン酸塩、クエン酸塩、ケイ酸塩、グリシン、グルタミン酸、アルギニン等）安定剤、溶解補助剤（ポリエチレングリコール、プロピレングリコール、グルタミン酸、アスパラギン酸等）、香味料（オレンジ、ストロベリー、ミント、レモン、バニラ等）等と混合され、常法に従って製剤化して用いられる。また、必要によりコーティング剤（白糖、ゼラチン、ヒドロキシプロピルセルロース、ヒドロキシプロピルメチルセルロースフタレート等）で被覆していてもよいし、また２以上の層で被覆していてもよい。また、必要に応じて常用される防腐剤、抗酸化剤、着色剤、甘味剤等の添加物を加えることもできる。

経口投与のための内服用液剤は、薬剤的に許容される水剤、懸濁剤、乳剤、シロップ剤、エリキシル剤等を含む。このような液剤においては、ひとつまたはそれ以上の活性物質が、一般的に用いられる希釈剤（精製水、エタノールまたはそれらの混液等）に溶解、懸濁または乳化される。さらにこの液剤は、湿潤剤、懸濁化剤、乳化剤、甘味剤、風味剤、芳香剤、保存剤、緩衝剤

等を含有していてもよい。

非経口投与のための外用剤の剤形には、例えば、軟膏剤、ゲル剤、クリーム剤、湿布剤、貼付剤、リニメント剤、噴霧剤、吸入剤、スプレー剤、エアゾル剤、点眼剤、および点鼻剤等が含まれる。これらはひとつまたはそれ以上の活性物質を含み、公知の方法または通常使用されている処方により製造される。

軟膏剤は公知または通常使用されている処方により製造される。例えば、ひとつまたはそれ以上の活性物質を基剤に研和、または溶融させて調製される。軟膏基剤は公知あるいは通常使用されているものから選ばれる。例えば、高級脂肪酸または高級脂肪酸エステル（アジピン酸、ミリスチン酸、パルミチン酸、ステアリン酸、オレイン酸、アジピン酸エステル、ミリスチン酸エステル、パルミチン酸エステル、ステアリン酸エステル、オレイン酸エステル等）、ロウ類（ミツロウ、鯨ロウ、セレシン等）、界面活性剤（ポリオキシエチレンアルキルエーテルリン酸エステル等）、高級アルコール（セタノール、ステアリルアルコール、セトステアリルアルコール等）、シリコン油（ジメチルポリシロキサン等）、炭化水素類（親水ワセリン、白色ワセリン、精製ラノリン、流動パラフィン等）、グリコール類（エチレングリコール、ジエチレングリコール、プロピレングリコール、ポリエチレングリコール、マクロゴール等）、植物油（ヒマシ油、オリーブ油、ごま油、テレピン油等）、動物油（ミンク油、卵黄油、スクワラン、スクワレン等）、水、吸収促進剤、かぶれ防止剤から選ばれるもの単独または２種以上を混合して用いられる。さらに、保湿剤、保存剤、安定化剤、抗酸化剤、着香剤等を含んでいてもよい。

ゲル剤は公知または通常使用されている処方により製造される。例えば、ひとつまたはそれ以上の活性物質を基剤に溶融させて調製される。ゲル基剤は公知あるいは通常使用されているものから選ばれる。例えば、低級アルコ

ール（エタノール、イソプロピルアルコール等）、ゲル化剤（カルボキシメチルセルロース、ヒドロキシエチルセルロース、ヒドロキシプロピルセルロース、エチルセルロース等）、中和剤（トリエタノールアミン、ジイソプロパノールアミン等）、界面活性剤（モノステアリン酸ポリエチレングリコール等）、ガム類、水、吸収促進剤、かぶれ防止剤から選ばれるもの単独または２種以上を混合して用いられる。さらに、保存剤、抗酸化剤、着香剤等を含んでいてもよい。

クリーム剤は公知または通常使用されている処方により製造される。例えば、ひとつまたはそれ以上の活性物質を基剤に溶融または乳化させて調製される。クリーム基剤は公知あるいは通常使用されているものから選ばれる。例えば、高級脂肪酸エステル、低級アルコール、炭化水素類、多価アルコール（プロピレングリコール、１，３－ブチレングリコール等）、高級アルコール（２－ヘキシルデカノール、セタノール等）、乳化剤（ポリオキシエチレンアルキルエーテル類、脂肪酸エステル類等）、水、吸収促進剤、かぶれ防止剤から選ばれるもの単独または２種以上を混合して用いられる。さらに、保存剤、抗酸化剤、着香剤等を含んでいてもよい。

湿布剤は公知または通常使用されている処方により製造される。例えば、ひとつまたはそれ以上の活性物質を基剤に溶融させ、練合物とし支持体上に展延塗布して製造される。湿布基剤は公知あるいは通常使用されているものから選ばれる。例えば、増粘剤（ポリアクリル酸、ポリビニルピロリドン、アラビアゴム、デンプン、ゼラチン、メチルセルロース等）、湿潤剤（尿素、グリセリン、プロピレングリコール等）、充填剤（カオリン、酸化亜鉛、タルク、カルシウム、マグネシウム等）、水、溶解補助剤、粘着付与剤、かぶれ防止剤から選ばれるもの単独または２種以上を混合して用いられる。さらに、保存剤、抗酸化剤、着香剤等を含んでいてもよい。

貼付剤は公知または通常使用されている処方により製造される。例えば、

ひとつまたはそれ以上の活性物質を基剤に溶融させ、支持体上に展延塗布して製造される。貼付剤用基剤は公知あるいは通常使用されているものから選ばれる。例えば、高分子基剤、油脂、高級脂肪酸、粘着付与剤、かぶれ防止剤から選ばれるもの単独または２種以上を混合して用いられる。さらに、保存剤、抗酸化剤、着香剤等を含んでいてもよい。

リニメント剤は公知または通常使用されている処方により製造される。例えば、ひとつまたはそれ以上の活性物を水、アルコール（エタノール、ポリエチレングリコール等）、高級脂肪酸、グリセリン、セッケン、乳化剤、懸濁化剤等から選ばれるもの単独または２種以上に溶解、懸濁または乳化させて調製される。さらに、保存剤、抗酸化剤、着香剤等を含んでいてもよい。

噴霧剤、吸入剤、およびスプレー剤は、一般的に用いられる希釈剤以外に亜硫酸水素ナトリウムのような安定剤と等張性を与えるような緩衝剤、例えば塩化ナトリウム、クエン酸ナトリウムあるいはクエン酸のような等張剤を含有していてもよい。

非経口投与のための注射剤としては、溶液、懸濁液、乳濁液および用時溶剤に溶解または懸濁して用いる固形の注射剤を包含する。注射剤は、ひとつまたはそれ以上の活性物質を溶剤に溶解、懸濁または乳化させて用いられる。溶剤として、例えば注射用蒸留水、生理食塩水、植物油、プロピレングリコール、ポリエチレングリコール、エタノールのようなアルコール類等およびそれらの組み合わせが用いられる。さらにこの注射剤は、安定剤、溶解補助剤（グルタミン酸、アスパラギン酸、ポリソルベート８０（登録商標）等）、懸濁化剤、乳化剤、無痛化剤、緩衝剤、保存剤等を含んでいてもよい。これらは最終工程において滅菌するか無菌操作法によって製造される。また無菌の固形剤、例えば凍結乾燥品を製造し、その使用前に無菌化または無菌の注射用蒸留水または他の溶剤に溶解して使用することもできる。

非経口投与のための吸入剤としては、エアロゾル剤、吸入用粉末剤又は吸

入用液剤が含まれ、当該吸入用液剤は用時に水又は他の適当な媒体に溶解又は懸濁させて使用する形態であってもよい。

これらの吸入剤は公知の方法に準じて製造される。

例えば、吸入用液剤の場合には、防腐剤（塩化ベンザルコニウム、パラベン等）、着色剤、緩衝化剤（リン酸ナトリウム、酢酸ナトリウム等）、等張化剤（塩化ナトリウム、濃グリセリン等）、増粘剤（カリボキシビニルポリマー等）、吸収促進剤などを必要に応じて適宜選択して調製される。

吸入用粉末剤の場合には、滑沢剤（ステアリン酸およびその塩等）、結合剤（デンプン、デキストリン等）、賦形剤（乳糖、セルロース等）、着色剤、防腐剤（塩化ベンザルコニウム、パラベン等）、吸収促進剤などを必要に応じて適宜選択して調製される。

吸入用液剤を投与する際には通常噴霧器（アトマイザー、ネブライザー）が使用され、吸入用粉末剤を投与する際には通常粉末薬剤用吸入投与器が使用される。

非経口投与のためその他の組成物としては、ひとつまたはそれ以上の活性物質を含み、常法により処方される直腸内投与のための坐剤および膣内投与のためのペッサリー等が含まれる。

スプレー剤は、一般的に用いられる希釈剤以外に亜硫酸水素ナトリウムのような安定剤と等張性を与えるような緩衝剤、例えば塩化ナトリウム、クエン酸ナトリウムあるいはクエン酸のような等張剤を含有していてもよい。スプレー剤の製造方法は、例えば、米国特許第2,868,691号明細書および米国特許第3,095,355号明細書に詳しく記載されている。

図面の簡単な説明

第１図（Ａ）は、Ｈ－２Ｌ^{d}特異的2C CTLクローンのＰＤ－１発現と、Ｐ８１５（肥満細胞種由来細胞株）のＰＤ－Ｌ１発現安定形質転換株でのＰＤ

－Ｌ１発現を示すフローサイトメトリー、（Ｂ）は、ＰＤ－Ｌ１発現Ｐ８１５細胞株に対する2C CTL細胞株の細胞傷害活性と抗ＰＤ－Ｌ１抗体（anti－ＰＤ－Ｌ１Ｆ（ａｂ')$_2$ＩｇＧ）の細胞傷害活性に対する効果を示す。

第２図は、同系マウスにおける移植ＰＤ－Ｌ１発現Ｐ８１５細胞株の腫瘍増殖性と浸潤性を示す。（Ａ）は移植ＰＤ－Ｌ１発現Ｐ８１５腫瘍の腫瘍容積（上）、移植後の生存率（下）であり、（Ｂ）は同系ＤＢＡ／２マウスにおける移植ＰＤ－Ｌ１発現Ｐ８１５腫瘍塊の組織染色像である（ａは腹壁および腹膜への腫瘍細胞の浸潤を示す４０倍像、ｂは同じく４００倍像、ｃは脾臓への転移、ｄは肝臓への転移を示す。）。

第３図は、同系マウスにおける移植ＰＤ－Ｌ１発現Ｐ８１５細胞株の腫瘍増殖性に対する抗ＰＤ－Ｌ１抗体のin vivo効果を示す。（Ａ）は当該マウスにおける腫瘍特異的ＣＤ８＋Ｔ細胞からのＩＦＮ－γ産生に対する抗ＰＤ－Ｌ１抗体のin vivo効果、（Ｂ）は当該マウスにおける移植ＰＤ－Ｌ１発現Ｐ８１５腫瘍の腫瘍容積(上)と生存率(下)に対する抗ＰＤ－Ｌ１抗体のin vivo効果を示す（図中、□は対照群（ラットＩｇＧ投与群）、○は抗ＰＤ－Ｌ１抗体（anti－ＰＤ－Ｌ１Ｆ（ａｂ')$_2$ＩｇＧ）投与群）。

第４図は、ＰＤ－１遺伝子ホモ欠損同系マウス（ＰＤ－１$^{-/-}$）でのＰＤ－Ｌ１発現Ｂ１６メラノーマの増殖抑制を示す。

第５図は、同系マウス（ＢＡＬＢ／ｃ）における移植ミエローマ細胞株の腫瘍増殖性に対する抗ＰＤ－Ｌ１抗体のin vivo効果とＰＤ－１の関与を示す。（Ａ）は各種ミエローマ細胞株でのＰＤ－Ｌ１発現を示すフローサイトメトリー、（Ｂ）は前記マウスにおける移植Ｊ５５８Ｌ腫瘍の腫瘍容積に対する抗ＰＤ－Ｌ１抗体のin vivo効果、（Ｃ）は野生型およびＰＤ－１遺伝子欠損ＰＤ－１$^{-/-}$の同系マウスにおける移植Ｊ５５８Ｌ腫瘍の腫瘍増殖の比較を示す。

第６図は、血管内皮でのＰＤ－Ｌ１発現を示す。（Ａ）はマウス心臓の血

管内皮細胞でのＰＤ－Ｌ１，ＰＤ－Ｌ２の発現を示す。（Ｂ）はマウス各組織でのＰＤ－Ｌ１発現の確認を組織染色を示す。（ａ）：眼球、（ｂ）：顎下腺、（ｃ）：心臓、（ｄ）：肺、（ｅ）：肝臓、（ｆ）：腎臓でのＰＤ－Ｌ１の発現を示す。図中、Ｃｈは脈絡膜、ＣＶは中心静脈、Ｇｌは糸球体、Ｒｅは網膜を示す。各矢印は、血管内皮細胞を示す。各染色像は４０倍拡大像である。

第７図は、肝臓非実質細胞でのＰＤ－Ｌ１発現を示す。（Ａ）はマウス肝臓でのＩＣＡＭ－１、（Ｂ）はＰＤ－Ｌ１の発現を示す（ＣＶ：中心静脈）。

第８図は、クップファー細胞（Kupffer Cell）および肝臓類洞周囲腔内皮細胞（ＬＳＥＣ）での細胞表面分子のフェノタイプを示す。

第９図は、ＰＤ－１遺伝子ホモ欠損マウス（ＰＤ－１$^{-/-}$）あるいは野生型マウス（ｗｔ）のＣＤ４陽性Ｔ細胞での細胞表面分子のフェノタイプを示す。

第１０図は、ＰＤ－１遺伝子ホモ欠損マウス（ＰＤ－１$^{-/-}$）あるいは野生型マウス（ｗｔ）のＣＤ８陽性Ｔ細胞での細胞表面分子のフェノタイプを示す。

第１１図は、Ｔ細胞増殖に対するＬＮＰＣのＰＤ－Ｌ１の効果を示す。（Ａ）はＰＤ－１$^{-/-}$マウスおよびｗｔマウスのそれぞれのナイーブＴ細胞刺激時の細胞増殖を示す。（Ｂ）はＰＤ－１$^{-/-}$マウスおよびｗｔマウス由来で既に活性化されたＴ細胞とＬＮＰＣとの共培養における細胞増殖に対する抗ＰＤ－Ｌ１抗体の効果を示す。

第１２図は、サイトカイン産生に対するＬＮＰＣのＰＤ－Ｌ１の効果を示す。（Ａ）はＰＤ－１$^{-/-}$マウスおよびｗｔマウスのそれぞれのナイーブＴ細胞刺激時のサイトカイン産生を示す。（Ｂ）は、ＰＤ－１$^{-/-}$マウスおよびｗｔマウス由来で既に活性化されたＴ細胞とＬＮＰＣとの共培養におけるサイトカイン産生に対する抗ＰＤ－Ｌ１抗体の効果を示す。（Ｃ）はＬＮＰＣとの共培養におけるＰＤ－１$^{-/-}$マウスおよびｗｔマウスからの活性化Ｔ細胞の細胞分裂を示す。

第１３図は、ウイルス感染したマウス肝臓におけるＴリンパ球細胞の増殖に対するＰＤ－１の関与を示す。（A）はアデノウイルス感染後０日目、（B）は同７日目でのＰＤ－１$^{-/-}$マウスおよびｗｔマウスの肝臓および脾臓でのＣＤ１９陽性およびＣＤ３陽性リンパ球細胞の増殖を示す。

第１４図は、ウイルス感染したマウス肝臓におけるＴリンパ球細胞の増殖に対するＰＤ－１の関与を示す。（A）はアデノウイルス感染後７日目でのＰＤ－１$^{-/-}$マウスおよびｗｔマウスの肝臓でのＣＤ４陽性およびＣＤ８陽性リンパ球細胞の増殖を示す。（B）は、感染後７日目での各種増殖性リンパ球の割合を示す。

第１５図は、ウイルス感染に対するＰＤ－１の関与を示す。図中（ａ）～（ｄ）は、アデノウイルス感染後０日および７日目でのＰＤ－１$^{-/-}$マウスおよびｗｔマウスの肝臓でのそれぞれの細胞増殖を示す組織染色像である。（ｅ）、（ｆ）はアデノウイルス感染後７日目でのＰＤ－１$^{-/-}$マウスでのＣＤ４陽性、ＣＤ８陽性Ｔ細胞の細胞増殖を示す。

第１６図は、ウイルス感染に対するＰＤ－１の関与を示す。図中（ｇ）～（ｎ）は、アデノウイルス感染後０日および７日目でのＰＤ－１$^{-/-}$マウスおよびｗｔマウスの肝臓のヘマトキシリン＆エオジン組織染色像である。図中（ｏ）～（ｒ）は、アデノウイルス感染後０日および７日目でのＰＤ－１$^{-/-}$マウスおよびｗｔマウスの肝臓のＸ－Ｇａｌ染色像である。

第１７図は、細胞傷害活性に対する各物質の増強作用を示す。図中（ａ）抗マウスＰＤ－１抗体の増強作用、（ｂ）抗マウスＰＤ－Ｌ１抗体の増強作用、（ｃ）マウスＰＤ－１Ｆｃの増強作用（ｄ）ヒトＰＤ－１Ｆｃの増強作用を示す。

発明を実施するための最良の形態

以下に実施例を挙げて本発明を、より具体的に説明するが、これらは本発

明の範囲を制限するものではない。

実施例１

マウスＰＤ－Ｌ１発現ベクターの作製は、マウス PD-L1cDNA（Journal of Experimental Medicine，2000年，第19巻，第7号，p.1027～1034）を制限酵素 EcoRI で消化して、発現ベクターpApuroXS（The EMBO Journal，1994年，第13巻，第6号，p.1341～1349）に挿入し、連結させることによって行った。作製した発現ベクターpApuroXS-PD-L1 のＰ８１５細胞への導入は、エレクトロポーレーション法（３６０Ｖ、５００μＦ）で行った。Ｐ８１５細胞の培養は、ＦＣＳ（１０%）、２－メルカプトエタノール（10^{-5}M）、各種抗生物質含 RPMI-1640 培地で培養できるが、さらに、抗生物質ピューロマイシン（Puromycin；３μｇ／ｍｌ）を含んだ培地の培養に対して耐性の同細胞株を継代培養することによって、マウスＰＤ－Ｌ１を安定的に発現する形質転換Ｐ８１５細胞株を取得した。ＰＤ－Ｌ１の発現は、フローサイトメトリー解析にて確認した。第１図（Ａ）にＨ－２Ｌ^{d}特異的2C CTL クローンのＰＤ－１発現（ｉ）と、Ｐ８１５（肥満細胞種由来細胞株）のＰＤ－Ｌ１発現安定形質転換株でのＰＤ－Ｌ１発現（ii）を示すフローサイトメトリーを示す。

同様の方法で、ＰＤ－Ｌ１を安定的に発現する形質転換Ｂ１６細胞株（Ｂ１６／ＰＤ－Ｌ１）を取得した（第１図（Ａ）（iii）～（ｖ）参照）。ここでは発現ベクターとして同様の方法で作製した pEFBOSneo-PD-L1（Nucleic Acid Research，1990年，第18巻，第17号，p.5322）を用い、細胞株の選択培養にはＧ４１８（0.5ｍｇ／ｍｌ）を使用した。

全長マウス PD-L1cDNA の3'末端側に６個のヒスチジンがタンデムに並んだペプチドタグ（His-Tag）を連結させたタンパク質をコードするｃＤＮＡを制限酵素 EcoRI および NotI で消化して、発現ベクターpVL1393（商品名：Clontech より購入）に挿入させた。続いて、この発現ベクターをＳＦ９昆虫細

胞（Invitrogen より購入）に導入して、封入体を回収した。この封入体ウイルスを HiFive 昆虫細胞（Invitrogen より購入）に２日間、２７℃下で培養することによって感染させた。溶解緩衝液（Tris-ＨＣｌ（５０mM，ｐＨ７，含１％ TritonX-100）、ＥＤＴＡ（１０mM）、ＮａＣｌ（１５０mM）、各種プロテアーゼ阻害剤）で溶解させた細胞溶解液を、Ｎｉ－セファロースカラムクロマトグラフィーで処理することによって、抗原となる精製ＰＤ－Ｌ１タンパク質を取得した。

透析した同ＰＤ－Ｌ１タンパク質を完全フロイントアジュバンドと共に８週令メス Whister ラット（SLC Japan より購入）に免疫して、数日後、末梢リンパ節から回収した２×１０^{8}細胞を、PEG1500（Amersham より購入）を用いて同数のＳＰ２／０細胞と細胞融合させた。さらに、RPMI1640 培地（ＨＡＴ（Sigma より購入）、Origen（１０%，Igen より購入）、ＦＣＳ（１０%）、２－メルカプトエタノール（１０$^{-5}$M）、各種抗生物質）で培養することによって選択し、産生抗体の存在をフローサイトメトリー解析にて確認した。これによって樹立されたハイブリドーマ（国際受託番号：FERM BP-8396 で認識されるハイブリドーマ）を Balb/C nu/nu マウスに移入して、後に腹水からの回収液をプロテインＧセファロースカラムクロマトグラフィーで精製することによって、ＰＤ－Ｌ１に対するモノクローナル抗体（１－１１１）を取得した。フローサイトメトリー等で使用される抗体は、Sulfo-NHS-LC-biotin（商品名：Pierce より購入）を用いてビオチン化したものを用いた。

また、同様の方法に従い、抗ヒトＰＤ－１抗体（国際受託番号：FERM BP-8392 で認識されるハイブリドーマから産生されるモノクローナル抗体）を作製した。

細胞傷害性アッセイは、^{51}Ｃｒ（クロム）遊離アッセイによって行った。

２Ｃ細胞（Journal of Immunology，1996 年，第 157 巻，第 2 号，p.670～678）は、２Ｃトランスジェニック Ｂ６マウス由来の（Ｈ－２Ｌ）^{d}アロ反応性の細

胞傷害性Ｔ細胞である。第１図（Ｂ）に、２Ｃ細胞（Ｅ：エフェクター）を^{51}Ｃｒラベル化したＰ８１５細胞（Ｔ：ターゲット）と共に（○）または３つのＰＤ－Ｌ１発現Ｐ８１５細胞株（Ｐ８１５／ＰＤ－Ｌ１）（□、◇、△）と共にあるいはさらに１０ｍｇ／ｍｌラット anti-PD-L1F(ab')$_2$IgG 存在下（▲）を、さまざまなＥ／Ｔ比で混合して、４時間で遊離される^{51}Ｃｒを測定した結果を示す。

抗ＰＤ－Ｌ１抗体（anti-PD-L1F(ab')$_2$）は、細胞傷害性Ｔリンパ球細胞の低下した細胞傷害活性を回復させた。これらの結果から、ＰＤ－Ｌ１の機能を阻害することによるＰＤ－１およびＰＤ－Ｌ１シグナルの阻害は、癌細胞に対する細胞傷害活性を増強させることができると考えられる。

実施例２

１×１０^{6}細胞のＰ８１５細胞（ｎ＝６）またはＰ８１５／ＰＤ－Ｌ１細胞（ｎ＝６）を同系ＤＢＡ／２マウスの皮下にそれぞれ移入し、腫瘍増殖とマウスの生存率を評価した。その結果を第２図（Ａ）に示す。図中、○はＰ８１５細胞株移植群、□、△はＰＤ－Ｌ１発現Ｐ８１５細胞株移植群である。さらにＰ８１５／ＰＤ－Ｌ１細胞を移入した群の組織学的解析を行った。移入２０日後のマウスの腹壁ならびに腹膜腔の組織切片を、１０％ホルムアルデヒドで固定、パラフィンで砲架して、ヘマトキシリン、エオジンで染色して得られた染色像を第２図（Ｂ）に示す。図中、ａは腹壁および腹膜への腫瘍細胞の浸潤を示す４０倍像、ｂは同じく４００倍像であり、ｃは脾臓への転移、ｄは肝臓への転移を示す。

Ｐ８１５細胞を移植した群では、Ｐ８１５細胞の増殖は抑えられており、６～７週目では３０％が生存したのに対して、ＰＤ－Ｌ１を発現するＰ８１５細胞（Ｐ８１５／ＰＤ－Ｌ１）を移植した群では、癌細胞の増殖は著しく、２～４週目までに全例が死亡した（第２図（Ａ））。Ｐ８１５／ＰＤ－Ｌ１

は、腹膜腔、さらに、腹腔へ浸潤しており、また、肝および脾臓への転移が認められた（第２図（Ｂ）ａ～ｄ参照）。

実施例３

Ｐ８１５細胞を移入し免疫したマウスから細胞傷害性Ｔ細胞ＣＴＬを調整し、２×１０^{6}個数のＣＴＬ細胞と、５×１０^{6}個数のＰ８１５細胞またはＰ８１５／ＰＤ－Ｌ１細胞のみ、あるいは anti-PD-L1F(ab')$_2$IgG（１０ｍｇ／ｍｌ）存在下でＰ８１５／ＰＤ－Ｌ１細胞をそれぞれ混合培養し、２４時間後の培養上清中のＩＦＮ－γをＥＬＩＳＡキット（Bioscience から購入）で測定した。その結果を第３図（Ａ）に示す。

また、第３図（Ｂ）に、３×１０^{6}個数のＰ８１５／ＰＤ－Ｌ１細胞を皮下移入した同系ＤＢＡ／２マウス（ｎ＝１０）に、ラットＩｇＧ（□）あるいは anti-PD-L1F(ab')$_2$IgG（0.1ｍｇ／一匹）（○）を、細胞移入後１、３、５、７日後にそれぞれ腹腔内投与し、腫瘍増殖とマウスの生存率を評価した結果を示す。

抗ＰＤ－Ｌ１抗体は、Ｐ８１５／ＰＤ－Ｌ１により抑制された細胞傷害性Ｔリンパ球細胞からのＩＦＮ－γ産生を回復させた（第３図（Ａ））。抗ＰＤ－Ｌ１抗体の投与は、癌細胞増殖を抑制し、明確な生存効果を示した（第３図（Ｂ））。この結果は、抗ＰＤ－Ｌ１抗体の投与が癌治療に有効であることを示している。

実施例４

１×１０^{6}個数のＢ１６メラノーマ（ｎ＝６）またはＢ１６／ＰＤ－Ｌ１細胞（ｎ＝６）をＢ６マウスにはぞれぞれ皮下移入し、同数のＢ１６／ＰＤ－Ｌ１細胞をＰＤ－１トランスジェニックＢ６マウス（ｎ＝５）およびＰＤ－１遺伝子ホモ欠損Ｂ６マウス（ＰＤ－１$^{-/-}$（ｎ＝４））（Science, 2001 年,

291巻，5502号，p.319～332）に移入し、以後２５日までそれぞれの腫瘍増殖を測定した。その結果を第４図に示す。

実施例５

2.5×１０^{8}個数のＪ５５８Ｌミエローマ細胞を皮下移入した同系Ｂａｌｂ／Ｃマウス（ｎ＝９）に、ラットＩｇＧあるいは anti-PD-L1F(ab')$_2$IgG（0.1ｍｇ／一匹）を細胞移入後３、５、７日後にそれぞれ腹腔内投与し、腫瘍増殖を評価した（第５図（Ｂ））。また、同様にＪ５５８Ｌミエローマ細胞を皮下移入したＰＤ－１ホモ欠損マウスとＢａｌｂ／Ｃ（ｎ＝４）での腫瘍増殖を比較した（第５図（Ｃ））。

抗ＰＤ－Ｌ１抗体の投与は、ＰＤ－Ｌ１を発現しているＪ５５８癌細胞（第５図（Ａ）に各種ミエローマ細胞株でのＰＤ－Ｌ１発現を示すフローサイトメトリーを示す。）の増殖を抑制した（第５図（Ｂ））。また、Ｊ５５８細胞を移植したＰＤ－１欠損マウスでは移植癌細胞の増殖は完全に阻害された（第５図（Ｃ））。これらの結果は、ＰＤ－Ｌ１もしくはＰＤ－１の阻害が癌治療に有効であることを示している。

実施例６

血管内皮細胞（以下、ＥＣｓと略す。）をマレリーバーグの方法（Journal Immunology Methods，2000年，第244巻，第1-2号，p.205～215）により、マウス心臓から取得した。具体的には、心臓組織をコラゲナーゼで消化した後、マウスＩｇとともに前培養、さらに、ＦＩＴＣ修飾した抗ＣＤ３１抗体、同修飾抗ＣＤ１０５抗体、同修飾抗 isolectinB4 抗体、および、抗ＦＩＴＣビーズを添加して培養した。この血管内皮細胞を Magnetic-activated cell-sorting separation columns（商品名：Miltenyi Biotec より購入）を用いて、ポジティブ選択によって精製した。

取得した血管内皮細胞でのＰＤ－Ｌ１、ＰＤ－Ｌ２の発現を、フローサイトメトリーで確認した。同細胞の標識には、抗ＰＤ－Ｌ１抗体（抗体名：１－１１１）、抗ＰＤ－Ｌ２抗体（抗体名：＃１２２）および蛍光標識した２次抗体を用いて行った（第６図（Ａ））。解析はCellQuestソフトウェア（Dickinsonより購入）を使用したFacscalibur（機器名：Becton Dickinsonより購入）で、１万回イベントで解析した。ＰＤ－Ｌ１あるいはＰＤ－Ｌ２の発現は、開口曲線で示され、コントロールＩｇは充填曲線で示される。

マウス各組織でのＰＤ－Ｌ１発現の確認を組織染色によって行った。各組織サンプリング１時間前に１００μｇビオチン標識化抗ＰＤ－Ｌ１抗体（１－１１１）を溶解した１００μｌＰＢＳをマウスに静脈投与した。続いて、５μｍ凍結切片を４％パラホルムアルデヒド（ＰＦＡ）で固定して、Streptavidin-FITCで染色した。さらに、各切片をPhalloidinで対比染色した（第６図（Ｂ）、（ａ）眼球、（ｂ）顎下腺、（ｃ）心臓、（ｄ）肺、（ｅ）肝臓、（ｆ）腎臓でのＰＤ－Ｌ１の発現を示す。図中、Ｃｈは脈絡膜、ＣＶは中心静脈、Ｇｌは糸球体、Ｒｅは網膜を示す。各矢印は、血管内皮細胞を示す。各染色像は４０倍拡大像である。）。ＰＤ－Ｌ１は、心臓、肺、腎臓、胃、小腸、顎下腺、眼球、肝臓の血管内皮で認められた。肝臓での発現は、肝臓洞様毛細血管に局在していた。

実施例７

肝臓非実質細胞（以下、ＬＮＰＣｓと略記する。）でのＰＤ－Ｌ１発現を組織染色（第７図（Ａ））ならびにフローサイトメトリー（第７図（Ｂ））で確認した。組織染色は、３％ＰＦＡで固定した５μｍ肝臓凍結切片をラット血清で前処理し、ビオチン標識化抗ＰＤ－Ｌ１抗体（１－１１１）あるいはビオチン標識化抗ＩＣＡＭ－１抗体（商品名：BD Pharmingenより購入）にて１時間、室温下で抗体反応を行ない、ビオチン抗体を tyramide signal

amplification（ＴＳＡ）fluorescence system（機器名：PerkinElmer Life Sciences より購入）にて視覚化した（第７図（Ａ）：ＩＣＡＭ－１の発現、同図（Ｂ）：ＰＤ－Ｌ１の発現、ＣＶ：中心静脈を示す。各染色像は４０倍拡大像である。）。

ＬＮＰＣｓは、pronaseE 法（Experimental Cell Research, 1976 年, 第 99 巻, p.444～449）によってマウス肝臓より単離した。具体的には、ＬＮＰＣｓは、肝臓を pronaseE 溶液（Merck）にて還流、培養し、密度勾配遠心法にて分離した。その細胞懸濁液中のクップファー細胞（Kupffer Cells）（ＣＤ５４$^{+}$、ＣＤ１１ｂ^{high}）の相対的分布は２０～２５％であり、肝臓類洞周囲腔内皮細胞（以下、ＬＳＥＣｓと略記する。）（ＣＤ５４＋、ＣＤ１１ｂ^{high}）は、７５～８０％である。

クップファー細胞とＬＳＥＣｓを、ＦＩＴＣ標識化抗ＣＤ１１ｂ抗体およびＩＣＡＭ－１、ＰＤ－Ｌ１、Ｂ７－１、Ｂ７－２に対するそれぞれのビオチン化モノクローナル抗体、それに続くＰＥ標識化 Streptavidin にて、それぞれ二重染色した。クップファー細胞とＬＳＥＣｓは、それぞれＣＤ１１ｂ^{high}とＣＤ１１ｂ^{low}細胞としてゲートされる（第８図）。

ＰＤ－Ｌ１は、クップファー細胞では、ＩＣＡＭ－１、Ｂ７－１、Ｂ７－２を共発現しているが、ＬＳＥＣｓではその発現は弱いものであった（第８図）。

実施例８

ＰＤ－１遺伝子ホモ欠損マウス（ＰＤ－１$^{-/-}$）あるいは野生型Ｃ５７ＢＬ／６マウス（ｗｔ）の脾臓ならびにリンパ組織から、T-cell enrichment column（商品名：Genzyme より購入）を用いたネガティブ選別によって、ナイーブＴ細胞（精製度９０％以上）を精製した。同細胞を１０μｇ／ｍｌ抗ＣＤ３モノクローナル抗体（２Ｃ１１）とともに４８時間培養して活性化させた。

上記方法によって活性化したナイーブＴ細胞を、ＦＩＴＣ標識化抗ＣＤ４

抗体あるいはＡＰＣ標識化抗ＣＤ８抗体ならびにＰＥ標識化抗ＣＤ２５抗体、ＰＥ標識化抗ＣＤ４４抗体、ＰＥ標識化抗ＣＤ６９抗体、ＰＥ標識化抗ＣＴＬＡ－４抗体、ビオチン標識化抗Ｂ７－１（ＣＤ８０）抗体、ビオチン標識化抗Ｂ７－２（ＣＤ８６）抗体、抗ＰＤ－１抗体（抗体名：Ｊ４３、国際受託番号 FERM BP-8118 で認識されるハイブリドーマから産生されるモノクローナル抗体）、抗ＰＤ－Ｌ１抗体（１－１１１）で二重染色して、フローサイトメトリーにてそれぞれの分子の発現を解析した（第９図、第１０図）。

なお、国際受託番号 FERM BP-8118 で識別されるハイブリドーマは、2001年 5 月 30 日付で日本国茨城県つくば市東１丁目１番地１ 中央第６（郵便番号 305-8566）、独立行政法人産業技術総合研究所特許生物寄託センターに受託番号 FERM P-18356 で寄託され、2002 年 7 月 16 日付で国際寄託に移管されている。

実施例９

ＰＤ－１遺伝子ホモ欠損マウス（ＰＤ－１$^{-/-}$）あるいは野生型マウス（ｗｔ）のナイーブＴ細胞の活性化は、上記実施例８に記載の方法で行った。活性化後の同細胞の増殖をＢｒｄＵ取りこみ法によって測定した（第１１図（Ａ））。ＢｒｄＵを４８時間の最後の６時間に添加して細胞を標識し、これを Proliferation ELISA kit（商品名：Roche より購入）を用いる測定によって細胞増殖を決定した。また、この時のＩＦＮ－γ産生量を ELISA Kit（商品名：Genzyme より購入）にて測定した（第１２図（Ａ））。

ＰＤ－１遺伝子ホモ欠損マウス（ＰＤ－１$^{-/-}$）あるいは野生型マウス（ｗｔ）由来のＴ細胞を上記実施例８で記載の方法であらかじめ活性化させた。続いて、既に活性化しているＴ細胞に対して、野生型マウスからのマイトマイシンＣ処理ＬＮＰＣｓの存在あるいは非存在下で、さらに、３０μｇ／ｍｌ抗ＰＤ－Ｌ１抗体（１－１１１）（コントロールとしたラットＩｇＧ）、２０

μｇ／ｍｌCTLA4-Ig（Genzyme）（コントロールとしたヒトＩｇＧ）の存在あるいは非存在下でそれぞれ６０時間培養して、最後の１２時間の同細胞の増殖をＢｒｄＵ取りこみ法によって測定した（第１１図（Ｂ））。また、４８時間時点でのＩＦＮ－γ産生量も測定した（第１２図（Ｂ））。

ナイーブＴ細胞の活性化時におけるＩＦＮ－γ産生量は、ＰＤ－１$^{-/-}$と野生型マウスの違いによる有意な差異は認められなかった。一方、既に活性化されたＴ細胞においては、野生型マウス由来のＴ細胞のＩＦＮ－γ産生量は、ＰＤ－１$^{-/-}$由来のものに比べ有意に低かった（第１２図）。このことから、既に活性化しているＴ細胞に対するＰＤ－１の抑制作用の効果は、ナイーブＴ細胞の活性化に対する効果よりも高いことが示唆された。

野生型マウス由来の活性化したＴ細胞は、ＬＮＰＣｓとの共培養では、Ｔ細胞の細胞増殖およびＩＦＮ－γ産生量に有意な変化は認められないが、ＰＤ－１$^{-/-}$由来の活性化したＴ細胞は、ＬＮＰＣｓとの共培養によって、その細胞増殖に有意な増加が認められた（第１１図（Ｂ）、第１２図（Ｂ））。また、野生型マウス由来の活性化したＴ細胞とＬＮＰＣｓとの共培養への抗ＰＤ－Ｌ１抗体の添加によって、Ｔ細胞の細胞増殖の増加が認められた（第１１図（Ｂ））。これらの結果は、ＬＮＰＣｓのＰＤ－１あるいはＰＤ－Ｌ１が、活性化Ｔ細胞の抑制に関与しており、ＰＤ－１の欠如あるいはＰＤ－１とＰＤ－Ｌ１間の相互作用の阻害が、Ｔ細胞を活性化することを示すものである。

ＰＤ－１遺伝子ホモ欠損マウス（ＰＤ－１$^{-/-}$）あるいは野生型マウス（ｗｔ）の活性化Ｔ細胞を、５μＭ CFSE(5-(6)-carboxy-fluorescein diacetate succinimidyl diester)（商品名：Molecular probes より購入）で標識して、ＬＮＰＣｓとともに４８時間共培養した。この時の細胞分裂を、ＦＡＣＳを用いたＣＦＳＥ活性測定によって決定した（第１２図（Ｃ））。

活性化Ｔ細胞の細胞増殖抑制は、細胞分裂の停止に起因しており、ＰＤ－

１シグナルがＴ細胞の細胞分裂を抑制することが示唆された（第１２図（Ｃ））。

実施例１０

ＰＤ－１遺伝子ホモ欠損マウス（ＰＤ－１$^{-/-}$）あるいは野生型マウス（ｗｔ）（３匹／群）に10^9-10^{10}PFU (plaque-forming units) Ad-lacZを静脈内投与して、マウスをアデノウイルスに感染させた。ここで使用したAd-lacZは、Ｅ１およびＥ３領域に欠損を有し、さらに、ｌａｃＺ遺伝子を持った５型アデノウイルスであり、２９３細胞で増殖させた後、塩化セシウム密度勾配遠心分離（Nucleic Acid Research, 1995年, 第234巻, 第19号, p.3816～3821）の記載によって精製したものである。

感染後０日目あるいは７日目に、同マウスに対して屠殺１時間前に0.5ｍｇＢｒｄＵ（商品名：Sigmaより購入）を静脈内投与して、採取した脾臓細胞および肝臓内リンパ球を抗ＢｒｄＵ抗体および抗ＣＤ１９抗体あるいは抗ＣＤ３抗体で二重標識した（第１３図）。

さらに、感染後７日目の同細胞に対しては、抗ＢｒｄＵ抗体、抗ＣＤ１９抗体、抗ＣＤ３抗体、抗ＣＤ４抗体、抗ＣＤ８抗体で二重標識した（第１４図（Ｂ）、それぞれの棒グラフはＢｒｄＵ陽性細胞の割合を示す。）。

アデノウイルス感染したＰＤ－１$^{-/-}$マウスの肝臓では、同様に感染した野生型マウスの肝臓に比較して、増殖性（ＢｒｄＵ陽性）の各リンパ球（ＣＤ１９陽性、ＣＤ３陽性、ＣＤ４陽性、ＣＤ８陽性）の割合が増加していた。一方、このような現象が脾臓では認められなかったことから、ＰＤ－１は、炎症を起した組織でのＴ細胞の増殖を阻害することが示唆された（第１４図（Ｂ））。

実施例１１

ＰＤ－１遺伝子ホモ欠損マウス（ＰＤ－１$^{-/-}$）あるいは野生型マウス（ｗｔ）

（３匹／群）に 10^9-10^{10}PFU Ad-lacZ を静脈内投与して、マウスをアデノウイルスに感染させ、さらに、感染後０日目あるいは７日目の屠殺１時間前に 0.5 ｍｇ ＢｒｄＵ（商品名：Sigma より購入）を静脈内投与して、採取した肝臓切片を抗ＢｒｄＵ抗体で標識した（第１５図（ａ）～（ｄ））、２０倍拡大像）。感染後７日目のＰＤ－１遺伝子ホモ欠損マウス（ＰＤ－$1^{-/-}$）の肝臓切片に対しては、抗ＢｒｄＵ抗体および抗ＣＤ４抗体あるいは抗ＣＤ８抗体で二重標識した（第１５図（ｅ）、（ｆ）、４０倍拡大像）。

感染後３０日目の野生型マウスの肝臓では、洞様毛細血管および非実質領域への中程度の局所的な細胞浸潤が認められたが、ＰＤ－$1^{-/-}$マウスでは、肝炎を示す症状は認められなかった（第１６図（ｈ）、（ｉ）および（ｊ）、（ｎ））。

感染後７日目あるいは３０日目のＰＤ－１遺伝子ホモ欠損マウス（ＰＤ－$1^{-/-}$）あるいは野生型マウス（ｗｔ）の肝臓切片に対して、ヘマトキシリン＆エオジン染色（第１６図（ｇ）～（ｊ），２０倍拡大像、（ｋ）～（ｎ），４０倍拡大像）およびＸ－Ｇａｌ染色を行った（第１６図（ｏ）～（ｒ），４０倍拡大像）。

感染後７日目および３０日目の野生型マウスの肝臓では、Ｘ－Ｇａｌ染色像が示すアデノウイルスの感染が認められたが、ＰＤ－$1^{-/-}$マウスでは、その感染は、３０日目にはほぼ排除されていた（第１６図（ｏ）、（ｐ）および（ｑ）、（ｒ））。これらの結果から、ＰＤ－１シグナルが、ウイルス感染による炎症組織におけるエフェクターＴ細胞の細胞増殖を誘導することによるウイルスの排除に関与することが示された。

実施例１２

マウスＰＤ－Ｌ１を強制発現させたＰ－８１５／ＰＤ－Ｌ１細胞は、５μｇ／ｍＬ puromycin（Sigma より購入）を含む通常培地（以下、選択培地と略

す。）を用いて、培養フラスコに播種し、３７℃、５％CO_2／９５％airの条件で５０％～９０％コンフルエントになるまで培養した。マウス cytotoxic T lymphocyte 2C 細胞は、ＭＭＣ（Mitomycin C）処理したＰ－８１５細胞およびＣｏｎＡ刺激ラット脾細胞培養上清とともに通常培地中で数日継代培養した。回収したＰ－８１５／ＰＤ－Ｌ１細胞に DELFIA EuTDA Cytotoxicity Reagents（PerkinElmer より購入）の BATDA Reagent を３μＬ加え、１５分間培養した。さらに、ＰＢＳで洗浄した。２Ｃ細胞は、Ｐ－８１５細胞を加えて継代した５～８日目の細胞を用いた。

被検物質である抗マウスＰＤ－１抗体（第１７図中、anti-mPD-1Ab（Ｊ４３））、抗マウスＰＤ－Ｌ１抗体（同図中、anti-mPD-L1Ab（１－１１１））、マウスＰＤ－１Ｆｃ（同図中、ｍＰＤ－１Ｆｃ）、ヒトＰＤ－１Ｆｃ（同図中、ｈＰＤ－１Ｆｃ）、マウスＩｇＧ２ａκ（同図中、ControlIg）またはＰＢＳを９６well plate に２０μＬ（１０ｎｇ／ｍＬ）ずつ分注し、Ｐ－８１５／ＰＤ－Ｌ１細胞または通常培地を５０μＬずつ加えた。さらに２Ｃ細胞、通常培地または１％TritonX100 を含む通常培地を５０μＬずつ加えた。通常培地を添加したウェルの上清５０μＬをバックグラウンド用として回収し、その他の上清を回収するまで、３７℃で保存した。残りの細胞は４時間培養した。続いて、同９６well plate を遠心し、上清を回収した。回収した上清に Cytotoxicity Reagents（PerkinElmer より購入）の DELFIA Europium Solution を２００μＬ添加して１５分間振とうした。振とう後、ARVOsx マルチラベルカウンター（WALLAC）にて時間分解蛍光測定を行った。なお、１％TritonX100 を含む通常培地を添加したウェルの上清を高コントロール、通常培地を添加したウェルの上清を低コントロールとした。

評価群の組成は、被検物質、Ｐ－８１５／ＰＤ－Ｌ１細胞及び２Ｃ細胞、高コントロール群の組成は、ＰＢＳ、Ｐ－８１５／ＰＤ－Ｌ１細胞および１％TritonX100 を含む通常培地、低コントロール群の組成は、ＰＢＳ、Ｐ－８１５

／ＰＤ－Ｌ１細胞および通常培地、２Ｃ細胞コントロール群の組成は、ＰＢＳ、通常培地および２Ｃ細胞、バックグラウンド群の組成は、ＰＢＳ、Ｐ－８１５／ＰＤ－Ｌ１細胞及び通常培地である。ＣＴＬ活性（％）は、以下の式にて算出した。すべての値は、バックグランドで得た平均値を引いたものを使用した。

CTL活性(%)＝([評価群測定値]－[2C細胞コントロール群測定値]－[低コントロール群測定値])÷([高コントロール群測定値]－[低コントロール群測定値])×100

抗ＰＤ－１抗体、抗ＰＤ－Ｌ１抗体およびＰＤ－１Ｆｃは、ＣＴＬ活性を有意に増強した（第１７図（ａ）～（ｄ）、図中、Ｅ：Tratio は、２Ｃ細胞とＰＤ－Ｌ１／Ｐ８１５細胞の混合比を示す。）。

実施例１３

Ｂ１６メラノーマ細胞を脾臓に移入したＣ５７ＢＬ／６マウスに、抗マウスＰＤ－１モノクローナル抗体を２日おきに腹腔内に投与して、移入後１８日目の肝臓重量を測定することによって、癌転移に対する抗ＰＤ－１抗体の抑制効果を評価した。

コントロールＩｇＧのみを投与したコントロール群に比べ、抗ＰＤ－１抗体投与群では、肝臓重量増加が有意に抑制された（肝臓重量／癌細胞非移入群：1.3ｇ、コントロール群：6.8ｇから抗ＰＤ－１抗体投与群：3.5ｇへ減少）。この重量増加の抑制は、Ｂ１６メラノーマ細胞の転移を抑制することを示すものである。

請 求 の 範 囲

1． ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を含有してなる免疫賦活組成物。

2． ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を含有してなる癌治療組成物。

3． 癌転移を抑制する組成物である請求の範囲２記載の癌治療組成物。

4． ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を含有してなる感染症治療組成物。

5． 免疫賦活を介して作用することを特徴とする請求の範囲２または３記載の癌治療組成物。

6． 免疫賦活を介して作用することを特徴とする請求の範囲４記載の感染症治療組成物。

7． ＰＤ－１とＰＤ－Ｌ１若しくはＰＤ－１とＰＤ－Ｌ２の相互作用阻害物質、ＰＤ－１の細胞内シグナル阻害物質、およびＰＤ－１、ＰＤ－Ｌ１若しくはＰＤ－Ｌ２の産生阻害物質から選択される一以上の免疫抑制シグナル阻害物質である請求の範囲１乃至６のいずれかに記載の組成物。

8． ＰＤ－１抗体、ＰＤ－Ｌ１抗体、可溶化ＰＤ－１、および可溶化ＰＤ－Ｌ１から選択される一以上のＰＤ－１とＰＤ－Ｌ１の相互作用阻害物質で

ある請求の範囲７記載の組成物。

９． 国際受託番号 FERM BP-8392 で識別されるハイブリドーマが産生する抗ヒトＰＤ－１抗体、非ヒト抗体をヒト化させた抗ＰＤ－１抗体、および完全ヒト型抗ヒトＰＤ－１抗体から選択されるＰＤ－１抗体である請求の範囲８記載の組成物。

１０． 遺伝子改変によりＰＤ－１発現が阻害されたリンパ球細胞が、免疫抑制シグナル阻害物質である請求の範囲１乃至６のいずれかに記載の組成物。

１１． ＰＤ－１とＰＤ－Ｌ１若しくはＰＤ－１とＰＤ－Ｌ２の相互作用阻害物質、ＰＤ－１の細胞内シグナル阻害物質、またはＰＤ－１、ＰＤ－Ｌ１若しくはＰＤ－Ｌ２の産生阻害物質が、タンパク質、ポリペプチド若しくはペプチド、ポリヌクレオチド若しくはポリヌクレオシド、抗体若しくはそれらの誘導体、有機合成化合物、無機化合物、および天然物から選択される一以上の物質である請求の範囲７記載の組成物。

１２． ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を投与することからなる免疫賦活方法。

１３． ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を投与することからなる癌治療方法。

１４． 癌転移を抑制する請求の範囲１３記載の癌治療方法。

１５． ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害

物質を投与することからなる感染症治療方法。

１６．　免疫賦活を介して作用することを特徴とする請求の範囲１３または１４記載の癌治療方法。

１７．　免疫賦活を介して作用することを特徴とする請求の範囲１５記載の感染症治療方法。

１８．　ＰＤ－１とＰＤ－Ｌ１若しくはＰＤ－１とＰＤ－Ｌ２の相互作用阻害物質、ＰＤ－１の細胞内シグナル阻害物質、およびＰＤ－１、ＰＤ－Ｌ１若しくはＰＤ－Ｌ２の産生阻害物質から選択される一以上の免疫抑制シグナル阻害物質である請求の範囲１２乃至１７のいずれかに記載の方法。

１９．　ＰＤ－１抗体、ＰＤ－Ｌ１抗体、可溶化ＰＤ－１、および可溶化ＰＤ－Ｌ１から選択される一以上のＰＤ－１とＰＤ－Ｌ１の相互作用阻害物質である請求の範囲１８記載の方法。

２０．　国際受託番号 FERM BP-8392 で識別されるハイブリドーマが産生する抗ヒトＰＤ－１抗体、非ヒト抗体をヒト化させたＰＤ－１抗体、および完全ヒト型抗ヒトＰＤ－１抗体から選択されるＰＤ－１抗体である請求の範囲１９記載の方法。

２１．　遺伝子改変によりＰＤ－１発現が阻害されたリンパ球細胞が、免疫抑制シグナル阻害物質である請求の範囲１２乃至１７のいずれかに記載の方法。

２２．　ＰＤ－１とＰＤ－Ｌ１若しくはＰＤ－１とＰＤ－Ｌ２の相互作用阻害物質、ＰＤ－１の細胞内シグナル阻害物質、またはＰＤ－１、ＰＤ－Ｌ１若しくはＰＤ－Ｌ２の産生阻害物質が、タンパク質、ポリペプチド若しくはペプチド、ポリヌクレオチド若しくはポリヌクレオシド、抗体若しくはそれらの誘導体、有機合成化合物、無機化合物、および天然物から選択される１つ以上の物質である請求の範囲１８記載の方法。

２３．　免疫賦活組成物を製造するためのＰＤ－1、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質の使用。

２４．　癌治療組成物を製造するためのＰＤ－1、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質の使用。

２５．　癌治療組成物が、癌転移抑制組成物である請求の範囲２４記載の物質の使用。

２６．　感染症治療組成物を製造するためのＰＤ－1、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質の使用。

２７．　ＰＤ－Ｌ１またはＰＤ－Ｌ２を発現するように形質転換されたスクリーニング用癌細胞株。

２８．　請求の範囲２７記載の細胞、リンパ球細胞および被験物質を接触させて、請求の範囲２７記載の細胞へのリンパ球細胞の免疫反応に対する被験物質の増強作用を評価することを特徴とする免疫賦活物質のスクリーニング方法。

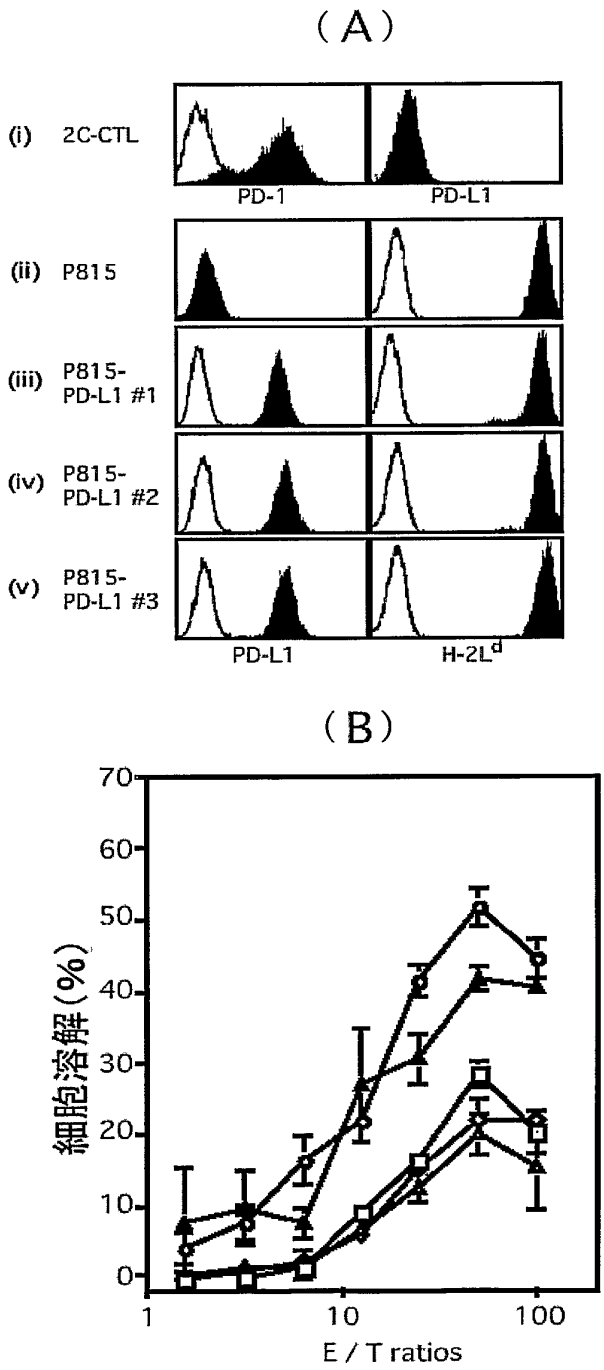
２９．　癌細胞である請求の範囲２７記載の細胞、リンパ球細胞および被験物質を接触させて、その癌細胞へのリンパ球細胞の免疫反応に対する被験物質の増強作用またはその腫瘍細胞の増殖に対する阻害作用を評価することを特徴とする癌治療物質のスクリーニング方法。

３０．　病原体を感染させた請求の範囲２７記載の細胞またはＰＤ－Ｌ１若しくはＰＤ－Ｌ２を発現する細胞、リンパ球細胞および被験物質を接触させて、その感染細胞へのリンパ球細胞の免疫反応に対する被験物質の増強作用または病原体増殖に対する阻害作用を評価することを特徴とする感染症治療物質のスクリーニング方法。

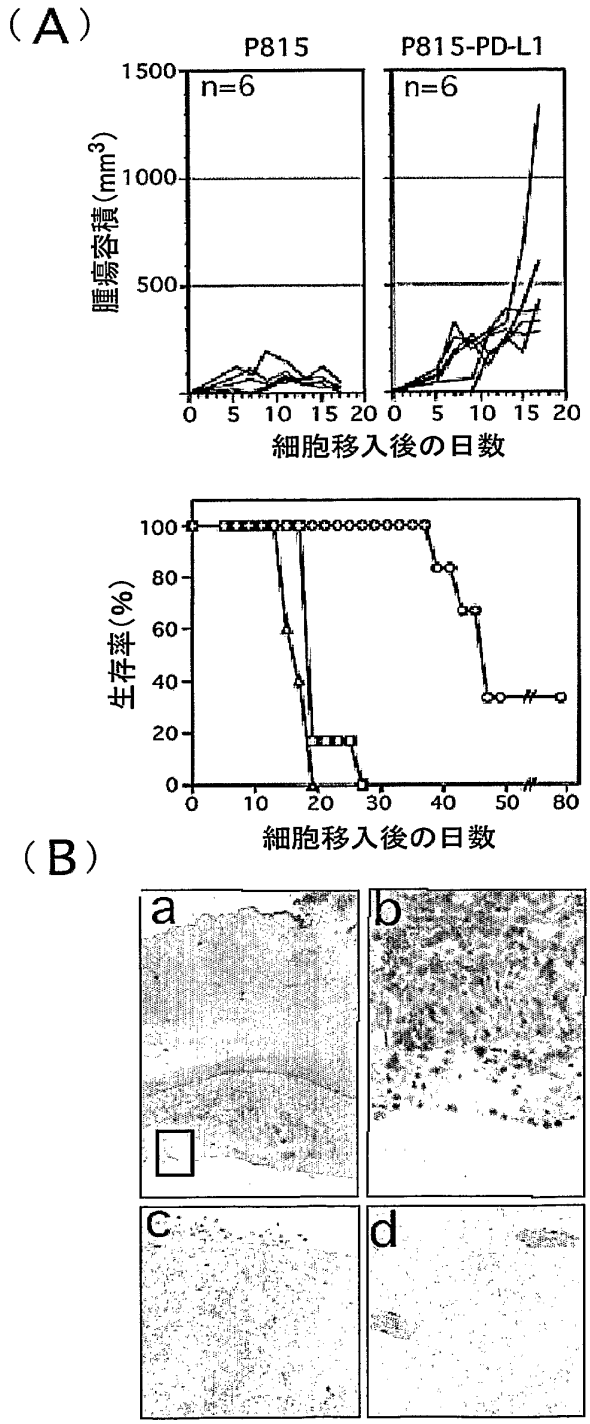
３１．　請求の範囲２７記載の癌細胞株を移植して作出した哺乳動物。

３２．　請求の範囲３１記載の哺乳動物に被験物質を投与し、被験物質による移植癌細胞の増殖に対する抑制率またはその被移植哺乳動物の生存率を評価することを特徴とする癌治療物質の選別方法。

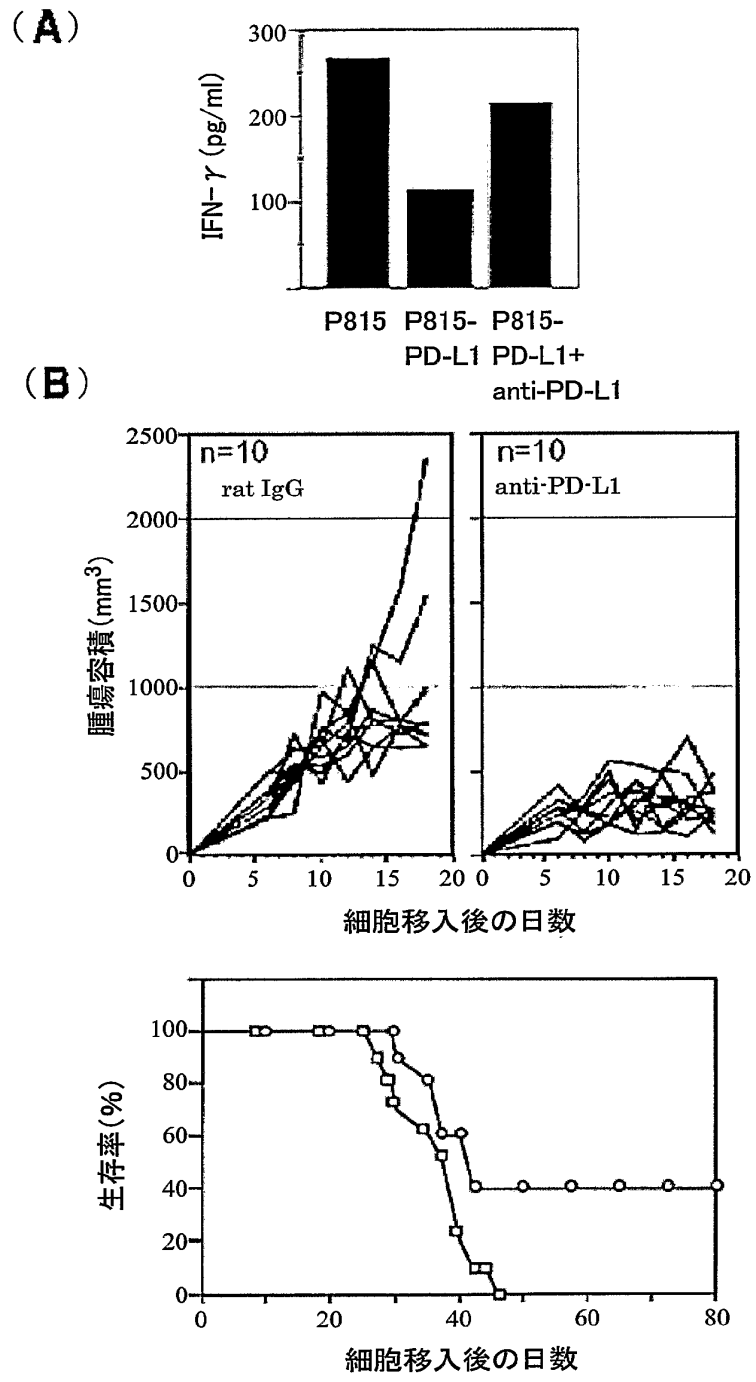
第１図


(A)
(i) 2C-CTL
PD-1
PD-L1
(ii) P815
(iii) P815-PD-L1 #1
(iv) P815-PD-L1 #2
(v) P815-PD-L1 #3
PD-L1
H-2L^d
(B)
細胞溶解(%)
70
60
50
40
30
20
10
0
1
10
100
E / T ratios

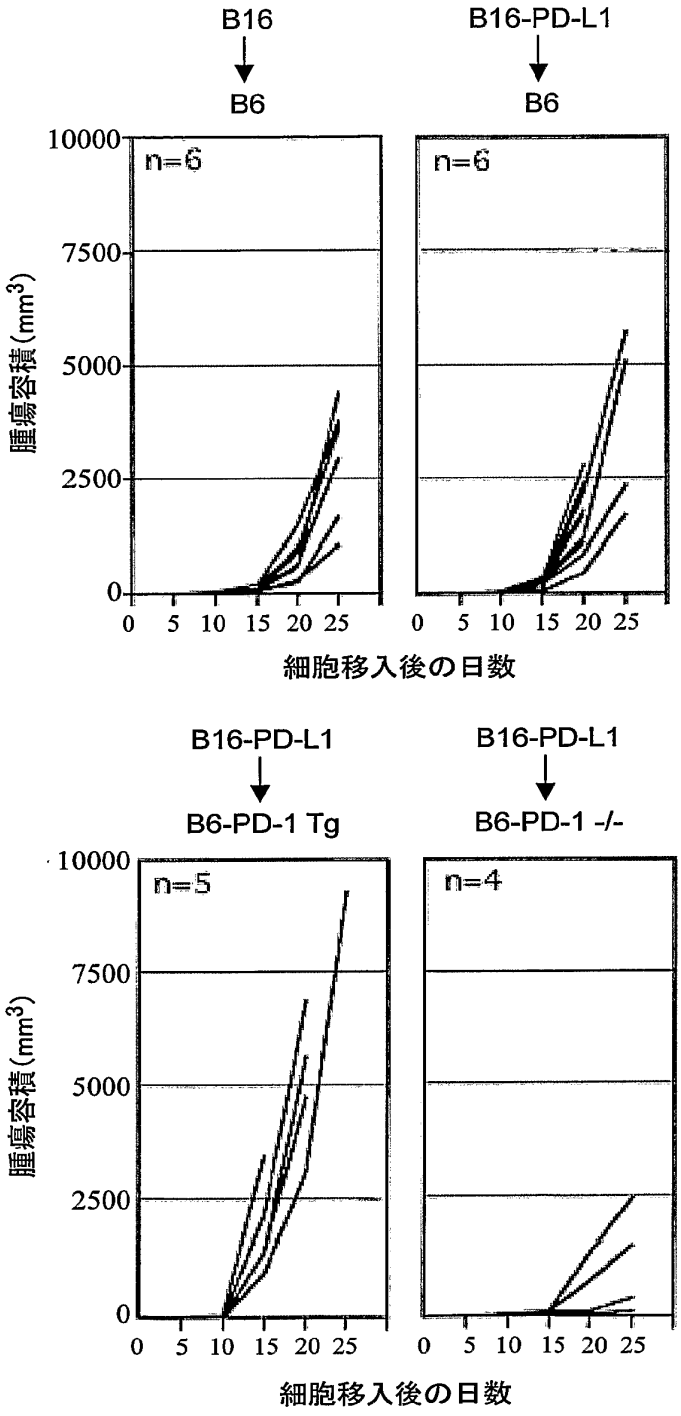
第２図


（A）
P815
P815-PD-L1
n=6
n=6
腫瘍容積(mm3)
1500
1000
500
0 5 10 15 20
0 5 10 15 20
細胞移入後の日数
生存率(%)
100
80
60
40
20
0
0 10 20 30 40 50 80
細胞移入後の日数
（B）
a
b
c
d

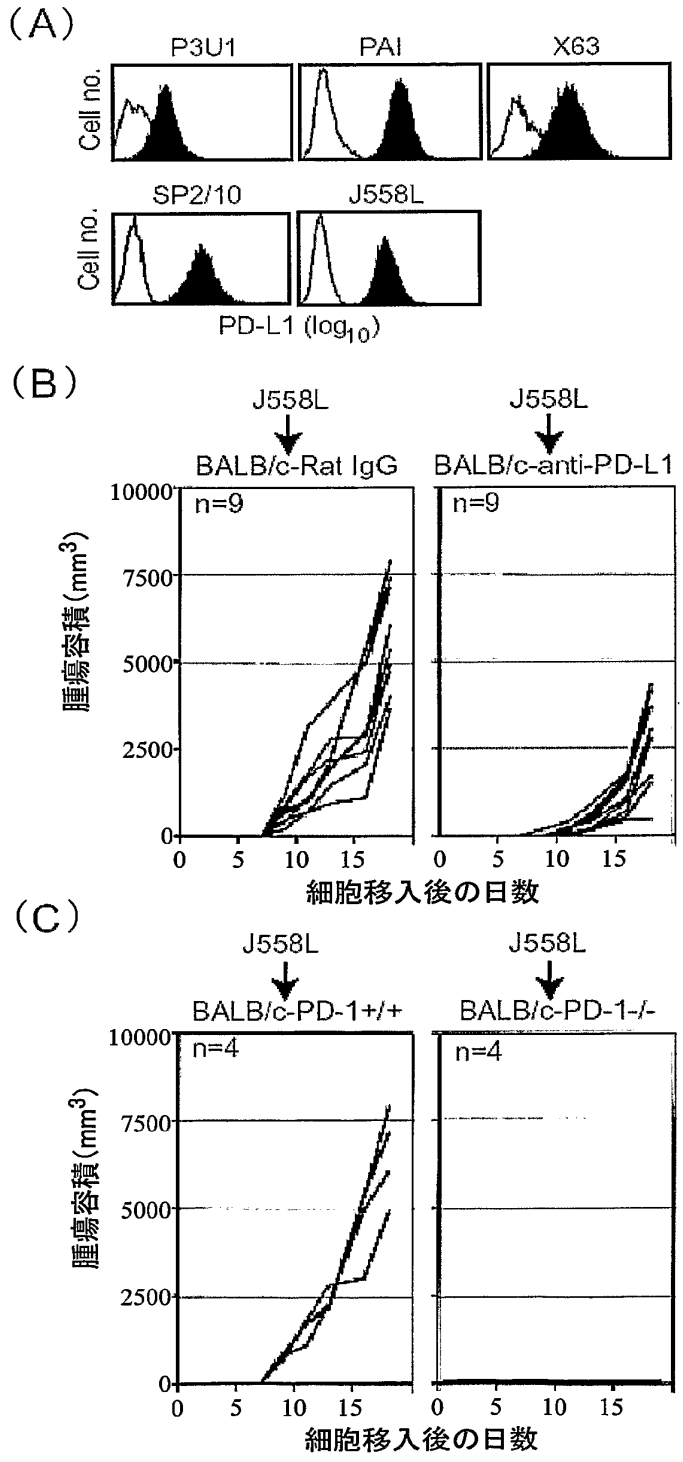
第３図


(A)
IFN-γ (pg/ml)
300
200
100
P815
P815-PD-L1
P815-PD-L1+anti-PD-L1
(B)
n=10
rat IgG
n=10
anti-PD-L1
腫瘍容積(mm3)
2500
2000
1500
1000
500
0
0 5 10 15 20
細胞移入後の日数
生存率(%)
100
80
60
40
20
0
0 20 40 60 80
細胞移入後の日数

第４図

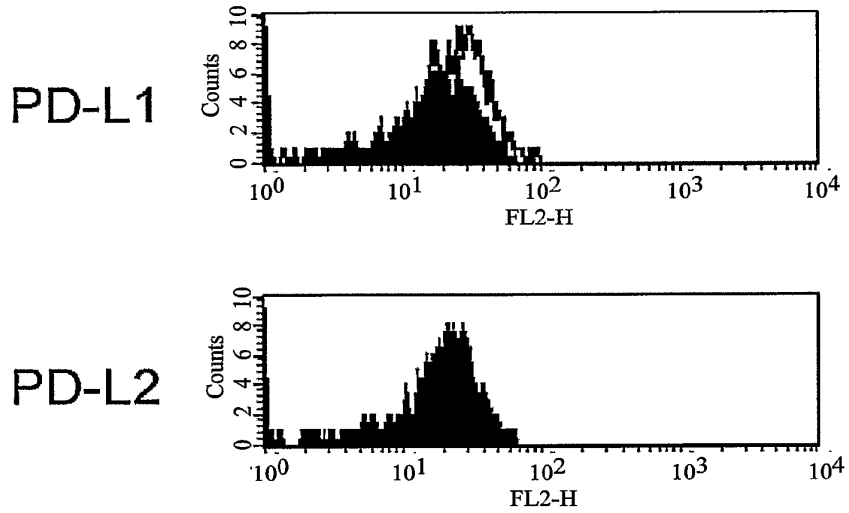

B16
B6
B16-PD-L1
B6
n=6
n=6
10000
7500
5000
2500
0
腫瘍容積(mm3)
0 5 10 15 20 25
0 5 10 15 20 25
細胞移入後の日数
B16-PD-L1
B6-PD-1 Tg
B16-PD-L1
B6-PD-1 -/-
n=5
n=4
10000
7500
5000
2500
0
腫瘍容積(mm3)
0 5 10 15 20 25
0 5 10 15 20 25
細胞移入後の日数

第５図

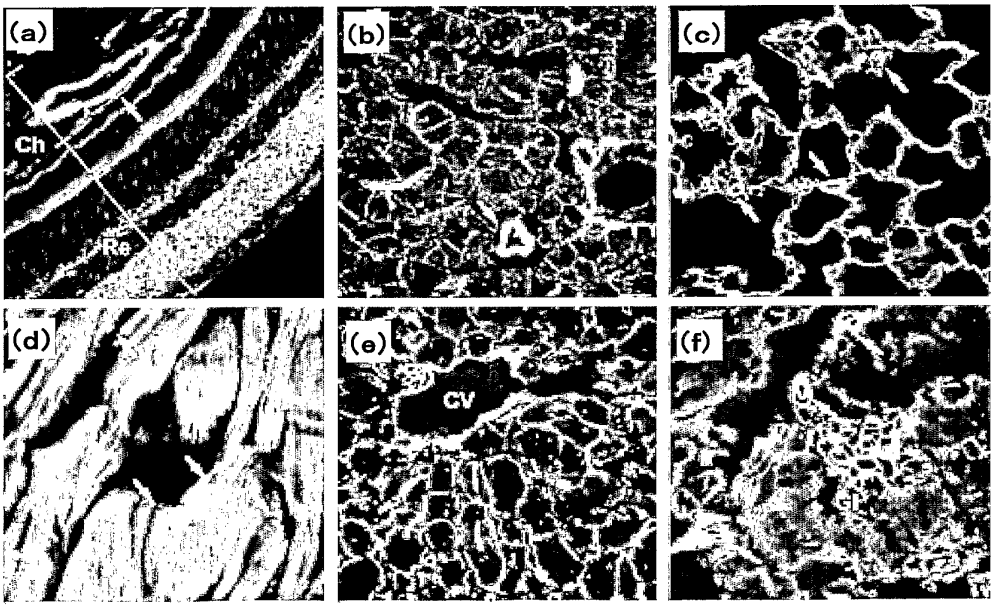

(A)
P3U1
PAI
X63
Cell no.
SP2/10
J558L
Cell no.
PD-L1 (log10)
(B)
J558L
J558L
BALB/c-Rat IgG
BALB/c-anti-PD-L1
n=9
n=9
腫瘍容積(mm3)
10000
7500
5000
2500
0
0 5 10 15
0 5 10 15
細胞移入後の日数
(C)
J558L
J558L
BALB/c-PD-1+/+
BALB/c-PD-1-/-
n=4
n=4
腫瘍容積(mm3)
10000
7500
5000
2500
0
0 5 10 15
0 5 10 15
細胞移入後の日数

第６図

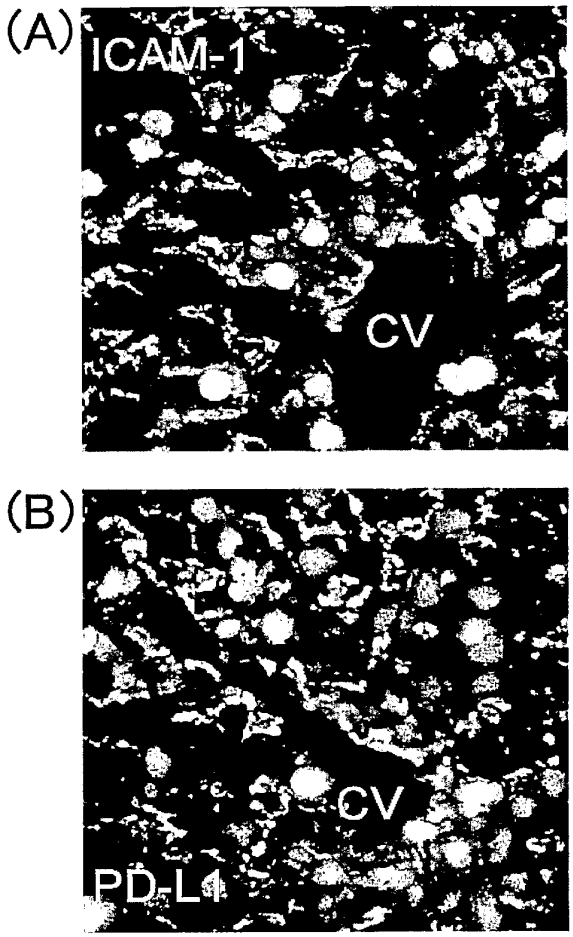
（A）


PD-L1
Counts
0 2 4 6 8 10
10^0 10^1 10^2 10^3 10^4
FL2-H
PD-L2
Counts
0 2 4 6 8 10
10^0 10^1 10^2 10^3 10^4
FL2-H

（B）

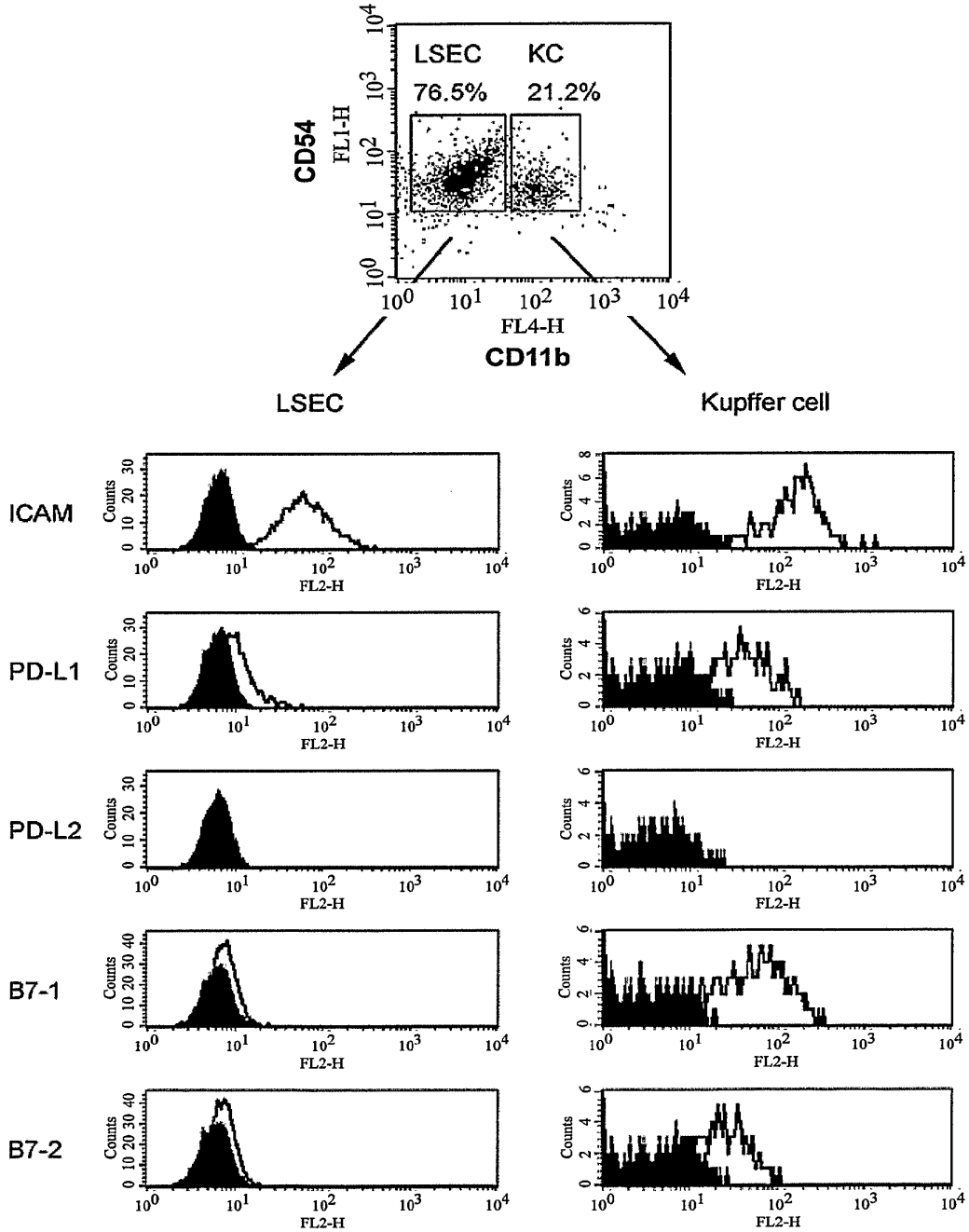

(a)
Ch
Rs
(b)
A
(c)
(d)
(e)
CV
(f)

第７図

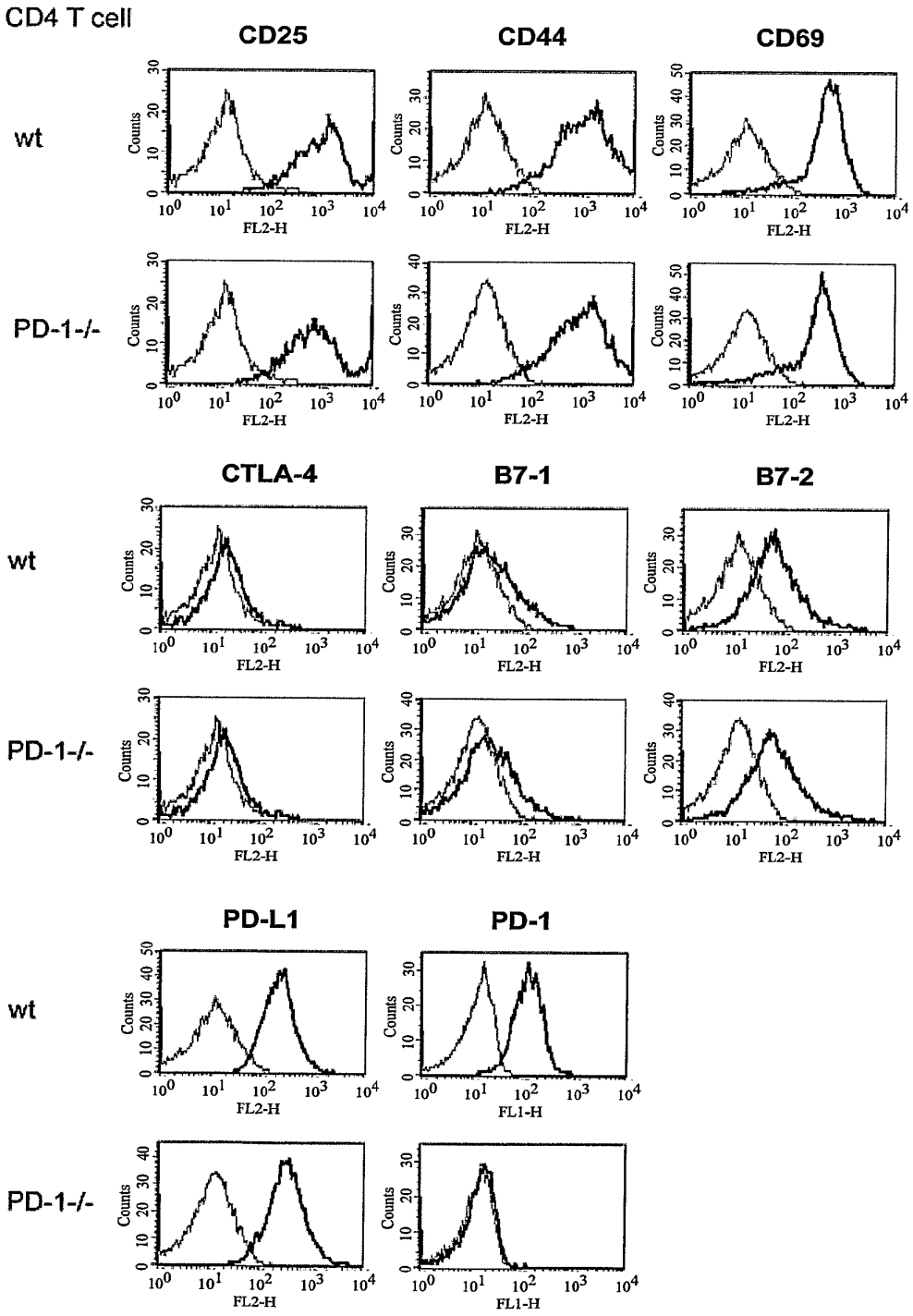


x40

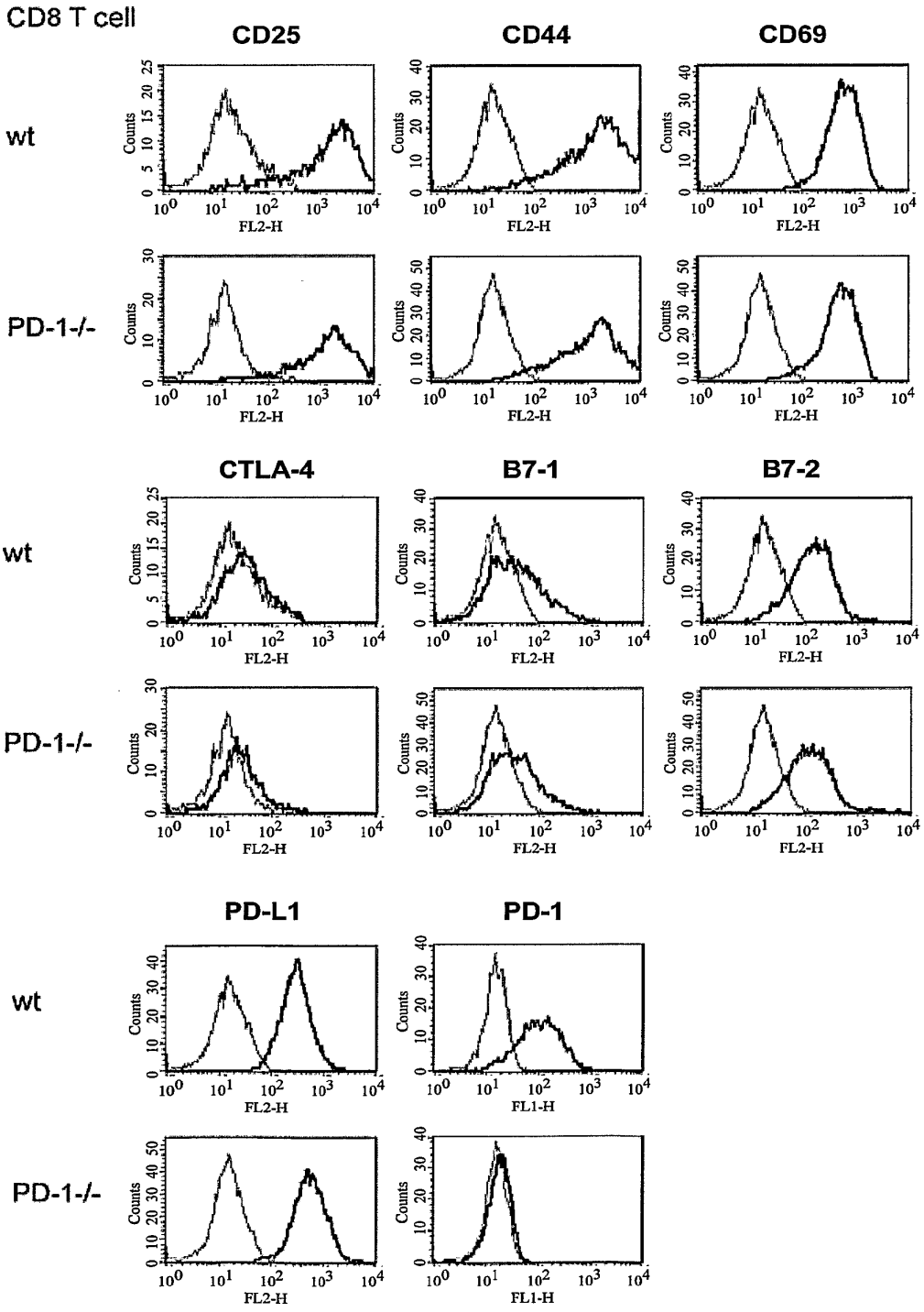
第８図


LSEC
KC
76.5%
21.2%
CD54
FL1-H
FL4-H
CD11b
LSEC
Kupffer cell
ICAM
PD-L1
PD-L2
B7-1
B7-2
Counts
FL2-H

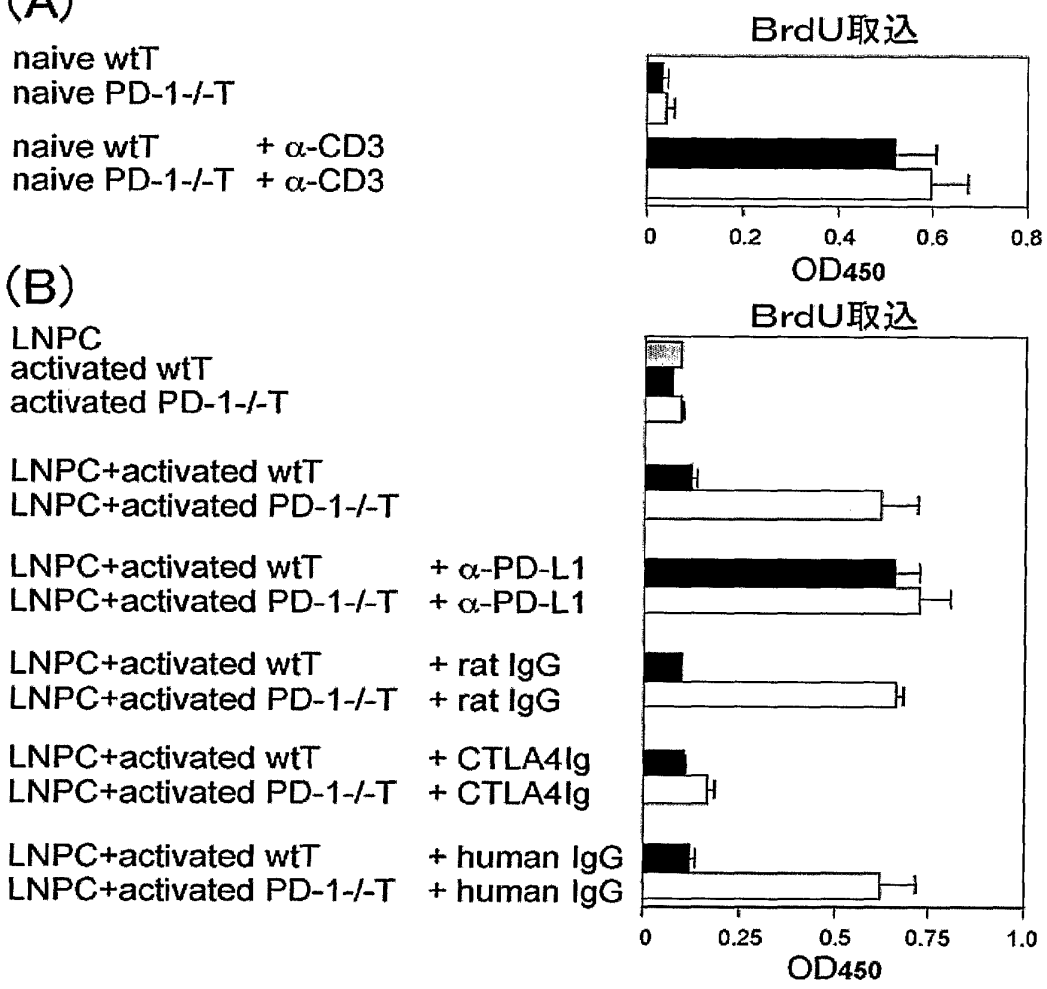
第９図


CD4 T cell
CD25
CD44
CD69
wt
PD-1-/-
CTLA-4
B7-1
B7-2
wt
PD-1-/-
PD-L1
PD-1
wt
PD-1-/-
Counts
FL2-H
FL1-H

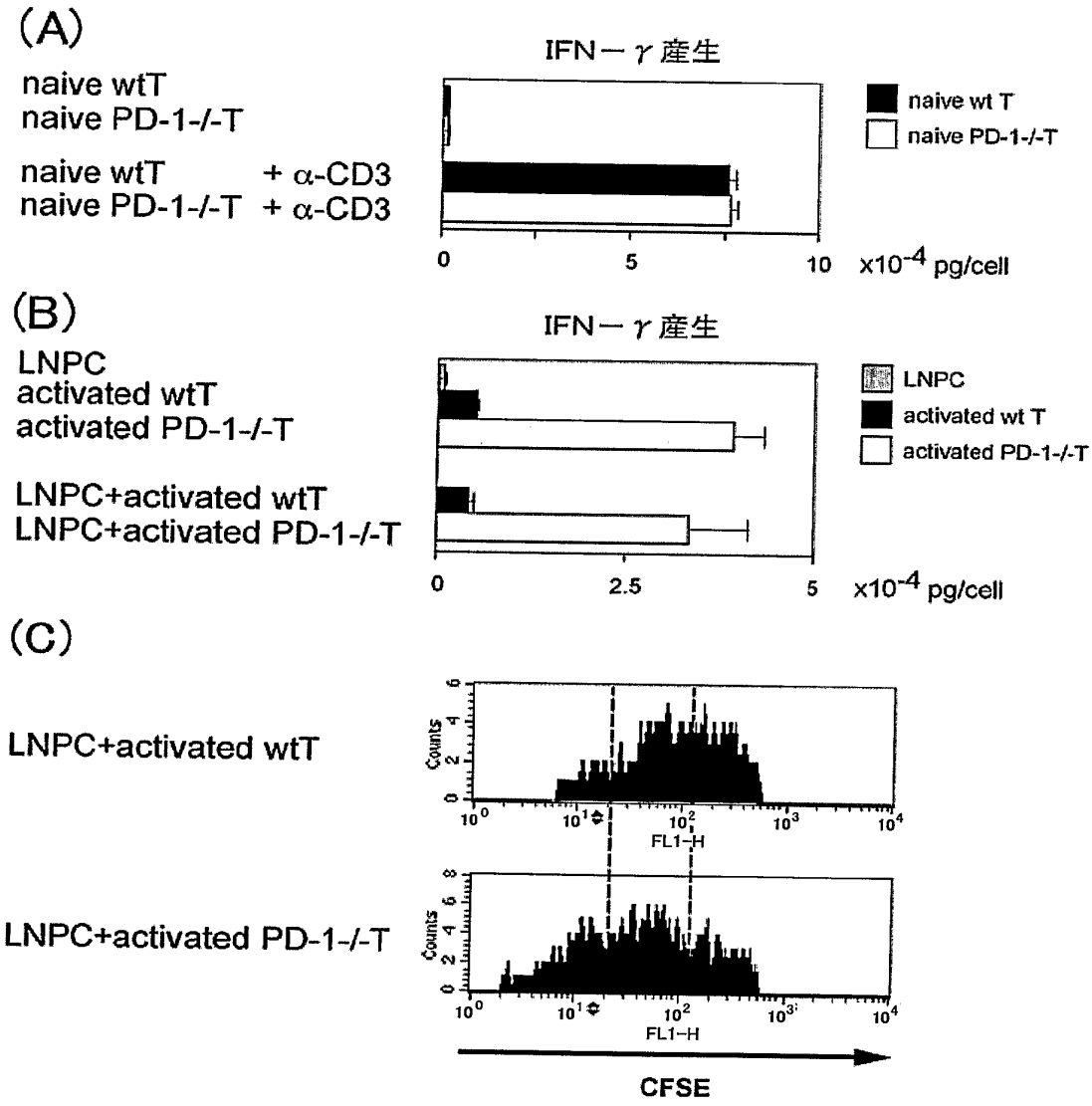
第１０図


CD8 T cell
CD25
CD44
CD69
wt
PD-1-/-
CTLA-4
B7-1
B7-2
wt
PD-1-/-
PD-L1
PD-1
wt
PD-1-/-
Counts
FL2-H
FL1-H

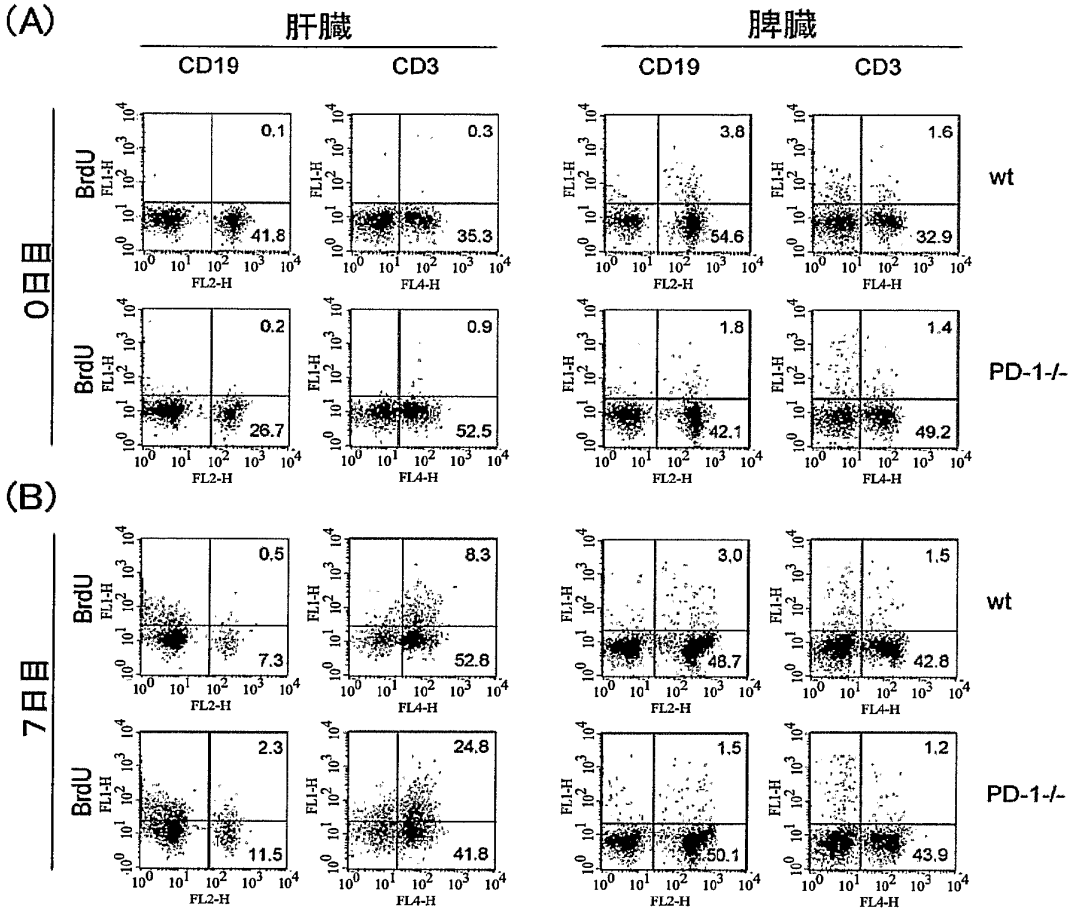
第１１図


(A)
naive wtT
naive PD-1-/-T
naive wtT + α-CD3
naive PD-1-/-T + α-CD3
BrdU取込
0
0.2
0.4
0.6
0.8
OD450
(B)
LNPC
activated wtT
activated PD-1-/-T
LNPC+activated wtT
LNPC+activated PD-1-/-T
LNPC+activated wtT + α-PD-L1
LNPC+activated PD-1-/-T + α-PD-L1
LNPC+activated wtT + rat IgG
LNPC+activated PD-1-/-T + rat IgG
LNPC+activated wtT + CTLA4Ig
LNPC+activated PD-1-/-T + CTLA4Ig
LNPC+activated wtT + human IgG
LNPC+activated PD-1-/-T + human IgG
BrdU取込
0
0.25
0.5
0.75
1.0
OD450

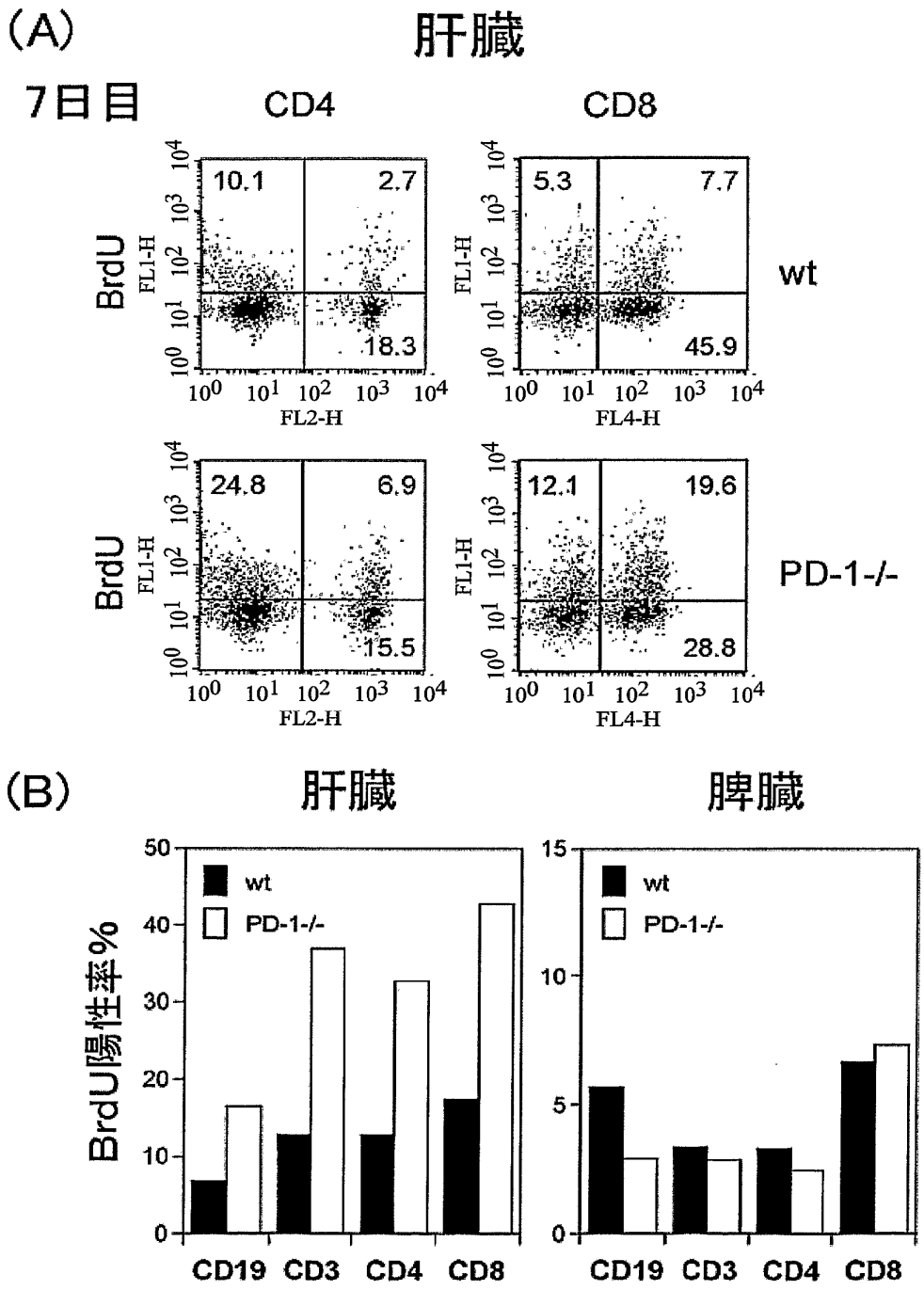
第１２図


(A)
naive wtT
naive PD-1-/-T
naive wtT + α-CD3
naive PD-1-/-T + α-CD3
IFNーγ産生
naive wt T
naive PD-1-/-T
0
5
10
x10-4 pg/cell
(B)
LNPC
activated wtT
activated PD-1-/-T
LNPC+activated wtT
LNPC+activated PD-1-/-T
IFNーγ産生
LNPC
activated wt T
activated PD-1-/-T
0
2.5
5
x10-4 pg/cell
(C)
LNPC+activated wtT
LNPC+activated PD-1-/-T
Counts
FL1-H
CFSE

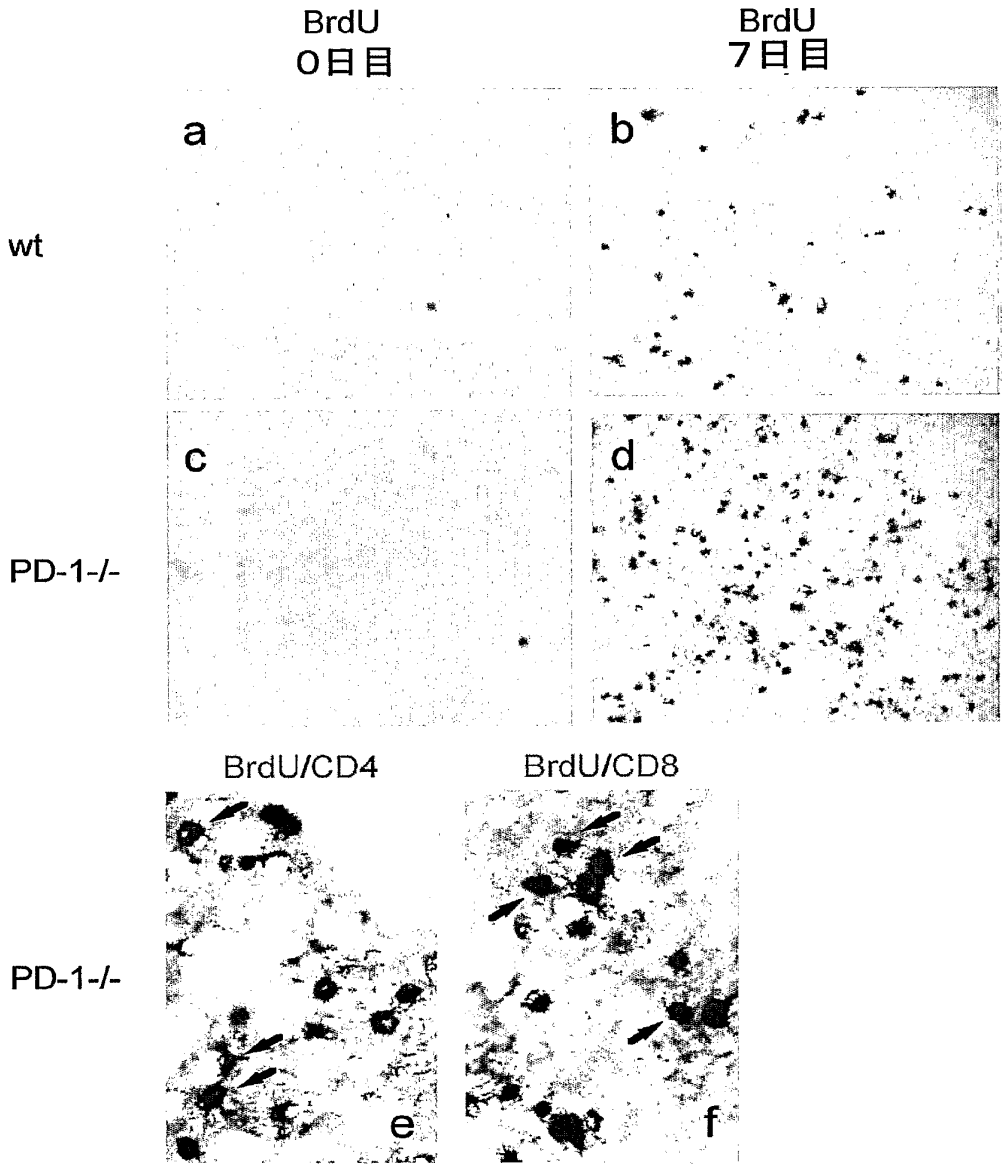
第１３図


(A)
肝臓
脾臓
CD19
CD3
CD19
CD3
0日目
BrdU
FL1-H
FL2-H
FL4-H
0.1
41.8
0.3
35.3
3.8
54.6
1.6
32.9
wt
0.2
26.7
0.9
52.5
1.8
42.1
1.4
49.2
PD-1-/-
(B)
7日目
0.5
7.3
8.3
52.8
3.0
48.7
1.5
42.8
wt
2.3
11.5
24.8
41.8
1.5
50.1
1.2
43.9
PD-1-/-

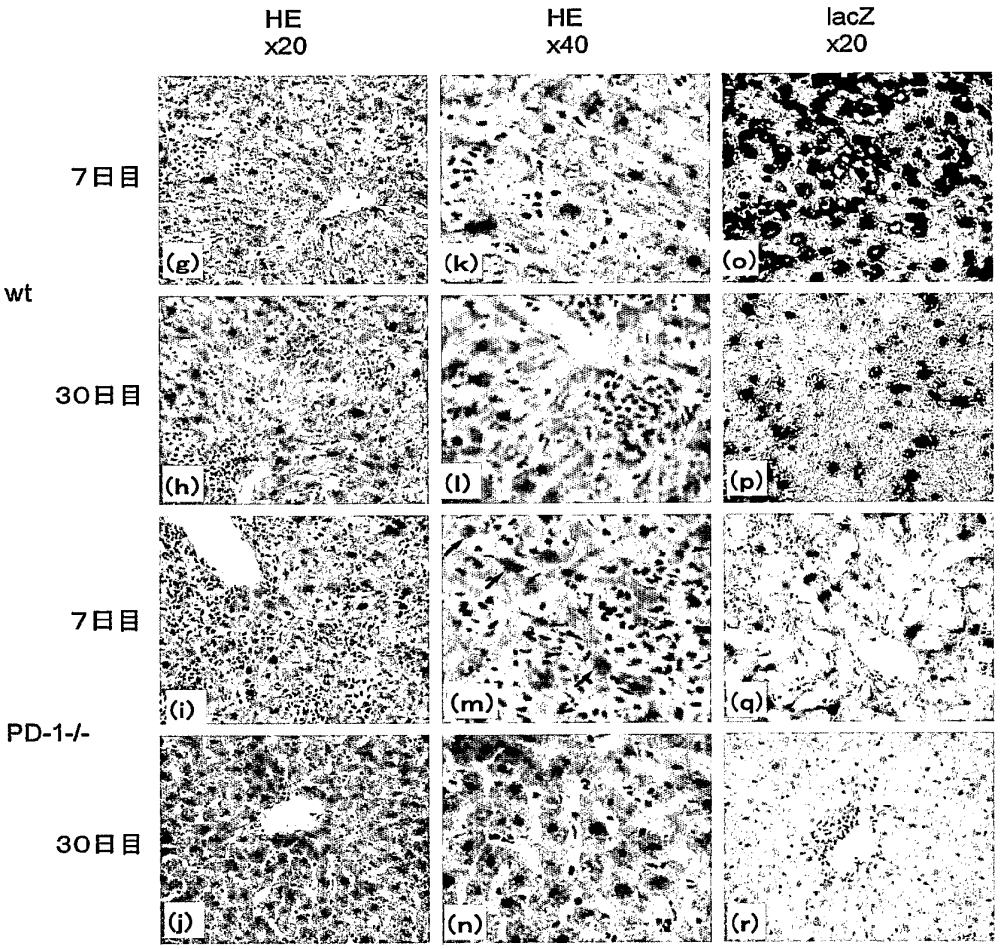
第１４図


(A)
肝臓
7日目
CD4
CD8
BrdU
FL1-H
FL2-H
FL4-H
10.1
2.7
18.3
5.3
7.7
45.9
wt
24.8
6.9
15.5
12.1
19.6
28.8
PD-1-/-
(B)
肝臓
脾臓
BrdU陽性率%
wt
PD-1-/-
CD19
CD3
CD4
CD8

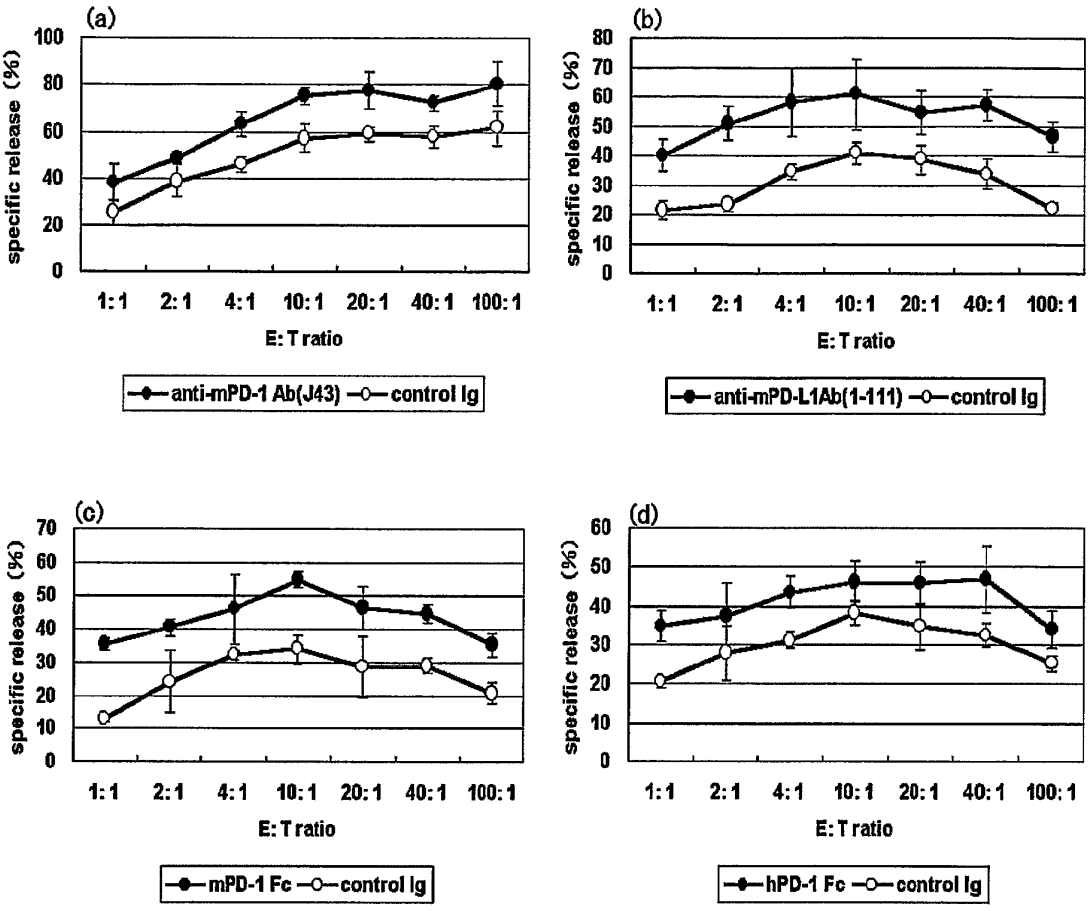
第１５図



第１６図



第１７図


(a)
specific release (%)
100
80
60
40
20
0
1:1 2:1 4:1 10:1 20:1 40:1 100:1
E:T ratio
anti-mPD-1 Ab(J43) control Ig
(b)
specific release (%)
80
70
60
50
40
30
20
10
0
1:1 2:1 4:1 10:1 20:1 40:1 100:1
E:T ratio
anti-mPD-L1Ab(1-111) control Ig
(c)
specific release (%)
70
60
50
40
30
20
10
0
1:1 2:1 4:1 10:1 20:1 40:1 100:1
E:T ratio
mPD-1 Fc control Ig
(d)
specific release (%)
60
50
40
30
20
10
0
1:1 2:1 4:1 10:1 20:1 40:1 100:1
E:T ratio
hPD-1 Fc control Ig

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP03/08420

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] A61K45/00, 38/17, 39/395, 31/7088, A61P31/00, 35/00, 35/04, 37/04, C12N5/10, C12Q1/02, G01N33/50, A01K6//027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[7] A61K45/00, 38/17, 39/395, 31/7088, A61P31/00, 35/00, 35/04, 37/04, C12N5/10, C12Q1/02, G01N33/50, A01K6//027

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAPLUS(STN), MEDLINE(STN), EMBASE(STN), BIOSIS(STN), JICST(JOIS)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/14557 A1 (DANA-FARBER CANCER INSTITUTE, INC.), 01 March, 2001 (01.03.01), Particularly, abstract; Claims; page 82, line 8 to page 86, line 10 & EP 1210428 A1 & US 2002/102651 A1 & US 2002/160000 A1 & BR 2000013581 A & NO 2002000859 A	1-11,23-33
X	WO 02/00730 A2 (GENETICS INSTITUTE, INC.), 03 January, 2002 (03.01.02), Particularly, abstract; Claims; page 104, line 9 to page 108, line 13 & WO 02/00730 A3 & WO 02/00730 C2 & EP 1297135 A2 & US 2002/110836 A1 & US 2002/164600 A1 & AU 2001071731 A5	1-11,23-33

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 October, 2003 (04.10.03)	21 October, 2003 (21.10.03)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP03/08420

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/00692 A2 (GENETICS INSTITUTE, INC.), 03 January, 2002 (03.01.02), Particularly, abstract; Claims; page 104, line 9 to page 108, line 13 & WO 02/00692 A3 & WO 02/00692 C2 & EP 1320599 A2 & US 2002/110836 A1 & US 2002/164600 A1	1-11,23-33
X	EP 670369 A2 (ONO PHARMACEUTICAL CO., LTD.), 06 September, 1995 (06.09.95), Particularly, abstract; Claims; page 5, line 52 to page 6, line 6 & EP 670369 A3 & US 5629204 A & US 5698520 A & JP 7-291996 A & JP 2003-230395 A & CA 2143491 A & CN 1113518 A & CN 1080311 B	1-11,23-33
X	BROWN, Julia A. et al., Blockade of PD-1 Ligands on dendritic cells enhances T cell activation and cytokine production., FASEB Journal, March 2002, Vol.16, No.4, pp.A710,517.4	1-11,23-33
P,X	WO 03/042402 A2 (DANA-FARBER CANCER INSTITUTE, INC.), 22 May, 2003 (22.05.03), Particularly, abstract; Claims; page 56 line 21 to page 59, line 2 (Family: none)	1-11,23-33
P,X	WO 03/011911 A1 (ONO PHARMACEUTICAL CO., LTD.), 13 February, 2003 (13.02.03), Particularly, abstract; Claims; page 14, lines 17 to 19 (Family: none)	1-11,23-33
P,X	WO 02/078731 A1 (WYETH), 10 October, 2002 (10.10.02), Particularly, abstract; Claims; page 74, line 27 to page 77, line 29 (Family: none)	1-11,23-33
P,X	BROWN, Julia A. et al., Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production., The Journal of Immunology., February 2003, Vol.170, No.3, 1257-66	1-11,23-33

Form PCT/ISA/210 (continuation of second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP03/08420

Box I Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 12-22

because they relate to subject matter not required to be searched by this Authority, namely:

Claims 12-22 pertain to methods for treatment of the human body by therapy and thus relates to a subject matter which this International Searching Authority is not required, under the provisions of Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. [ ] Claims Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

国際調査報告

国際出願番号　ＰＣＴ／ＪＰ０３／０８４２０

Ａ．　発明の属する分野の分類（国際特許分類（ＩＰＣ））

Int.Cl[7] A61K 45/00, 38/17, 39/395, 31/7088, A61P31/00, 35/00, 35/04, 37/04, C12N5/10, C12Q1/02, G01N33/50, A01K6//027

Ｂ．　調査を行った分野

調査を行った最小限資料（国際特許分類（ＩＰＣ））

Int.Cl[7] A61K 45/00, 38/17, 39/395, 31/7088, A61P31/00, 35/00, 35/04, 37/04, C12N5/10, C12Q1/02, G01N33/50, A01K6//027

最小限資料以外の資料で調査を行った分野に含まれるもの

国際調査で使用した電子データベース（データベースの名称、調査に使用した用語）

CAPLUS(STN), MEDLINE(STN), EMBASE(STN), BIOSIS(STN), JICST(JOIS)

Ｃ．　関連すると認められる文献

引用文献のカテゴリー＊	引用文献名　及び一部の箇所が関連するときは、その関連する箇所の表示	関連する請求の範囲の番号
X	WO 01/14557 A1(DANA-FARBER CANCER INSTITUTE, INC.) 2001.03.01 特に、Abstract, Claims, 第82ﾍﾟｰｼﾞ第8行-第86ﾍﾟｰｼﾞ第10行 & EP 1210428 A1 & US 2002/102651 A1 & US 2002/160000 A1 & BR 2000013581 A & NO 2002000859 A	1-11, 23-33

☒ Ｃ欄の続きにも文献が列挙されている。　　☐ パテントファミリーに関する別紙を参照。

＊　引用文献のカテゴリー
「Ａ」特に関連のある文献ではなく、一般的技術水準を示すもの
「Ｅ」国際出願日前の出願または特許であるが、国際出願日以後に公表されたもの
「Ｌ」優先権主張に疑義を提起する文献又は他の文献の発行日若しくは他の特別な理由を確立するために引用する文献（理由を付す）
「Ｏ」口頭による開示、使用、展示等に言及する文献
「Ｐ」国際出願日前で、かつ優先権の主張の基礎となる出願の日の後に公表された文献
「Ｔ」国際出願日又は優先日後に公表された文献であって出願と矛盾するものではなく、発明の原理又は理論の理解のために引用するもの
「Ｘ」特に関連のある文献であって、当該文献のみで発明の新規性又は進歩性がないと考えられるもの
「Ｙ」特に関連のある文献であって、当該文献と他の１以上の文献との、当業者にとって自明である組合せによって進歩性がないと考えられるもの
「＆」同一パテントファミリー文献

国際調査を完了した日	国際調査報告の発送日
０４．１０．０３	21.10.03

国際調査機関の名称及びあて先	特許庁審査官（権限のある職員）	４Ｃ	９２８４
日本国特許庁（ＩＳＡ／ＪＰ） 郵便番号１００－８９１５ 東京都千代田区霞が関三丁目４番３号	瀬下　浩一 印 電話番号　０３－３５８１－１１０１　内線　３４５２		

様式ＰＣＴ／ＩＳＡ／２１０（第２ページ）（１９９８年７月）

Ｃ（続き）．　関連すると認められる文献

引用文献のカテゴリー＊	引用文献名　及び一部の箇所が関連するときは、その関連する箇所の表示	関連する請求の範囲の番号
X	WO 02/00730 A2(GENETICS INSTITUTE, INC.)2002.01.03 特に、Abstract, Claims, 第104ﾍﾟｰｼﾞ第9行-第108ﾍﾟｰｼﾞ第13行 & WO 02/00730 A3 & WO 02/00730 C2 & EP 1297135 A2 & US 2002/110836 A1 & US 2002/164600 A1 & AU 2001071731 A5	1-11, 23-33
X	WO 02/00692 A2(GENETICS INSTITUTE, INC.)2002.01.03 特に、Abstract, Claims, 第104ﾍﾟｰｼﾞ第9行-第108ﾍﾟｰｼﾞ第13行 & WO 02/00692 A3 & WO 02/00692 C2 & EP 1320599 A2 & US 2002/110836 A1 & US 2002/164600 A1	1-11, 23-33
X	EP 670369 A2(ONO PHARMACEUTICAL CO., LTD.)1995.09.06 特に、Abstract, Claims, 第5ﾍﾟｰｼﾞ第52行-第6ﾍﾟｰｼﾞ第6行 & EP 670369 A3 & US 5629204 A & US 5698520 A & JP 7-291996 A & JP 2003-230395 A & CA 2143491 A & CN 1113518 A & CN 1080311 B	1-11, 23-33
X	BROWN, Julia A. et al, Blockade of PD-1 Ligands on dendritic cells enhances T cell activation and cytokine production., FASEB Journal, Mar. 2002, Vol.16, No.4, pp. A710, 517.4	1-11, 23-33
ＰX	WO 03/042402 A2(DANA-FARBER CANCER INSTITUTE,INC.) 2003.05.22 特に、Abstract, Claims, 第56ﾍﾟｰｼﾞ第21行-第59ﾍﾟｰｼﾞ第2行 （ファミリーなし）	1-11, 23-33
ＰX	WO 03/011911 A1(小野薬品工業株式会社)2003.02.13 特に、Abstract, 請求の範囲, 第14ﾍﾟｰｼﾞ第17-19行 （ファミリーなし）	1-11, 23-33
ＰX	WO 02/078731 A1(WYETH)2002.10.10 特に、Abstract, Claims, 第74ﾍﾟｰｼﾞ第27行-第77ﾍﾟｰｼﾞ第29行 （ファミリーなし）	1-11, 23-33
ＰX	BROWN, Julia A. et al, Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production., The Jounrnal of Immunology. Feb. 2003 , Vol.170, No.3, 1257-66	1-11, 23-33

国際調査報告

国際出願番号　ＰＣＴ／ＪＰ０３／０８４２０

第Ⅰ欄　請求の範囲の一部の調査ができないときの意見（第１ページの２の続き）

法第８条第３項（ＰＣＴ１７条(2)(a)）の規定により、この国際調査報告は次の理由により請求の範囲の一部について作成しなかった。

1．[X] 請求の範囲　１２－２２　は、この国際調査機関が調査をすることを要しない対象に係るものである。
つまり、
請求の範囲１２－２２は治療による人体の処置方法に関するものであって、ＰＣＴ１７条(2)(a)(i)及びＰＣＴ規則39.1(iv)の規定により、この国際調査機関が調査をすることを要しない対象に係るものである。

2．[ ] 請求の範囲　＿＿＿＿＿＿＿＿　は、有意義な国際調査をすることができる程度まで所定の要件を満たしていない国際出願の部分に係るものである。つまり、

3．[ ] 請求の範囲　＿＿＿＿＿＿＿＿　は、従属請求の範囲であってＰＣＴ規則6.4(a)の第２文及び第３文の規定に従って記載されていない。

第Ⅱ欄　発明の単一性が欠如しているときの意見（第１ページの３の続き）

次に述べるようにこの国際出願に二以上の発明があるとこの国際調査機関は認めた。

1．[ ] 出願人が必要な追加調査手数料をすべて期間内に納付したので、この国際調査報告は、すべての調査可能な請求の範囲について作成した。

2．[ ] 追加調査手数料を要求するまでもなく、すべての調査可能な請求の範囲について調査することができたので、追加調査手数料の納付を求めなかった。

3．[ ] 出願人が必要な追加調査手数料を一部のみしか期間内に納付しなかったので、この国際調査報告は、手数料の納付のあった次の請求の範囲のみについて作成した。

4．[ ] 出願人が必要な追加調査手数料を期間内に納付しなかったので、この国際調査報告は、請求の範囲の最初に記載されている発明に係る次の請求の範囲について作成した。

追加調査手数料の異議の申立てに関する注意
[ ] 追加調査手数料の納付と共に出願人から異議申立てがあった。
[ ] 追加調査手数料の納付と共に出願人から異議申立てがなかった。

様式ＰＣＴ／ＩＳＡ／２１０（第１ページの続葉（１））（１９９８年７月）

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 March 2015 (26.03.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/042246 A1

(51) International Patent Classification:
C07K 16/28 (2006.01) *A61P 35/00* (2006.01)
A61K 39/395 (2006.01)

(21) International Application Number:
PCT/US2014/056277

(22) International Filing Date:
18 September 2014 (18.09.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/880,606 20 September 2013 (20.09.2013) US
62/014,471 19 June 2014 (19.06.2014) US

(71) Applicant: **BRISTOL-MYERS SQUIBB COMPANY** [US/US]; Route 206 & Province Line Road, Princeton, NJ 08543 (US).

(72) Inventors: **KORMAN, Alan, J.**; 700 Bay Road, Redwood City, CA 94063 (US). **LONBERG, Nils**; 700 Bay Road, Redwood City, CA 94063 (US). **FONTANA, David, J.**; 1201 Eastlake Avenue E., Seattle, WA 98102 (US). **GUTIERREZ, Andres, A.**; Route 206 & Province Line Road, Princeton, NJ 08543 (US). **SELBY, Mark, J.**; 700 Bay Road, Redwood City, CA 94063 (US). **LEWIS, Katherine, E.**; 1201 Eastlake Avenue E., Seattle, WA 98102 (US).

(74) Agents: **REMILLARD, Jane, E.** et al.; Nelson Mullins Riley & Scarborough LLP, One Post Office Square, Boston, MA 02109-2127 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2015/042246 A1

(54) Title: COMBINATION OF ANTI-LAG-3 ANTIBODIES AND ANTI-PD-1 ANTIBODIES TO TREAT TUMORS

(57) Abstract: Provided are methods for clinical treatment of tumors (e.g., advanced solid tumors) using an anti-LAG-3 antibody in combination with an anti-PD-1 antibody.

COMBINATION OF ANTI-LAG-3 ANTIBODIES AND ANTI-PD-1 ANTIBODIES TO TREAT TUMORS

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 61/880,606, filed September 20, 2013 and U.S. Provisional Application No. 62/014,471, filed June 19, 2014. The contents of any patents, patent applications, and references cited throughout this specification are hereby incorporated by reference in their entireties.

BACKGROUND

Lymphocyte activation gene-3 (LAG-3; CD223) is a type I transmembrane protein that is expressed on the cell surface of activated $CD4^+$ and $CD8^+$ T cells and subsets of NK and dendritic cells (Triebel F, et *al.*, *J. Exp. Med.* 1990; 171:1393-1405; Workman CJ, *et al.*, *J. Immunol.* 2009; 182(4):1885-91). LAG-3 is closely related to CD4, which is a co-receptor for T helper cell activation. Both molecules have 4 extracellular Ig-like domains and require binding to their ligand, major histocompatibility complex (MHC) class II, for their functional activity. In contrast to CD4, LAG-3 is only expressed on the cell surface of activated T cells and its cleavage from the cell surface terminates LAG-3 signaling. LAG-3 can also be found as a soluble protein but it does not bind to MHC class II and its function is unknown.

It has been reported that LAG-3 plays an important role in promoting regulatory T cell (Treg) activity and in negatively regulating T cell activation and proliferation (Workman CJ, *et al.*, *J. Immunol.* 2005; 174:688-695). Both natural and induced Treg express increased LAG-3, which is required for their maximal suppressive function (Camisaschi C, *et al.*, *J. Immunol.* 2010; 184:6545-6551 and Huang CT, *et al.*, *Immunity*. 2004; 21:503–513). Furthermore, ectopic expression of LAG-3 on $CD4^+$ effector T cells reduced their proliferative capacity and conferred on them regulatory potential against third party T cells (Huang CT, *et al.*, *Immunity*. 2004; 21:503-513). Recent studies have also shown that high LAG-3 expression on exhausted lymphocytic choriomeningitis virus (LCMV)-specific $CD8^+$ T cells contributes to their unresponsive state and limits $CD8^+$ T cell antitumor responses (Blackburn SD, *et al.*, *Nat. Immunol.* 2009; 10:29-37 and Grosso JF, *et al.*, *J. Clin.*

Invest. 2007; 117:3383-3392). In fact, LAG-3 maintained tolerance to self and tumor antigens via direct effects on $CD8^+$ T cells in 2 murine models (Grosso JF, *et al.*, *J. Clin. Invest.* 2007; 117:3383-3392).

Immune tolerance observed in the setting of tumor development and tumor recurrence, however, seems to be mediated by the co-expression of various T cell negative regulatory receptors, not solely from LAG-3. Data from chronic viral infection models (Blackburn SD, *et al.*, *Nat. Immunol.* 2009; 10:29-37, Grosso JF, *et al.*, *J. Clin. Invest.* 2007; 117:3383-3392, and Lyford-Pike S, *et al.*, *Cancer Res.* 2013;73(6):1733-41), knock-out mice (Woo SR, *et al.*, *Cancer Res.* 2012;72:917-927; Okazaki T, *et al., J. Exp Med.* 2011; 208:395-407, and Bettini M. *et al.*, *J. Immunol.* 2011;187:3493-3498), tumor recurrence models (Goding SR, *et al.*, *J. Immunol.* 2013; 190(9):4899-4909) and, to a more limited extent, human cancer patients (Goding SR, *et al.*, *J. Immunol.* 2013; 190(9):4899-4909**,** Matsuzaki J, *et al., Proc. Natl. Acad. Sci.*, U S A. 2010;107:7875-7880, and Gandhi MK, *et al.*, *Blood.* 2006;108:2280-2289) support a model wherein T cells that are continuously exposed to antigen become progressively inactivated through a process termed "exhaustion." Exhausted T cells are characterized by the expression of T cell negative regulatory receptors, predominantly CTLA-4, PD-1, and LAG-3, whose action is to limit the cell's ability to proliferate, produce cytokines, and kill target cells and/or to increase Treg activity. However, the timing and sequence of expression of these molecules in the development and recurrence of tumors have not been fully characterized.

Programmed Cell Death 1 (PD-1) is a cell surface signaling receptor that plays a critical role in the regulation of T cell activation and tolerance (Keir ME, et al., *Annu Rev Immunol* 2008; 26:677-704). It is a type I transmembrane protein and together with BTLA, CTLA-4, ICOS and CD28, comprise the CD28 family of T cell co-stimulatory receptors. PD-1 is primarily expressed on activated T cells, B cells, and myeloid cells (Dong H, *et al.*, *Nat Med.* 1999; 5:1365-1369). It is also expressed on natural killer (NK) cells (Terme M, *et al.*, *Cancer Res* 2011; 71:5393-5399). Binding of PD-1 by its ligands, PD-L1 and PD-L2, results in phosphorylation of the tyrosine residue in the proximal intracellular immune receptor tyrosine inhibitory domain, followed by recruitment of the phosphatase SHP-2, eventually resulting in down-regulation of T cell activation. One important role of PD-1 is to limit the activity of T cells in peripheral tissues at the time of an inflammatory response to infection, thus limiting the development of autoimmunity (Pardoll DM., *Nat Rev*

Cancer 2012; 12:252-264). Evidence of this negative regulatory role comes from the finding that PD-1-deficient mice develop lupus-like autoimmune diseases including arthritis and nephritis, along with cardiomyopathy (Nishimura H, *et al.*, *Immunity*, 1999; 11:141-151; and Nishimura H, *et al.*, *Science*, 2001; 291:319-322). In the tumor setting, the consequence is the development of immune resistance within the tumor microenvironment. PD-1 is highly expressed on tumor-infiltrating lymphocytes, and its ligands are up-regulated on the cell surface of many different tumors (Dong H, *et al.*, *Nat Med* 2002; 8:793-800). Multiple murine cancer models have demonstrated that binding of ligand to PD-1 results in immune evasion. In addition, blockade of this interaction results in anti-tumor activity (Topalian SL, *et a*l. *NEJM* 2012; 366(26):2443-2454; Hamid O, *et al.*, *NEJM* 2013; 369:134-144). Moreover, it has been shown that inhibition of the PD-1/PD-L1 interaction mediates potent antitumor activity in preclinical models (U.S. Patent Nos. 8,008,449 and 7,943,743).

Patients with metastatic or refractory solid tumors have very poor prognosis (Rosenberg SA, *et al.*, *Cancer immunotherapy in Cancer: Principles & Practice of Oncology* (Eds DeVita VT, Lawrence TS and Rosenberg SA) 2011; 332-344 (Lippincott Williams & Wilkins, Philadelphia PA)). Despite advances in multimodal therapy, increases in overall survival in this patient population have been limited. Accordingly, it is an object of the present invention to provide improved methods for treating subjects with such tumors (*e.g.*, advanced refractory solid tumors).

SUMMARY

Provided herein are methods for treating tumors in a human patient, particularly solid tumors (e.g., advanced refractory solid tumors), comprising administering to the patient a combination of an anti-LAG-3 antibody and an anti-PD-1 antibody, wherein the combination is administered (or is for administration) according to a particular clinical dosage regimen (*i.e.*, at a particular dose amount and according to a specific dosing schedule). In one embodiment, the human patient suffers from melanoma, non-small cell lung cancer (NSCLC), virally-related cancer, head and neck cancer (HNC) or gastric adenocarcinoma.

An exemplary anti-LAG-3 antibody is BMS-986016 comprising heavy and light chains comprising the sequences shown in SEQ ID NOs:1 and 2, respectively, or antigen binding fragments and variants thereof. In other embodiments, the antibody comprises

the heavy and light chain complementarity determining regions (CDRs) or variable regions (VRs) of BMS-986016. Accordingly, in one embodiment, the antibody comprises CDR1, CDR2, and CDR3 domains of the heavy chain variable (VH) region of BMS-986016 having the sequence shown in SEQ ID NO:3, and CDR1, CDR2 and CDR3 domains of the light chain variable (VL) region of BMS-986016 having the sequence shown in SEQ ID NO:5. In another embodiment, the antibody comprises CDR1, CDR2 and CDR3 heavy chain sequences set forth in SEQ ID NOs:7, 8, and 9, respectively, and CDR1, CDR2 and CDR3 light chain sequences as set forth in SEQ ID NOs:10, 11, and 12, respectively. In another embodiment, the antibody has VH and/or VL regions comprising the amino acid sequences set forth in SEQ ID NO:3 and/or SEQ ID NO:5, respectively. In another embodiment, the antibody comprises the VH and/or VL regions encoded by the nucleic acid sequences set forth in SEQ ID NO:4 and/or SEQ ID NO:6, respectively. In another embodiment, the antibody competes for binding with, and/or binds to the same epitope on LAG-3 as, the above-mentioned antibodies. In another embodiment, the antibody has at least about 90% variable region amino acid sequence identity with the above-mentioned antibodies (*e.g.*, at least about 90%, 95% or 99% variable region identity with SEQ ID NO:3 or SEQ ID NO:5).

An exemplary anti-PD-1 antibody is Nivolumab (also referred to as "5C4" in WO 2006/121168; and known as BMS-936558, MDX-1106 and ONO-4538) comprising heavy and light chains comprising the sequences shown in SEQ ID NOs:17 and 18, respectively, or antigen binding fragments and variants thereof. In other embodiments, the antibody comprises the heavy and light chain CDRs or VRs of BMS-936558. Accordingly, in one embodiment, the antibody comprises CDR1, CDR2, and CDR3 domains of the VH region of BMS-936558 having the sequence shown in SEQ ID NO:19, and CDR1, CDR2 and CDR3 domains of the VL region of BMS-936558 having the sequence shown in SEQ ID NO:21. In another embodiment, the antibody comprises heavy chain CDR1, CDR2 and CDR3 domains comprising the sequences set forth in SEQ ID NOs:23, 24, and 25, respectively, and light chain CDR1, CDR2 and CDR3 domains comprising the sequences set forth in SEQ ID NOs: 26, 27, and 28, respectively. In another embodiment, the antibody comprises VH and/or VL regions comprising the amino acid sequences set forth in SEQ ID NO: 19 and/or SEQ ID NO:21, respectively. In another embodiment, the antibody comprises the heavy chain variable (VH) and/or light chain variable (VL) regions encoded by the nucleic acid sequences set forth in SEQ ID NO:20 and/or SEQ ID NO:22, respectively. In another embodiment, the antibody

competes for binding with, and/or binds to the same epitope on PD-1 as, the above-mentioned antibodies. In another embodiment, the antibody has at least about 90% variable region amino acid sequence identity with the above-mentioned antibodies (*e.g.*, at least about 90%, 95% or 99% variable region identity with SEQ ID NO:19 or SEQ ID NO:21).

Accordingly, in one aspect, methods of treating solid tumors (*e.g.*, advanced refractory solid tumors) in a human patient are provided, the methods comprising administering to the patient, an effective amount of each of:

(a) an anti-LAG-3 antibody comprising CDR1, CDR2 and CDR3 domains of the heavy chain variable region having the sequence set forth in SEQ ID NO:3, and CDR1, CDR2 and CDR3 domains of the light chain variable region having the sequence set forth in SEQ ID NO:5,

(b) an anti-PD-1 antibody comprising CDR1, CDR2 and CDR3 domains of the heavy chain variable region having the sequence set forth in SEQ ID NO:19, and CDR1, CDR2 and CDR3 domains of the light chain variable region having the sequence set forth in SEQ ID NO:21,

wherein the method comprises at least one administration cycle, wherein the cycle is a period of eight weeks, wherein for each of the at least one cycles, four doses of the anti-LAG-3 antibody are administered at a dose of 3, 20, 80, or 240 mg and four doses of the anti-PD-1 antibody are administered at a dose of 80 or 240 mg. In another embodiment, four doses of the anti-LAG-3 antibody are administered at a dose of about 0.03, 0.25, 1, or 3 mg/kg body weight and four doses of the anti-PD-1 antibody are administered at a dose of 1 or 3 mg/kg body weight.

In one embodiment, the anti-LAG-3 antibody and anti-PD-1 antibody are administered at the following doses:

(a) 3 mg anti-LAG-3 antibody and 80 mg of anti-PD-1 antibody;

(b) 3 mg anti-LAG-3 antibody and 240 mg of anti-PD-1 antibody;

(c) 20 mg anti-LAG-3 antibody and 240 mg of anti-PD-1 antibody;

(d) 80 mg anti-LAG-3 antibody and 240 mg of anti-PD-1 antibody; or

(e) 240 mg anti-LAG-3 antibody and 240 mg of anti-PD-1 antibody.

In another embodiment, the anti-LAG-3 antibody and anti-PD-1 antibody are administered at the following doses:

(a) 0.03 mg/kg anti-LAG-3 antibody and 1 mg/kg of anti-PD-1 antibody;

(b) 0.03 mg/kg anti-LAG-3 antibody and 3 mg/kg of anti-PD-1 antibody;

(c) 0.25 mg/kg anti-LAG-3 antibody and 3 mg/kg of anti-PD-1 antibody;

(d) 1 mg/kg anti-LAG-3 antibody and 3 mg/kg of anti-PD-1 antibody; or

(e) 3 mg/kg anti-LAG-3 antibody and 3 mg/kg of anti-PD-1 antibody.

In one embodiment, the dose of the anti-LAG-3 and/or anti-PD-1 antibody is calculated per mg/kg body weight. In another embodiment, the dose of the anti-LAG-3 and/or anti-PD-1 antibody is a flat-fixed dose. In another embodiment, an intermediate dose of LAG-3 and/or PD-1 is used. For example, LAG-3 could be administered at 0.4 mg/kg and PD-1 could be administered at 90 mg/kg. In another embodiment, dosage regimens are adjusted to provide the optimum desired response (*e.g.*, an effective response).

In another embodiment, the anti-PD-1 antibody is administered on Days 1, 15, 29, and 43 of each cycle. In another embodiment, the anti-LAG-3 antibody is administered on Days 1, 15, 29, and 43 of each cycle. In another embodiment, the anti-PD-1 antibody is administered prior to administration of the anti-LAG-3 antibody. In another embodiment, the anti-PD-1 antibody is administered after administration of the anti- LAG-3 antibody. In another embodiment, the treatment consists of up to 12 cycles.

In one embodiment, the anti-PD-1 antibody and anti-LAG-3 antibody are administered as a first ("front") line of treatment (*e.g.*, the initial or first treatment). In another embodiment, the anti-PD-1 antibody and anti-LAG-3 antibody are administered as a second line of treatment (*e.g.*, after initial treatment with the same or a different therapeutic, including after relapse and/or where the first treatment has failed). The anti-LAG-3 and anti-PD-1 antibodies can be administered to a subject by any suitable means. In one embodiment, the antibodies are formulated for intravenous administration. In another embodiment, the antibodies are administered simultaneously (*e.g.*, formulated together in a single formulation or concurrently as separate formulations). Alternatively, in another embodiment, the antibodies are administered sequentially (*e.g.*, as separate formulations). In another embodiment the anti-LAG-3 antibody is administered within about 30 minutes (e.g., within about 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, or less minutes) prior to administration of the anti-PD-1 antibody.

The efficacy of the treatment methods provided herein can be assessed using any suitable means. In one embodiment, the treatment produces at least one therapeutic effect selected from the group consisting of reduction in size of a tumor,

reduction in number of metastatic lesions over time, complete response, partial response, and stable disease.

Also provided are kits that include a pharmaceutical composition containing an anti-LAG-3 antibody, such as BMS-986016, and an anti-PD-1 antibody, such as BMS-936558, and a pharmaceutically-acceptable carrier, in a therapeutically effective amount adapted for use in the methods described herein. In one embodiment, the kit comprises:

(a) a dose of an anti-LAG-3 antibody comprising CDR1, CDR2 and CDR3 domains of the heavy chain variable region having the sequence set forth in SEQ ID NO:3, and CDR1, CDR2 and CDR3 domains of the light chain variable region having the sequence set forth in SEQ ID NO:5;

(b) a dose of an anti-PD-1 antibody comprising CDR1, CDR2 and CDR3 domains of the heavy chain variable region having the sequence set forth in SEQ ID NO:19, and CDR1, CDR2 and CDR3 domains of the light chain variable region having the sequence set forth in SEQ ID NO:21; and

(c) instructions for using the anti-LAG-3 antibody and anti-PD-1 antibody in a method of the invention.

In another aspect, an anti-LAG-3 antibody is provided, the anti-LAG-3 antibody comprising CDR1, CDR2 and CDR3 domains of the heavy chain variable region having the sequence set forth in SEQ ID NO:3, and CDR1, CDR2 and CDR3 domains of the light chain variable region having the sequence set forth in SEQ ID NO:5, for co-administration with an anti-PD-1 antibody comprising CDR1, CDR2 and CDR3 domains of the heavy chain variable region having the sequence set forth in SEQ ID NO:19, and CDR1, CDR2 and CDR3 domains of the light chain variable region having the sequence set forth in SEQ ID NO:21, in at least one cycle, wherein for each cycle four doses of the anti-LAG-3 antibody are administered at a dose of 3, 20, 80, or 240 mg and four doses of the anti-PD-1 antibody are administered at a dose of 80 or 240 mg. In another embodiment, four doses of the anti-LAG-3 antibody are administered at a dose of 0.03, 0.25, 1, or 3 mg/kg body weight and four doses of the anti-PD-1 antibody are administered at a dose of 1 or 3 mg/kg body weight.

In another aspect of the invention, the anti-PD-1 antibody in any of the aforementioned embodiments is replaced by, or combined with, an anti-PD-L1 or anti-PD-L2 antibody. Accordingly, the invention also features methods, compositions

and kits for treating tumors in human patients using the above-described clinically effective dosages of an anti-LAG-3 antibody combined with the above-described clinically effective dosages of an anti-PD-1 antibody, wherein the dosage of the PD-1 antibody is replaced with the same dosage of an anti-PD-L1 or anti-PD-L2 antibody.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows inhibition of tumor growth *in vivo* using a combination treatment of an anti-LAG-3 antibody and an anti-PD-1 antibody in a murine tumor model.

Figures 2A and 2B are schematics illustrating the parts of a phase I clinical trial.

Figure 3 is a schematic illustrating the Screening, Treatment, Clinical Follow-up, and Survival Follow-up phases of the clinical trial.

DETAILED DESCRIPTION

I. Definitions

As used herein, the term "subject" or "patient" is a human cancer patient (*e.g.*, a patient having an advanced solid tumor, such as an advanced refractory solid tumor).

As used herein, "effective treatment" refers to treatment producing a beneficial effect, *e.g.*, amelioration of at least one symptom of a disease or disorder. A beneficial effect can take the form of an improvement over baseline, *i.e.*, an improvement over a measurement or observation made prior to initiation of therapy according to the method. A beneficial effect can also take the form of arresting, slowing, retarding, or stabilizing of a deleterious progression of a marker of solid tumor. Effective treatment may refer to alleviation of at least one symptom of a solid tumor. Such effective treatment may, *e.g.,* reduce patient pain, reduce the size and/or number of lesions, may reduce or prevent metastasis of a tumor, and/or may slow tumor growth.

The term “effective amount” refers to an amount of an agent that provides the desired biological, therapeutic, and/or prophylactic result. That result can be reduction, amelioration, palliation, lessening, delaying, and/or alleviation of one or more of the signs, symptoms, or causes of a disease, or any other desired alteration of

a biological system. In reference to solid tumors, an effective amount comprises an amount sufficient to cause a tumor to shrink and/or to decrease the growth rate of the tumor (such as to suppress tumor growth) or to prevent or delay other unwanted cell proliferation. In some embodiments, an effective amount is an amount sufficient to delay tumor development. In some embodiments, an effective amount is an amount sufficient to prevent or delay tumor recurrence. An effective amount can be administered in one or more administrations. The effective amount of the drug or composition may: (i) reduce the number of cancer cells; (ii) reduce tumor size; (iii) inhibit, retard, slow to some extent and may stop cancer cell infiltration into peripheral organs; (iv) inhibit (*i.e.*, slow to some extent and may stop tumor metastasis; (v) inhibit tumor growth; (vi) prevent or delay occurrence and/or recurrence of tumor; and/or (vii) relieve to some extent one or more of the symptoms associated with the cancer. In one example, an "effective amount" is the amount of anti-LAG-3 antibody and the amount of anti-PD-1 antibody, in combination, clinically proven to affect a significant decrease in cancer or slowing of progression of cancer, such as an advanced solid tumor. As used herein, the terms "fixed dose", "flat dose" and "flat-fixed dose" are used interchangeably and refer to a dose that is administered to a patient without regard for the weight or body surface area (BSA) of the patient. The fixed or flat dose is therefore not provided as a mg/kg dose, but rather as an absolute amount of the agent (*e.g.*, the anti-LAG-3 antibody and/or anti-PD-1 antibody).

As used herein, a "body surface area (BSA)-based dose" refers to a dose (*e.g.*, of the anti-LAG-3 antibody and/or anti-PD-1 antibody) that is adjusted to the body-surface area (BSA) of the individual patient. A BSA-based dose may be provided as mg/kg body weight. Various calculations have been published to arrive at the BSA without direct measurement, the most widely used of which is the Du Bois formula (see Du Bois D, Du Bois EF (Jun 1916) *Archives of Internal Medicine* 17 (6): 863-71; and Verbraecken, J. et al. (Apr 2006). *Metabolism — Clinical and Experimental* 55 (4): 515–24). Other exemplary BSA formulas include the Mosteller formula (Mosteller RD. *N Engl J Med*., 1987; 317:1098), the Haycock formula (Haycock GB, et al., *J Pediatr* 1978, 93:62-66), the Gehan and George formula (Gehan EA, George SL, *Cancer Chemother Rep* 1970, 54:225-235), the Boyd formula (Current, JD (1998), *The Internet Journal of Anesthesiology* **2** (2); and Boyd, Edith (1935), University of Minnesota. The Institute of Child Welfare, Monograph Series, No. x.

London: Oxford University Press), the Fujimoto formula (Fujimoto S, *et al.*, Nippon Eiseigaku Zasshi 1968;5:443-50), the Takahira formula (Fujimoto S, *et al.*, Nippon Eiseigaku Zasshi 1968;5:443-50), and the Schlich formula (Schlich E, *et al.*, Ernährungs Umschau 2010;57:178-183).

The term "antibody" describes polypeptides comprising at least one antibody-derived antigen binding site (*e.g.*, VH/VL region or Fv, or CDR). Antibodies include known forms of antibodies. For example, the antibody can be a human antibody, a humanized antibody, a bispecific antibody, or a chimeric antibody. The antibody also can be a Fab, Fab'2, ScFv, SMIP, Affibody®, nanobody, or a domain antibody. The antibody also can be of any of the following isotypes: IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgAsec, IgD, and IgE. The antibody may be a naturally occurring antibody or may be an antibody that has been altered (*e.g.,* by mutation, deletion, substitution, conjugation to a non-antibody moiety). For example, an antibody may include one or more variant amino acids (compared to a naturally occurring antibody) which changes a property (*e.g.*, a functional property) of the antibody. For example, numerous such alterations are known in the art which affect, *e.g.,* half-life, effector function, and/or immune responses to the antibody in a patient. The term antibody also includes artificial polypeptide constructs which comprise at least one antibody-derived antigen binding site.

The term "LAG-3" refers to Lymphocyte Activation Gene-3. The term "LAG-3" includes variants, isoforms, homologs, orthologs and paralogs. For example, antibodies specific for a human LAG-3 protein may, in certain cases, cross-react with a LAG-3 protein from a species other than human. In other embodiments, the antibodies specific for a human LAG-3 protein may be completely specific for the human LAG-3 protein and may not exhibit species or other types of cross-reactivity, or may cross-react with LAG-3 from certain other species, but not all other species (*e.g.*, cross-react with monkey LAG-3 but not mouse LAG-3). The term "human LAG-3" refers to human sequence LAG-3, such as the complete amino acid sequence of human LAG-3 having Genbank Accession No. NP_002277 (SEQ ID NO:13). The term "mouse LAG-3" refers to mouse sequence LAG-3, such as the complete amino acid sequence of mouse LAG-3 having Genbank Accession No. NP_032505. LAG-3 is also known in the art as, for example, CD223. The human LAG-3 sequence may differ from human LAG-3 of Genbank Accession No. NP_002277 by having, *e.g.*,

conserved mutations or mutations in non-conserved regions and the LAG-3 has substantially the same biological function as the human LAG-3 of Genbank Accession No. NP_002277. For example, a biological function of human LAG-3 is having an epitope in the extracellular domain of LAG-3 that is specifically bound by an antibody of the instant disclosure or a biological function of human LAG-3 is binding to MHC Class II molecules.

The term "monkey LAG-3" is intended to encompass LAG-3 proteins expressed by Old World and New World monkeys, including but not limited to cynomolgus monkey LAG-3 and rhesus monkey LAG-3. A representative amino acid sequence for monkey LAG-3 is the rhesus monkey LAG-3 amino acid sequence which is also deposited as Genbank Accession No. XM_001108923. Another representative amino acid sequence for monkey LAG-3 is the alternative rhesus monkey sequence of clone pa23-5 as described in US 2011/0150892 A1. This alternative rhesus sequence exhibits a single amino acid difference, at position 419, as compared to the Genbank-deposited sequence.

A particular human LAG-3 sequence will generally be at least 90% identical in amino acid sequence to human LAG-3 of Genbank Accession No. NP_002277 and contains amino acid residues that identify the amino acid sequence as being human when compared to LAG-3 amino acid sequences of other species (*e.g.*, murine). In certain cases, a human LAG-3 can be at least 95%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to LAG-3 of Genbank Accession No. NP_002277. In certain embodiments, a human LAG-3 sequence will display no more than 10 amino acid differences from the LAG-3 sequence of Genbank Accession No. NP_002277. In certain embodiments, the human LAG-3 can display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the LAG-3 sequence of Genbank Accession No. NP_002277. Percent identity can be determined as described herein.

As used herein, the terms “Programmed Death 1,” “Programmed Cell Death 1,” “Protein PD-1,” “PD-1,” PD1,” “PDCD1,” “hPD-1” and “hPD-I” are used interchangeably, and include variants, isoforms, species homologs of human PD-1, and analogs having at least one common epitope with PD-1. The complete PD-1 sequence can be found under GenBank Accession No. U64863 (SEQ ID NO:29).

The protein Programmed Death 1 (PD-1) is an inhibitory member of the CD28 family of receptors, that also includes CD28, CTLA-4, ICOS and BTLA. PD-1 is

expressed on activated B cells, T cells, and myeloid cells (Agata *et al.*, *supra*; Okazaki *et al.* (2002) *Curr. Opin. Immunol.* 14: 391779-82; Bennett *et al.* (2003) *J Immunol* 170:711-8). The initial members of the family, CD28 and ICOS, were discovered by functional effects on augmenting T cell proliferation following the addition of monoclonal antibodies (Hutloff *et al. Nature* (1999); 397:263-266; Hansen *et al. Immunogenics* (1980); 10:247-260). PD-1 was discovered through screening for differential expression in apototic cells (Ishida *et al. EMBO J* (1992); 11:3887-95). The other members of the family, CTLA-4 and BTLA, were discovered through screening for differential expression in cytotoxic T lymphocytes and TH1 cells, respectively. CD28, ICOS and CTLA-4 all have an unpaired cysteine residue allowing for homodimerization. In contrast, PD-1 is suggested to exist as a monomer, lacking the unpaired cysteine residue characteristic in other CD28 family members.

The PD-1 gene is a 55 kDa type I transmembrane protein that is part of the Ig gene superfamily (Agata *et al.* (1996) *Int Immunol* 8:765-72). PD-1 contains a membrane proximal immunoreceptor tyrosine inhibitory motif (ITIM) and a membrane distal tyrosine-based switch motif (ITSM) (Thomas, M.L. (1995) *J Exp Med* 181:1953-6; Vivier, E and Daeron, M (1997) *Immunol Today* 18:286-91). Although structurally similar to CTLA-4, PD-1 lacks the MYPPPY motif that is critical for B7-1 and B7-2 binding. Two ligands for PD-1 have been identified, PD-L1 and PD-L2, that have been shown to downregulate T cell activation upon binding to PD-1 (Freeman *et al.* (2000) *J Exp Med* 192:1027-34; Latchman *et al.* (2001) *Nat Immunol* 2:261-8; Carter *et al.* (2002) *Eur J Immunol* 32:634-43). Both PD-L1 and PD-L2 are B7 homologs that bind to PD-1, but do not bind to other CD28 family members. PD-L1 is abundant in a variety of human cancers (Dong *et al.* (2002) *Nat. Med.* 8:787-9). The interaction between PD-1 and PD-L1 results in a decrease in tumor infiltrating lymphocytes, a decrease in T-cell receptor mediated proliferation, and immune evasion by the cancerous cells (Dong *et al.* (2003) *J. Mol. Med.* 81:281-7; Blank *et al.* (2005) *Cancer Immunol. Immunother.* 54:307-314; Konishi *et al.* (2004) *Clin. Cancer Res.* 10:5094-100). Immune suppression can be reversed by inhibiting the local interaction of PD-1 with PD-L1, and the effect is additive when the interaction of PD-1 with PD-L2 is blocked as well (Iwai *et al.* (2002) *Proc. Nat'l. Acad. Sci. USA* 99:12293-7; Brown *et al.* (2003) *J. Immunol.* 170:1257-66).

Consistent with PD-1 being an inhibitory member of the CD28 family, PD-1 deficient animals develop various autoimmune phenotypes, including autoimmune

cardiomyopathy and a lupus-like syndrome with arthritis and nephritis (Nishimura *et al.* (1999) *Immunity* 11:141-51; Nishimura *et al.* (2001) *Science* 291:319-22). Additionally, PD-1 has been found to play a role in autoimmune encephalomyelitis, systemic lupus erythematosus, graft-versus-host disease (GVHD), type I diabetes, and rheumatoid arthritis (Salama *et al.* (2003) *J Exp Med* 198:71-78; Prokunina and Alarcon-Riquelme (2004) *Hum Mol Genet* 13:R143; Nielsen *et al.* (2004) *Lupus* 13:510). In a murine B cell tumor line, the ITSM of PD-1 was shown to be essential to block BCR-mediated Ca^{2+}-flux and tyrosine phosphorylation of downstream effector molecules (Okazaki *et al.* (2001) *PNAS* 98:13866-71).

"Programmed Death Ligand-1 (PD-L1)" is one of two cell surface glycoprotein ligands for PD-1 (the other being PD-L2) that downregulate T cell activation and cytokine secretion upon binding to PD-1. The term "PD-L1" as used herein includes human PD-L1 (hPD-L1), variants, isoforms, and species homologs of hPD-L1, and 5 analogs having at least one common epitope with hPD-L1. The complete hPD-L1 sequence can be found under GenBank Accession No. Q9NZQ7.

IIa. Anti-LAG-3 Antibodies

Anti-human-LAG-3 antibodies (or VH/VL domains derived therefrom) suitable for use in the invention can be generated using methods well known in the art. Alternatively, art recognized anti-LAG-3 antibodies can be used. For example, the anti-human LAG-3 antibody described in US2011/0150892 A1, the teachings of which are hereby incorporated by reference, and referred to as monoclonal antibody 25F7 (also known as "25F7" and "LAG3.1) can be used. Other art recognized anti-LAG-3 antibodies that can be used include IMP731 described in US 2011/007023, the teachings of which also are hereby incorporated by reference.

Antibodies that compete with any of the above-referenced art-recognized antibodies for binding to LAG-3 also can be used.

An exemplary anti-LAG-3 antibody is BMS-986016 comprising heavy and light chains comprising the sequences shown in SEQ ID NOs:1 and 2, respectively, or antigen binding fragments and variants thereof, as described in PCT/US13/48999, the teachings of which are hereby incorporated by reference.

In other embodiments, the antibody has the heavy and light chain CDRs or variable regions of BMS-986016. Accordingly, in one embodiment, the antibody comprises CDR1, CDR2, and CDR3 domains of the VH region of BMS-986016

having the sequence set forth in SEQ ID NO:3, and CDR1, CDR2 and CDR3 domains of the VL region of BMS-986016 having the sequence set forth in SEQ ID NO:5. In another embodiment, the antibody comprises CDR1, CDR2 and CDR3 domains comprising the sequences set forth in SEQ ID NOs:7, 8, and 9, respectively, and CDR1, CDR2 and CDR3 domains comprising the sequences set forth in SEQ ID NOs:10, 11, and 12, respectively. In another embodiment, the antibody comprises VH and/or VL regions comprising the amino acid sequences set forth in SEQ ID NO:3 and/or SEQ ID NO: 5, respectively. In another embodiment, the antibody comprises heavy chain variable (VH) and/or light chain variable (VL) regions encoded by the nucleic acid sequences set forth in SEQ ID NO:4 and/or SEQ ID NO:6, respectively. In another embodiment, the antibody competes for binding with and/or binds to the same epitope on LAG-3 as the above-mentioned antibodies. In another embodiment, the antibody binds an epitope of human LAG-3 comprising the amino acid sequence PGHPLAPG (SEQ ID NO:14). In another embodiment, the antibody binds an epitope of human LAG-3 comprising the amino acid sequence HPAAPSSW (SEQ ID NO:15) or PAAPSSWG (SEQ ID NO:16).

In another embodiment, the antibody has at least about 90% variable region amino acid sequence identity with the above-mentioned antibodies (*e.g.*, at least about 90%, 95% or 99% variable region identity with SEQ ID NO:3 or SEQ ID NO:5).

IIb. Anti-PD-1 Antibodies

Anti-human-PD-1 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the invention can be generated using methods well known in the art. Alternatively, art recognized anti-PD-1 antibodies can be used. For example, monoclonal antibodies 5C4 (referred to herein as Nivolumab or BMS-936558), 17D8, 2D3, 4H1, 4A11, 7D3, and 5F4, described in WO 2006/121168, the teachings of which are hereby incorporated by reference, can be used. Other known PD-1 antibodies include Lambrolizumab (MK-3475) described in WO 2008/156712, and AMP-514 described in WO 2012/145493, the teachings of which are hereby incorporated by reference. Further known PD-1 antibodies and other PD-1 inhibitors include those described in WO 2009/014708, WO 03/099196, WO 2009/114335 and WO 2011/161699, the teachings of which are hereby incorporated by reference.

Antibodies that compete with any of these art-recognized antibodies or inhibitors for binding to PD-1 also can be used.

An exemplary anti-PD-1 antibody is BMS-936558 comprising heavy and light chains comprising the sequences shown in SEQ ID NOs:17 and 18, respectively, or antigen binding fragments and variants thereof.

In other embodiments, the antibody has heavy and light chain CDRs or variable regions of BMS-936558. Accordingly, in one embodiment, the antibody comprises CDR1, CDR2, and CDR3 domains of the VH of BMS-936558 having the sequence set forth in SEQ ID NO:19, and CDR1, CDR2 and CDR3 domains of the VL of BMS-936558 having the sequence set forth in SEQ ID NO:21. In another embodiment, the antibody comprises CDR1, CDR2 and CDR3 domains comprising the sequences set forth in SEQ ID NOs:23, 24, and 25, respectively, and CDR1, CDR2 and CDR3 domains comprising the sequences set forth in SEQ ID NOs:26, 27, and 28, respectively. In another embodiment, the antibody comprises VH and/or VL regions comprising the amino acid sequences set forth in SEQ ID NO: 19 and/or SEQ ID NO: 21, respectively. In another embodiment, the antibody comprises heavy chain variable (VH) and/or light chain variable (VL) regions encoded by the nucleic acid sequences set forth in SEQ ID NO:20 and/or SEQ ID NO:22, respectively. In another embodiment, the antibody competes for binding with and/or binds to the same epitope on PD-1 as the above-mentioned antibodies. In another embodiment, the antibody has at least about 90% variable region amino acid sequence identity with the above-mentioned antibodies (*e.g.*, at least about 90%, 95% or 99% variable region identity with SEQ ID NO:19 or SEQ ID NO:21).

IIc. Anti-PD-L1 Antibodies

Anti-human-PD-L1 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the invention can be generated using methods well known in the art. Alternatively, art recognized anti-PD-L1 antibodies can be used. For example, human anti-PD-L1 antibodies disclosed in U.S. Patent No 7,943,743, the contents of which are hereby incorporated by reference, can be used. Such anti-PD-L1 antibodies include 3G10, 12A4 (also referred to as BMS-936559), 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4. Other art recognized anti-PD-L1 antibodies which can be used include those described in, for example, U.S. Patent Nos. 7,635,757 and 8,217,149, U.S. Publication No. 2009/0317368, and PCT Publication Nos.

WO 2011/066389 and WO 2012/145493, the teachings of which also are hereby incorporated by reference. Antibodies that compete with any of these art-recognized antibodies or inhibitors for binding to PD-L1 also can be used.

III. Pharmaceutical Compositions

Pharmaceutical compositions suitable for administration to human patients are typically formulated for parenteral administration, *e.g.*, in a liquid carrier, or suitable for reconstitution into liquid solution or suspension for intravenous administration.

In general, such compositions typically comprise a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable" means approved by a government regulatory agency or listed in the U.S. Pharmacopeia or another generally recognized pharmacopeia for use in animals, particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil, glycerol polyethylene glycol ricinoleate, and the like. Water or aqueous solution saline and aqueous dextrose and glycerol solutions may be employed as carriers, particularly for injectable solutions (*e.g.*, comprising an anti-LAG-3 or anti-PD-1 antibody). Liquid compositions for parenteral administration can be formulated for administration by injection or continuous infusion. Routes of administration by injection or infusion include intravenous, intraperitoneal, intramuscular, intrathecal and subcutaneous. In one embodiment, the anti-LAG-3 and/or anti-PD-1 antibodies are administered intravenously (*e.g.*, in separate formulations or together (in the same formulation or in separate formulations)).

IV. Patient Populations

Provided herein are clinical methods for treating solid tumors cancer (*e.g.*, advanced refractory solid tumors) in human patients using a combination of an anti-LAG-3 antibody and an anti-PD-1 antibody.

Examples of cancers that may be treated using the methods of the invention, include liver cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, breast cancer, lung cancer, cutaneous or intraocular malignant melanoma, renal cancer, uterine cancer, ovarian cancer, colorectal cancer, colon cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular 20 cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumors of 25 childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, environmentally induced cancers including those induced by asbestos, hematologic malignancies 30 including, for example, multiple myeloma, B-cell lymphoma, Hodgkin lymphoma/primary mediastinal B-cell lymphoma, non-Hodgkin's lymphomas, acute myeloid lymphoma, chronic myelogenous leukemia, chronic lymphoid leukemia, follicular lymphoma, diffuse large B-cell lymphoma, Burkitt's lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, mantle cell lymphoma, acute lymphoblastic leukemia, mycosis fungoides, anaplastic large cell lymphoma, T-cell lymphoma, and precursor T-lymphoblastic lymphoma, and any combinations of said cancers. The present invention is also applicable to treatment of metastatic cancers.

In one embodiment, the human patient suffers from non-small cell lung cancer (NSCLC) or a virally-related cancer (e.g., a human papilloma virus (HPV)-related tumor) or gastric adenocarcinoma. In a particular embodiment, the HPV-related tumor is HPV+ head and neck cancer (HNC). In another particular embodiment, the gastric adenocarcinoma is associated with Epstein-Barr virus (EBV) infection.

Patients can be tested or selected for one or more of the above described clinical attributes prior to, during or after treatment.

V. Combination Therapy

Combination therapies provided herein involve administration of an anti-LAG-3 antibody and another antibody that blocks an inhibitory immune receptor (*e.g.,* a receptor, which upon binding to its natural ligand, inhibits/neutralizes activity, such as cytotoxic activity), particularly an anti-PD-1 antibody, to treat subjects having solid tumors (*e.g.,* advanced refractory solid tumors).

In one embodiment, the invention provides an anti-LAG-3 antibody and an anti-PD-1 antibody in combination according to a defined clinical dosage regimen, to treat subjects having a solid tumor (*e.g.,* an advanced refractory solid tumor). In a particular embodiment, the anti-LAG-3 antibody is BMS-986016. In another embodiment, the anti-PD-1 antibody is BMS-936558. In another embodiment, dosage regimens are adjusted to provide the optimum desired response (*e.g.*, an effective response).

As used herein, adjunctive or combined administration (coadministration) includes simultaneous administration of the compounds in the same or different dosage form, or separate administration of the compounds (*e.g.*, sequential administration). Thus, the anti-LAG-3 and anti-PD-1 antibodies can be simultaneously administered in a single formulation. Alternatively, the anti-LAG-3 and anti-PD-1 antibodies can be formulated for separate administration and are administered concurrently or sequentially (e.g., one antibody is administered within about 30 minutes prior to administration of the second antibody).

For example, the anti-PD1 antibody can be administered first followed by (*e.g.,* immediately followed by) the administration of the anti-LAG-3 antibody, or vice versa. In one embodiment, the anti-PD-1 antibody is administered prior to administration of the anti-LAG-3 antibody. In another embodiment, the anti-PD-1 antibody is administered after administration of the anti-LAG-3 antibody. In another embodiment, the anti-LAG-3 antibody and anti-PD-1 antibody are administered concurrently. Such concurrent or sequential administration preferably results in both antibodies being simultaneously present in treated patients.

VI. Treatment Protocols

Suitable treatment protocols for treating a solid tumor in a human patient include, for example, administering to the patient an effective amount of each of:

(a) an anti-LAG-3 antibody comprising CDR1, CDR2 and CDR3 domains of the heavy chain variable region having the sequence set forth in SEQ ID NO:3, and

CDR1, CDR2 and CDR3 domains of the light chain variable region having the sequence set forth in SEQ ID NO:5,

(b) an anti-PD-1 antibody comprising CDR1, CDR2 and CDR3 domains of the heavy chain variable region having the sequence set forth in SEQ ID NO:19, and CDR1, CDR2 and CDR3 domains of the light chain variable region having the sequence set forth in SEQ ID NO:21,

wherein the method comprises at least one administration cycle, wherein the cycle is a period of eight weeks, wherein for each of the at least one cycles, at least four doses of the anti-LAG-3 antibody are administered at a flat dose of about 1, 3, 10, 20, 50, 80, 100, 130, 150, 180, 200, 240 or 280 mg and at least four doses of the anti-PD-1 antibody are administered at flat dose of about 50, 80, 100, 130, 150, 180, 200, 240 or 280 mg. In another embodiment, four doses of the anti-LAG-3 antibody are administered at a dose of 0.01, 0.03, 0.25, 0.1, 0.3, 1 or 3, 5, 8 or 10 mg/kg body weight and four doses of the anti-PD-1 antibody are administered at a dose of 0.1, 0.3, 1, 3, 5, 8 or 10 mg/kg body weight.

In one embodiment, the anti-LAG-3 antibody and anti-PD-1 antibody are administered at the following doses:

(a) 3 mg anti-LAG-3 antibody and 80 mg of anti-PD-1 antibody;

(b) 3 mg anti-LAG-3 antibody and 240 mg of anti-PD-1 antibody;

(c) 20 mg anti-LAG-3 antibody and 240 mg of anti-PD-1 antibody;

(d) 80 mg anti-LAG-3 antibody and 240 mg of anti-PD-1 antibody; or

(e) 240 mg anti-LAG-3 antibody and 240 mg of anti-PD-1 antibody.

In another embodiment, the anti-LAG-3 antibody and anti-PD-1 antibody are administered at the following doses:

(a) 0.3 mg/kg anti-LAG-3 antibody and 1 mg/kg of anti-PD-1 antibody;

(b) 0.3 mg/kg anti-LAG-3 antibody and 3 mg/kg of anti-PD-1 antibody;

(c) 0.25 mg/kg anti-LAG-3 antibody and 3 mg/kg of anti-PD-1 antibody;

(d) 1 mg/kg anti-LAG-3 antibody and 3 mg/kg of anti-PD-1 antibody; or

(e) 3 mg/kg anti-LAG-3 antibody and 3 mg/kg of anti-PD-1 antibody.

In one embodiment, the dose of the anti-LAG-3 and/or anti-PD-1 antibody is calculated per body weight, *e.g.*, mg/kg body weight. In another embodiment, the dose of the anti-LAG-3 and/or anti-PD-1 antibody is a flat-fixed dose. In another embodiment, the dose of the anti-LAG-3 and/or anti-PD-1 antibody is varied over time. For example, the anti-LAG-3 antibody and/or anti-PD-1 antibody may be

initially administered at a high dose and may be lowered over time. In another embodiment, the anti-LAG-3 antibody and/or anti-PD-1 antibody is initially administered at a low dose and increased over time.

In another embodiment, the amount of the anti-LAG-3 and/or anti-PD-1 antibodies administered is constant for each dose. In another embodiment, the amount of antibody administered varies with each dose. For example, the maintenance (or follow-on) dose of the antibody can be higher or the same as the loading dose which is first administered. In another embodiment, the maintenance dose of the antibody can be lower or the same as the loading dose.

In another embodiment, the anti-LAG-3 and/or anti-PD-1 antibodies are formulated for intravenous administration. In one embodiment, the anti-PD-1 antibody is administered on Days 1, 15, 29, and 43 of each cycle. In another embodiment, the anti-LAG-3 antibody is administered on Days 1, 15, 29, and 43 of each cycle.

In other embodiments, the anti-LAG-3 and/or anti-PD-1 antibodies are administered once per week, once every or three two weeks, once per month or as long as a clinical benefit is observed or until there is a complete response, confirmed progressive disease or unmanageable toxicity.

In another embodiment, a cycle of administration is eight weeks, which can be repeated, as necessary. In another embodiment, the treatment consists of up to 12 cycles.

In another embodiment, 4 doses of the anti-PD-1 antibody are administered per eight week cycle. In another embodiment, 4 doses of the anti-LAG-3 antibody are administered per eight week cycle.

In another embodiment, the anti-PD-1 antibody and anti-LAG-3 antibody are administered as a first line of treatment (*e.g.*, the initial or first treatment). In another embodiment, the anti-PD-1 antibody and anti-LAG-3 antibody are administered as a second line of treatment (*e.g.*, after the initial or first treatment, including after relapse and/or where the first treatment has failed).

In another aspect, the invention features any of the aforementioned embodiments, wherein the anti-PD-1 antibody is replaced by, or combined with, an anti-PD-L1 or anti-PD-L2 antibody.

VII. Outcomes

With respect to target lesions, responses to therapy may include:

Complete Response (CR) (RECIST V1.1)	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.
Partial Response (PR) (RECIST V1.1)	At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.
Progressive Disease (PD) (RECIST V1.1)	At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).
Stable Disease (SD) (RECIST V1.1)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.
Immune-related Complete Response (irCR) (irRECIST)	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.
Immune-related Partial Response (irPR) (irRECIST)	At least a 30% decrease in the sum of diameters of target lesions and all new measurable lesions (ie Percentage Change in Tumor Burden), taking as reference the baseline sum diameters. Note: the appearance of new measurable lesions is factored into the overall Tumor Burden, but does not automatically qualify as progressive disease until the sum of the diameters increases by ≥ 20% when compared to nadir.
Immune-related Progressive Disease (irPD) (irRECIST)	At least a 20% increase in Tumor Burden (ie the sum of diameters of target lesions, and any new measurable lesions) taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. Tumor assessments using

	immune-related criteria for progressive disease incorporates the contribution of new measurable lesions. Each net percentage change in tumor burden per assessment accounts for the size and growth kinetics of both old and new lesions as they appear.
Immune-related Stable Disease (irSD) (irRECIST)	Neither sufficient shrinkage to qualify for irPR nor sufficient increase to qualify for irPD, taking as reference the smallest sum diameters while on study.

With respect to non-target lesions, responses to therapy may include:

Complete Response (CR) (RECIST V1.1)	Disappearance of all non-target lesions. All lymph nodes must be non-pathological in size (<10 mm short axis).
Non-CR/Non-PD (RECIST V1.1)	Persistence of one or more non-target lesion(s).
Progressive Disease (PD) (RECIST V1.1)	*Unequivocal progression* of existing non-target lesions. The appearance of one or more new lesions is also considered progression.
Immune-related Complete Response (irCR) (irRECIST)	Disappearance of all non-target lesions. All lymph nodes must be non-pathological in size (< 10 mm short axis).
Immune-related Progressive Disease (irPD) (irRECIST)	Increases in number or size of non-target lesion(s) does not constitute progressive disease unless/until Tumor Burden increases by 20% (ie the sum of the diameters at nadir of target lesions and any new measurable lesions increases by the required amount). Non-target lesions are not considered in the definition of Stable Disease and Partial Response.

Patients treated according to the methods disclosed herein preferably experience improvement in at least one sign of cancer. In one embodiment, improvement is measured by a reduction in the quantity and/or size of measurable tumor lesions. In another embodiment, lesions can be measured on chest x-rays or CT or MRI films. In another embodiment, cytology or histology can be used to evaluate responsiveness to a therapy.

In one embodiment, the patient treated exhibits a complete response (CR), a partial response (PR), stable disease (SD), immune-related complete disease (irCR), immune-related partial response (irPR), or immune-related stable disease (irSD). In another embodiment, the patient treated experiences tumor shrinkage and/or decrease in growth rate, *i.e.*, suppression of tumor growth. In another embodiment, unwanted cell proliferation is reduced or inhibited. In yet another embodiment, one or more of the following can occur: the number of cancer cells can be reduced; tumor size can be reduced; cancer cell infiltration into peripheral organs can be inhibited, retarded, slowed, or stopped; tumor metastasis can be slowed or inhibited; tumor growth can be inhibited; recurrence of tumor can be prevented or delayed; one or more of the symptoms associated with cancer can be relieved to some extent.

In other embodiments, administration of effective amounts of the anti-LAG-3 antibody and anti-PD-1 antibody according to any of the methods provided herein produces at least one therapeutic effect selected from the group consisting of reduction in size of a tumor, reduction in number of metastatic lesions appearing over time, complete remission, partial remission, or stable disease. In still other embodiments, the methods of treatment produce a comparable clinical benefit rate (CBR = CR+ PR+ SD $\geq$ 6 months) better than that achieved by an anti-LAG-3 antibody or anti-PD-1 antibody alone. In other embodiments, the improvement of clinical benefit rate is about 20% 20%, 30%, 40%, 50%, 60%, 70%, 80% or more compared to an anti-LAG-3 antibody or anti-PD-1 antibody alone.

VIII. Kits and Unit Dosage Forms

Also provided herein are kits which include a pharmaceutical composition containing an anti-LAG-3 antibody, such as BMS-986016, and an anti-PD-1 antibody, such as BMS-936558, and a pharmaceutically-acceptable carrier, in a therapeutically effective amount adapted for use in the preceding methods. The kits optionally also can include instructions, *e.g.*, comprising administration schedules, to allow a practitioner (*e.g.*, a physician, nurse, or patient) to administer the composition contained therein to administer the composition to a patient having cancer (*e.g.,* a solid tumor). The kit also can include a syringe.

Optionally, the kits include multiple packages of the single-dose pharmaceutical compositions each containing an effective amount of the anti-LAG-3 or anti-PD-1 antibody for a single administration in accordance with the methods provided above.

Instruments or devices necessary for administering the pharmaceutical composition(s) also may be included in the kits. For instance, a kit may provide one or more pre-filled syringes containing an amount of the anti-LAG-3 or anti-PD-1 antibody.

In one embodiment, the present invention provides a kit for treating a solid tumor in a human patient, the kit comprising:

(a) a dose of an anti-LAG-3 antibody comprising CDR1, CDR2 and CDR3 domains of the heavy chain variable region having the sequence set forth in SEQ ID NO:3, and CDR1, CDR2 and CDR3 domains of the light chain variable region having the sequence set forth in SEQ ID NO:5;

(b) a dose of an anti-PD-1 antibody comprising CDR1, CDR2 and CDR3 domains of the heavy chain variable region having the sequence set forth in SEQ ID NO:19, and CDR1, CDR2 and CDR3 domains of the light chain variable region having the sequence set forth in SEQ ID NO:21; and

(c) instructions for using the anti-LAG-3 antibody and anti-PD-1 antibody in the methods described herein.

The following examples are merely illustrative and should not be construed as limiting the scope of this disclosure in any way as many variations and equivalents will become apparent to those skilled in the art upon reading the present disclosure.

The contents of all references, GenBank entries, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

EXAMPLES

Example 1: Pre-Clinical Pharamacology of Anti-PD-1 Antibody (BMS-936558)

BMS-936558 is a fully human, IgG4 (kappa) isotype monoclonal antibody that binds to PD-1 with nanomolar affinity as measured by surface plasmon resonance using a Biacore® biosensor system and a high degree of specificity, thus precluding binding to its ligands PD-L1 and PD-L2. BMS-936558 does not bind other related family members, such as BTLA, CTLA-4, ICOS or CD28. Pre-clinical testing of BMS-936558 demonstrated that binding to PD-1 results in enhanced T cell proliferation and release of interferon-gamma (IFN-gamma) *in vitro*. The heavy and light chain amino acid sequences of BMS-936558 are provided in SEQ ID NOs:1 and 2, respectively.

Example 2: *In Vivo* Toxicity of Anti-PD-1 Antibody Novolumab (BMS-936558)

Toxicology studies in cynomolgus monkeys confirmed that BMS-936558 was well tolerated at doses up to 50 mg/kg given twice weekly for 27 doses. Drug-related findings were limited to a reversible decrease in triiodothyronine (T3) by 28%, without concomitant abnormalities in other markers of thyroid function (data not shown).

Example 3: Clinical Pharmacology and Safety of Anti-PD-1 Antibody (BMS-936558)

The overall safety experience with BMS-936558, as a monotherapy or in combination with other therapeutics, is based on experience in approximately 1500 subjects treated to date. In general for monotherapy, the safety profile is similar across tumor types. The one exception is pulmonary inflammation adverse events (AEs) which may be numerically greater in subjects with NSCLC because in some cases, it can be difficult to distinguish between BMS-936558-related and unrelated causes of pulmonary symptoms and radiographic changes. The safety profile is generally consistent across completed and ongoing clinical trials with no maximum tolerated dose reached at any dose tested up to 10 mg/kg. There was no pattern in the incidence, severity, or causality of adverse events to BMS-936558 dose level.

Study CA209003 has contributed to most of the clinical experience with BMS-936558 in subjects with NSCLC and other solid malignancies to date. CA209003 was a Phase 1 multi-dose escalation study in subjects with previously treated advanced or metastatic melanoma, RCC, NSCLC, colorectal cancer, or hormone-refractory prostate cancer. In CA209003, subjects were administered BMS-936558 intravenously every 2 weeks with doses of 0.1, 0.3, 1, 3, or 10 mg/kg. No maximum tolerated dose was identified in CA209003. The maximum dose level evaluated was 10 mg/kg. The incidence, severity, and relationship of adverse events were generally similar across dose levels and tumor types.

As of 03 July 2012, 296 (97.4%) out of 304 subjects treated with BMS-936558 had at least 1 reported adverse event regardless of causality. There was no pattern in the incidence, severity, or relationship of adverse events to the BMS-936558 dose level. Treatment-related adverse events of any grade occurred in 220 (72.4%) of subjects. The most frequent drug-related adverse events occurring in > 5% of subjects included fatigue (25.7%), rash (13.5%), diarrhea (11.8%), pruritus (10.2%), nausea (7.9%), decreased appetite (7.9%), hemoglobin decreased (5.9%),

and pyrexia (5.3%). Most treatment-related adverse events were low grade (Grade 1 or 2). Treatment-related high-grade (Grade 3 or 4) adverse events were reported in 45 (14.8%) of subjects, with the most common being fatigue (1.6%), decreased appetite (1.0%), and diarrhea (1.0%). At least one serious adverse event (SAE) was reported for 150 (49.3%) of the 304 subjects at all dose levels. Grade 3-4 SAEs were reported for 23 subjects (7.6%). Drug-related SAEs of any grade occurred in 11.5% of subjects. Grade 3-4 drug-related SAEs reported in at least 2 subjects included diarrhea (3 subjects [1.0%]), pneumonitis (3 subjects [1.0%]), pneumonia (2 subjects [0.7%]), and lipase increased (2 subjects [0.7%]). Similar to the overall adverse event profile, there was no apparent relationship in the incidence or severity of drug-related adverse events to BMS-936558 dose level. There were no apparent differences in the frequency of adverse events based on subjects' tumor type.

Selected treatment-related adverse events have occurred with low frequency (< 5%), but are considered clinically meaningful because they require greater vigilance for early recognition and prompt intervention. These adverse events include alanine aminotransferase (ALT) increased (4.3%), aspartate aminotransferase (AST) increased (3.6%), pneumonitis (3.3%), hypothyroidism (3.0%), hyperthyroidism (1.3%), adrenal insufficiency (0.7%), and colitis (0.7%). Grade 3-4 events of pneumonitis were reported in 3 subjects (1.0%) as described above (1 event was Grade 4). Grade 3 events of colitis, ALT increased, and AST increased were reported in 2 subjects (0.7%) each. Grade 3 events of adrenal insufficiency, hyperthyroidism, and hypothyroidism were reported in 1 subject (0.3%) each. Because of the potential for clinically meaningful BMS-936558-related adverse events requiring early recognition and prompt intervention, management algorithms have been developed for suspected pulmonary toxicity, diarrhea or suspected colitis, hepatotoxicity, endocrinopathy, and nephrotoxicity.

Treatment-related adverse events leading to discontinuation were reported in 18 (5.9%) of the 304 treated subjects on CA209003. The only events reported in more than 1 subject were pneumonitis (4 subjects [1.3%]) and hepatitis (2 subjects [0.7%]). There were 3 (1.0%) drug-related deaths; each occurred after the development of pneumonitis.

The safety of BMS-936558 in combination with other therapeutics is being explored in several ongoing clinical trials.

Example 4: Pharmacokinetics of Anti-PD-1 Antibody (BMS-936558)

Single-dose pharmacokinetics (PK) of BMS-936558 were evaluated in 39 subjects with multiple tumor types in CA209001 in the dose range of 0.3 to 10 mg/kg. The median Tmax across dose levels ranged from 1.6 to 3.1 hours with individual values ranging from 0.9 to 7 hours. The PK of BMS-936558 was linear in the range of 0.3 to 10 mg/kg with dose-proportional increase in Cmax and AUC(INF) and low to moderate intersubject variability was observed at each dose level (*i.e.*, coefficient of variation [CV] ranging from 16 to 45%). Geometric mean clearance (CLT) after a single IV dose ranged from 0.13 to 0.19 mL/h/kg, while mean volume of distribution (Vz) varied between 83 to 113 mL/kg across doses. The mean terminal T-HALF of BMS-936558 was 17 to 25 days, consistent with half-life of endogenous IgG4, indicating that the elimination mechanism of BMS-936558 may be similar to IgG4. Both elimination and distribution of BMS-936558 appeared to be independent of dose within the dose range studied. In a multiple dose study of multiple tumor types (CA209003), available data from 128 subjects, mean T-HALF was 21 - 24 hours and median T-max ranged from 0.6 to 3.3 across dose levels, which aligns with the single dose data.

Example 5: Phase I Clinical Trial with Anti-PD-1 Antibody (BMS-936558)

BMS-936558 has demonstrated clinical activity in a completed Phase 1 single-dose study and 2 ongoing multiple-dose escalation studies (Phase 1 monotherapy: CA209003 and Phase 1b combination therapy with ipilimumab) in subjects with NSCLC, melanoma, RCC, and other malignancies. Tumor response was determined by modified Response Evaluation Criteria in Solid Tumors (RECIST) established by the NCI. The evaluable population consists of 294 subjects with a variety of solid tumor malignancies (melanoma, n=138; NSCLC, n=122; RCC, n=34) who are currently being treated with nivolumab.

In CA209003, an objective response rate (ORR) of 31.1% (33 of 106 response-evaluable subjects) was reported in subjects with melanoma treated with BMS-936558 monotherapy every 2 weeks (Q2W) at doses ranging from 0.1 to 10 mg/kg. The majority of responses were durable and exceeded 6 months.

In the most active dose range (3 to 10 mg/kg), an ORR of 13.5% to 27.8% was reported among subjects with NSCLC with a 24-week progression-free survival rate (PFSR) of 23% to 51%. Durable responses were observed in both squamous and non-squamous subtypes.

Of the 34 response-evaluable RCC subjects in CA209003, responses were reported in both the 1-mg/kg (5 of 18 subjects, 27.8%) and 10-mg/kg (5 of 16 subjects, 31.3%) treatment groups. Estimated progression-free survival rate (PFSR) at 24 weeks was 50% in the 1-mg/kg and 67% in the 10-mg/kg BMS-936558 treatment groups.

Preliminary results from the Phase 1b study of combination therapy with BMS-936558 and ipilimumab suggest an advantage in combining two T cell-targeted therapies for subjects with melanoma. In the 0.3 mg/kg BMS-936558 + 3 mg/kg ipilimumab treatment group, responses were observed in 5 out of 14 evaluable subjects (35.7%, 1 complete response and 2 partial responses by conventional modified World Health Organization [mWHO] criteria, and 2 partial responses by immune-related mWHO criteria). In the 1 mg/kg BMS-936558 + 3 mg/kg ipilimumab treatment group, responses were observed in 9 out of 15 evaluable subjects (60%, 3 CRs and 6 PRs; all by conventional mWHO criteria). In the 3 mg/kg BMS-936558 + 3 mg/kg ipilimumab treatment group, objective responses were observed in 4 out of 6 evaluable subjects (66.7%, 3 partial responses by conventional mWHO criteria and 1 partial response by immune-related mWHO criteria). Further details are provided by Wolchok et al. (2013) NEJM 369(2):122-33, and/or PCT/US2013/040764.

Example 6: Pre-Clinical Pharamacology of Anti-LAG-3 Antibody (BMS-986016)

BMS-986016 is a fully human antibody specific for human LAG-3 that was isolated from immunized transgenic mice expressing human immunoglobulin genes. It is expressed as an IgG4 isotype antibody that includes a stabilizing hinge mutation (S228P) for attenuated Fc receptor binding in order to reduce or eliminate the possibility of antibody- or complement-mediated target cell killing. The heavy and light chain amino acid sequences of BMS-986016 are provided in SEQ ID NOs:17 and 18, respectively.

The ability of BMS-986016 to bind recombinant human LAG-3 antigen was determined using Biacore and enzyme-linked immunosorbent assay (ELISA). Binding to human and primate LAG-3+ transfectants and to activated human or primate T cells was measured using flow cytometric and Scatchard analyses. BMS-986016 binds to human LAG-3 with high affinity (K_D = 0.12-0.5 nM), and inhibits the binding of LAG-3 to cells expressing its ligand, MHC class II (IC50, 0.67 nM). BMS-986016 binds to cynomolgus LAG-3 on transfected CHO cells and on activated cynomolgus T cells with a lower

affinity (EC50, 21.5-34.3 nM) than to activated human T cells. A high concentration of BMS-986016, in the absence of secondary co-stimulation, elicits no measurable cytokine response from cultured human peripheral blood cells nor does the drug mediate measurable antibody-dependent or complement-dependent killing of target cells. BMS-986016 promotes the activation of an antigen-specific mouse T cell hybridoma expressing human LAG-3 in co-culture with an MHC class II-positive antigen-presenting cell. In addition, BMS-986016 enhances activation of human T cells in superantigen stimulation assays when added alone or in combination with BMS-936558 (anti-PD-1 antibody).

Example 7: Toxicity of Anti-LAG-3 Antibody (BMS-986016) Alone or in Combination with Anti-PD-1 Antibody (BMS-936558)

The following preclinical toxicology studies were performed:

A. *Four-Week Intermittent (QW) Intravenous Exploratory Combination Pharmacodynamic and Toxicity Study in Cynomolgus Monkeys with Anti-LAG3.1 Antibody (a precursor of BMS-986016) and BMS-936558*

The key results were as follows. Anti-LAG3.1 administered at 50 mg/kg/week, alone or in combination with 50 mg/kg/week BMS-936558, did not result in any adverse changes. No-observed-adverse-effect level (NOAEL) for single-agent anti-LAG3.1 was considered to be 50 mg/kg/week (AUC[0-168h] = 231,000 μg·h/mL), and NOAEL for anti-LAG3.1 in combination with 50 mg/kg/week BMS-936558 was considered to be 50 mg/kg/week (mean anti-LAG3.1 AUC[0-168h] = 210,000 μg·h/mL; mean BMS-936558AUC[0-168h] = 159,500 μg·h/mL).

B. *GLP-Compliant Four-Week Intravenous Combination Toxicity Study in Cynomolgus Monkeys with a 6-Week Recovery with BMS-986016 and BMS-936558*

The key results were as follows. Single-agent BMS-986016 administered at up to 100 mg/kg/week did not result in adverse changes. Single-agent BMS-936558 administered at 50 mg/kg/week resulted in slight to minimal non-reversible lymphoplasmacytic inflammation of the choroid plexus of the brain, which was considered non-adverse given the lower severity and incidence of the

lymphoplasmacytic inflammation compared to combination treatment with BMS-986016 and BMS-936558, lack of vasculitis or tissue destruction, and absence of clinical manifestations during the course of treatment. Combined administration of BMS-986016 and BMS-936558 (100 and 50 mg/kg/week, respectively) resulted in moribundity of 1 male out of 9 monkeys on study Day 29. From Days 26 to 29, this monkey presented with elevated body temperature, shivers, red or clear nasal discharge, fecal changes (unformed, scant or absent feces), decreased feeding behavior, mild dehydration, sneezing, decreased activity, and hunched posture. After 2 days of veterinary care and antibiotic treatment, this animal did not show any improvement and was euthanized on Day 29 for poor clinical condition.

There were no remarkable gross necropsy findings. Histopathological findings in this monkey included: slight lymphoplasmacytic inflammation of the choroid plexus; minimal to moderate lymphohistocytic inflammation of the vasculature of the brain parenchyma, meninges, spinal cord (cervical and lumbar); and minimal to moderate mixed cell inflammation of the epididymes, seminal vesicles and testes. Clinical pathology changes indicated decreases in red blood cell count, hemoglobin concentration and hematocrit whose cause was unclear, and an increase in fibrinogen correlating with the inflammation observed in the central nervous system (CNS) and male reproductive tract.

Additional histopathological findings upon combination administration of BMS-986016 and BMS-936558 (100 and 50 mg/kg/week, respectively) were limited to minimal to slight non-reversible lymphoplasmacytic inflammation of the choroid plexus in the brain in 7 of 8 remaining monkeys, and minimal lymphohistiocytic inflammation of the vasculature of the brain parenchyma in 1 of 8 remaining monkeys, whose reversibility could not be assessed.

NOAEL for single-agent BMS-986016 was considered to be 100 mg/kg/week (mean AUC[0-168h] = 474,000 μg·h/mL); NOAEL for single-agent BMS-936558 was considered to be 50 mg/kg/week (mean AUC[0-168h] = 193,000 μg·h/mL); NOAEL for combination of BMS-986016 and BMS-936558 was not determined. However, the combination therapy was generally well tolerated and clinical signs of toxicity were observed in only 1 of 9 monkeys (approximately 10%). Therefore, 100/50 mg/kg/week BMS-986016/nivolumab (mean BMS-986016 AUC[0-168h] =

514,000 μg·h/mL; mean nivolumab AUC[0-168h] = 182,000 μg·h/mL) was considered the STD10.

The doses administered (100 mg/kg BMS-986016 and 50 mg/kg BMS-936558) are ≥ 10 times higher than the maximum doses proposed for the current study. The starting dose of 20 mg (0.25 mg/kg) for BMS-986016 monotherapy (Part A) is less than 1/10 of the human equivalent of the cynomolgus monkey NOAEL (636 mg; 8.0 mg/kg), and is below the HED after a linear adjustment of the NOAEL target exposure for the highest affinity difference estimate of 265-fold (24 mg; 0.30 mg/kg). The calculated safety multiple for exposures at the 20 mg (0.25 mg/kg) starting dose is 315-fold based on the cynomolgus monkey NOAEL of 100 mg/kg/week without accounting for affinity differences.

The starting dose of 3 mg (0.03 mg/kg) for BMS-986016 for the combination therapy (Part B) is based on a linear adjustment of the cynomolgus monkey STD10 for the 265-fold highest affinity difference estimate with an added 10-fold safety factor. The maximum recommended starting dose (MRSD) for BMS-986016 based on a 100 mg/kg/week STD10 is 0.03 mg/kg in humans. The starting dose of 80 mg (1 mg/kg) for BMS-936558 for the combination therapy (Part B) is based on known human BMS-936558 PK parameters with an added 10-fold safety factor. The MRSD for BMS-936558 based on the 50 mg/kg/week cynomolgus monkey STD10 is 4.3 mg/kg in humans, and has been further reduced to identify a dose with acceptable levels of adverse events.

C. GLP-Compliant Tissue Cross Reactivity Study in Human and Select Cynomolgus Monkey Tissues with BMS-986016.

Positive staining with BMS-986016-FITC was observed in the plasma membrane or plasma membrane granules following human tissues: mononuclear leukocytes of the urinary bladder, blood cells, colon - large intestine, eye, esophagus, small intestine, stomach, kidney, lung, lymph node, placenta, salivary gland, skin, spleen, thymus, tonsil, uterus - cervix, and uterus - endometrium; and hematopoetic cells of the bone marrow. In addition, staining with BMS-986016-FITC was observed in the cytoplasm of the human pituitary endocrine cell epithelium. Within the limited panel of cynomolgus monkey tissues evaluated, staining with BMS-986016-FITC was observed in the plasma membrane or plasma membrane granules of the mononuclear

leukocytes of the spleen. With scientific reports of LAG-3-expressing cells in germinal centers and interfollicular T-cell areas of normal human lymphoid tissues (lymph node, tonsil, spleen, thymus, bone marrow and mucosal-associated lymphoid tissue) and having the morphology and distribution of lymphocytes, the staining of mononuclear leukocytes and hematopoietic cells with BMS-986016-FITC in this study (in the human and cynomolgus monkey tissues) was anticipated. Given that LAG-3 mRNA is expressed in the human pituitary and LAG3.1-G4P-FITC staining was observed in adenohypophysis of the human pituitary in a pilot tissue cross reactivity study, BMS-986016-FITC staining of human pituitary endocrine cell epithelium cytoplasm and cytoplasmic granules was also anticipated. Although BMS-986016 is not expected to have access to the cytoplasmic compartment *in vivo* and the repeat-dose toxicology studies in monkeys showed no effects on the pituitary gland, these findings may be of clinical significance.

D. In Vitro Cytokine Release and Lymphocyte Activation Assessment with BMS-986016 using Human Peripheral Blood Mononuclear Cells.

BMS-986016 did not induce cytokine release when presented to human PBMCs regardless of concentration, donor, or incubation time. The levels of cytokines observed were either at or near the assay lower limits of quantification with no evidence of dose-dependence or pattern across donors (IL-1β, IL-2, IL-5, IL-10, IL-12p70, and IFN-γ) or were generally overlapping with cytokine levels from PBMCs incubated with negative controls (IL-6, IL-8, TNF-α).

Consistent with the lack of cytokine release, there was no evidence that BMS-986016 induced T or NK cell activation, as measured by surface expression of CD25 and CD69. Expression levels of these markers on T and NK cells following stimulation with BMS-986016 were similar to those observed upon stimulation with negative controls.

Overall, these data indicate that BMS-986016 does not possess agonistic potential to induce either T or NK cellular activation or cytokine release.

Example 8: Preclinical Pharmacokinetics of Anti-LAG-3 Antibody (BMS-986016)

In accordance with regulatory guidelines for biotechnology-derived pharmaceuticals (ICH Harmonised Tripartite Guideline, S6(R1) Preclinical Safety

Evaluation of Biotechnology-Derived Pharmaceuticals. International Conference on Harmonisation, 2011), no metabolism studies with BMS-986016 have been conducted in animals. The expected *in vivo* degradation of monoclonal antibodies (mAbs) is to small peptides and amino acids via biochemical pathways that are independent of cytochrome P450 enzymes.

BMS-986016 demonstrated favorable pharmacokinetic (PK) properties in cynomolgus monkeys. From both single-dose and repeat-dose IV PK studies, BMS-986016 decayed bi-exponentially and the exposure was approximately dose-proportional. The systemic clearance (CLTp) ranges from 0.12 to 0.22 mL/h/kg and a terminal half-life (T-HALF) 133 to 414 hours. The volume of distribution at steady state (Vss) was 62 to 72 mL/kg, suggesting limited distribution outside the plasma. Anti–BMS-986016 antibodies were detected in some monkeys but the presence of anti–BMS-986016 antibodies appeared to have no impact on BMS-986016 exposure.

Example 9: Inhibition of Tumor Growth *In Vivo* by Combination Treatment with Anti-LAG-3 Antibody and Anti-PD-1 Antibody

An experiment was conducted in a murine tumor model to test the hypothesis that the combination of anti-LAG-3 and anti-PD-1 would potentiate anti-tumor efficacy. These studies evaluated tumor growth inhibition in syngeneic tumor models (Sa1N fibrosarcoma and MC38 colon adenocarcinoma) and monitored acceleration of autoimmunity in the non-obese diabetic (NOD) model. Anti-LAG-3 antibody administration resulted in both overall tumor growth inhibition and an increase in the number of tumor-free (TF) mice in those treatment groups, as shown in Figure 1. Anti-LAG-3 antibody administered in combination with anti-PD-1 antibody provided enhanced anti-tumor activity above the activity of either agent alone. For example, in multiple Sa1N tumor models, anti-LAG-3 antibody resulted in 20%-30% TF mice compared to control and anti-PD-1 antibody-treated mice (0%-10% TF mice), while the combination of anti-LAG-3 and anti-PD-1 antibodies resulted in 60%-90% TF mice. In the MC38 model, anti–LAG-3 antibody showed modest tumor growth inhibition alone, but when administered in combination with anti–PD-1 antibody, resulted in enhanced antitumor activity above that observed for anti–PD-1 antibody alone (80% vs. 40% TF mice, respectively).

Example 10: Phase 1 Trial in Patients Having Solid Tumors

A phase 1 trial of anti-LAG-3 antibody (BMS-986016) and anti-PD-1 antibody (BMS-936558) is conducted in patients having advanced solid tumors to demonstrate the efficacy of administering BMS-986016 and BMS-936558 as a combination treatment.

1. Objectives

The primary objective of the study is to assess the safety and tolerability of BMS-986016 given in combination with BMS-936558 and to identify dose limiting toxicities (DLTs) and the maximally tolerated dose (MTD) of the combination, in subjects with advanced solid tumors.

Secondary objectives include assessing the preliminary anti-tumor activity of the combination of BMS-986016 and BMS-936558 in subjects with advanced solid tumors, characterizing the pharmacokinetics (PK) of BMS-986016 and BMS-936558 when co-administered, monitoring immunogenicity of BMS-986016 and BMS-936558 administered as combination therapy, and assessing the effect of BMS-986016 and BMS-936558 on corrected QT ("QTc"). Additional exploratory objectives include assessing the pharmacodynamic effects of BMS-986016 and BMS-936558 combination therapy based on select biomarkers in the peripheral blood and tumor biopsy specimens, characterizing T cell function during BMS-986016 and BMS-936558 combination therapy, assessing the 2-year landmark overall survival in subjects treated with BMS-986016 and BMS-936558, exploring preliminary antitumor activity of BMS-986016 and BMS-936558 combination therapy in subjects with advanced solid tumors, characterizing pharmacokinetics and exposure-response relationships in subjects treated with BMS-986016 and BMS-936558, and investigating the relationship between clinical efficacy and selected peripheral and tumor biomarkers.

2. Study Design and Duration

This is a Phase 1, open-label study of BMS-986016 administered as a single agent and in combination with BMS-936558 (nivolumab) to subjects with advanced solid tumors. The study is conducted in 3 parts. Part A and Part B consist of a 3 + 3 + 3 dose escalation design with BMS-986016 administered as a single agent (Part A) or in combination with BMS-936558 (Part B) in subjects with advanced solid tumors. Treatment in Part B is initiated upon the decision to escalate to the third dose cohort

in Part A (in accordance with dose escalation rules). Subsequently, escalation in the 2 parts proceeds in parallel. At no point does the dose of BMS-986016 administered in combination with BMS-936558 (Part B) exceed doses of BMS-986016 that have been demonstrated previously to be safe on the monotherapy dose escalation arm (Part A). Part C consists of cohort expansion in 6 disease-restricted populations of approximately 16 subjects each, with BMS-986016 administered in combination with BMS-936558. Treatment in Part C is initiated when the maximum tolerated dose (MTD) (or maximum administered dose (MAD) if no MTD is determined) for Part B has been determined. The doses selected for Part C do not exceed the Part B MTD or MAD, but dose determination may incorporate assessment of other data, including toxicities and PK and pharmacodynamic data from Parts A and B. A schematic of the study is provided in Figure 2.

Subjects complete up to 4 periods of the study as follows: Screening (up to 28 days), Treatment (up to a maximum of twelve 8-week cycles of study therapy), Clinical Follow-up (135 days), and Survival Follow-up (up to 2 years following the first dose of study drug; a longer follow-up period could be considered in selected cases if an efficacy signal is apparent). During this period, diagnostic imaging may be performed every 12 weeks until progression in subjects who discontinue due to CR, and in subjects with PR at the end of Cycle 12.

The Treatment Period consists of up to twelve 8-week treatment cycles. Each treatment cycle is comprised of 4 doses of either BMS-986016 alone (Part A) or in combination with BMS-936558 (Parts B and C), administered on Days 1, 15, 29, and 43 of each treatment cycle. In Parts B and C when both antibodies are administered in combination, nivolumab will be given first followed by BMS-986016 within 30 minutes of completing the infusion of nivolumab. Tumor response is evaluated using RECIST v1.1. Subjects are allowed to continue study therapy until the occurrence of either: (1) confirmed complete response (CR), (2) completion of the maximum number of twelve 8-week cycles, (3) progressive disease (PD), (4) clinical deterioration, and/or (5) meeting other criteria for discontinuation. Treatment beyond progression is allowed in select subjects with initial RECIST v1.1-defined PD who are receiving clinical benefit and tolerating treatment. Subjects who discontinue treatment enter a 135-day Clinical Follow-up period.

After completion of the Clinical Follow-up period, subjects enter the Survival Follow-up period. During this period, clinic visits or telephone contact every 12

weeks are performed to assess survival status. The duration of this period is up to 2 years following the first dose of study drug, although a longer follow-up period is considered in selected cases if an efficacy signal is apparent. Subjects in the Survival Follow-up period who have disease progression are allowed to receive tumor-directed therapy as required. A study schematic is depicted in Figure 3.

Assessments, including physical examinations, vital sign measurements, 12-lead ECG, and clinical laboratory evaluations are performed at selected times throughout the dosing interval. Subjects are closely monitored for adverse events throughout the study. Blood samples are collected for up to 4 hours following the start of study drug administration for pharmacokinetic analysis.

Subjects are allowed to continue on therapy for up to twelve 8-week cycles or until confirmed complete response, progressive disease, clinical deterioration, or meeting criteria for discontinuation. Subjects may be on study for a total of up to approximately 2.3 years, including a 28-day screening period, up to twelve 8-week cycles of treatment, a 135-day clinical follow-up period, and up to 2 years of follow-up for survival (beginning from the first dose of study drug). The total duration of the study is expected to be approximately 5 years from the time of the first visit of the first subject to the required survival follow-up of the last subject enrolled.

3. Dose Escalation

Part A

In Part A, a 3 + 3 + 3 design is used to assess the safety of BMS-986016 given as single agent. A fourth subject may be enrolled at the beginning of a dose escalation cohort, if subject is able to start the first day of dosing within approximately one week of the third subject in the same dose escalation cohort. The dosages during dose escalation are provided in Figures 2A and 2B and Table 1 (set forth below). Three subjects (or 4, if applicable) are initially treated in each dose cohort. In Dose Cohort 1, each of the first 3 subjects (or 4, if applicable) is designated as sentinel subjects and begin a treatment at least 5 days apart. Subjects in subsequent cohorts are not required to observe the 5-day interval between treatment start dates. Dose escalation in Part A proceeds as follows:

- If 0 of the first 3 subjects (or 4, if applicable) experience a dose-limiting toxicity (DLT) within the DLT evaluation interval, a new cohort of 3 subjects (or 4, if applicable) is treated at the next higher dose level.
- If 1 of 3 subjects (or 4, if applicable) experience a DLT within the DLT evaluation interval, that cohort is expanded to 6 subjects.
- If 2 of 6 subjects experience a DLT within the DLT evaluation interval, that cohort is expanded to 9 subjects.
- If ≥ 2 of 3 (or 4, if applicable), ≥ 3 of 6, or ≥ 3 of 9 subjects experience DLTs within a dose cohort during the DLT evaluation interval, then that dose level is determined to have exceeded the MTD.

Table 1: Dose Escalation Schedule for Part A — BMS-986016 Monotherapy

Dose Cohort Number	Total Subjects	BMS-986016 Dose (IV; mg)
1	n = approximately 3-9	20
2	n = approximately 3-9	80
3	n = approximately 3-9	240
4	n = approximately 3-9	800
Total	N = approximately 12-36	

Prior to declaring the MTD (or MAD), any cohort previously established to be safe is expanded to obtain additional experience or to investigate dose levels intermediate to those defined in the protocol. Dose escalation rules (cohort size, DLT evaluation interval, cohort expansion criteria, etc.) apply to these expanded or additional cohorts. A maximum of 9 subjects are enrolled in any additional or expanded dose cohorts.

No within-subject dose escalations are permitted. If a dose level is found to exceed the MTD, subjects enrolled in that dose level are treated at a lower dose.

Part B

Treatment in Part B is initiated after the decision is made to escalate to the third dose cohort in Part A (in accordance with dose escalation rules). Subsequently, escalation in the 2 parts proceeds in parallel. At no point does the dose of BMS-986016 administered in combination with BMS-936558 (Part B) exceed doses of

BMS-986016 that have been demonstrated previously to be safe on the monotherapy dose escalation arm (Part A). Treatment assignments for subjects eligible for both Part A and Part B alternates between the 2 parts, with consecutively treated subjects assigned to different parts through interactive voice response system (IVRS) whenever possible. If there are no openings available in the part to which the subject is assigned by this algorithm, the subject is assigned to the next open cohort or part. As in Part A, a 3 + 3 + 3 design is also be used in Part B to assess the safety of BMS-986016 given in combination with nivolumab. A fourth subject may be enrolled at the beginning of a dose escalation cohort, if subject is able to start the first day of dosing within approximately one week of the third subject in the same dose escalation cohort. The dosages evaluated during dose escalation are provided in Figures 2A and 2B and Table B (set forth below). As in Part A, each of the first 3 subjects (or 4, if applicable) in the first dose cohort in Part B will be designated as sentinel subjects and will begin treatment at least 5 days apart.

Table 2: Dose Escalation Schedule for Part B — BMS-986016 in Combination with BMS-936558

Dose Cohort Number	Total Subjects	BMS-986016 Dose (IV; mg)	BMS-936558 Dose (IV; mg)
1	n = approximately 3-9	3	80
2	n = approximately 3-9	3	240
3	n = approximately 3-9	20	240
4	n = approximately 3-9	80	240
5	n = approximately 3-9	240	240
Total	N = approximately 15-45		

Three subjects are treated initially in each dose cohort. In Dose Cohort 1, each of the first 3 subjects, designated as sentinel subjects, begins treatment at least 5 days apart. Subjects in subsequent cohorts are not be required to observe the 5-day interval between treatment start dates.

Dose escalation in Part B proceeds as described for Part A. If the MTD is exceeded in Dose Cohort 2, the subsequent cohort is treated with 20 mg of BMS-986016 and 80 mg of BMS-936558. If this dose combination is found to be safe, escalation proceeds at the previously defined BMS-986016 doses, maintaining the BMS-936558 dose at 80 mg.

If no MTD is reached through Dose Cohort 5, then additional cohorts of BMS-986016 given in combination with BMS-936558 are considered based on the aggregate safety experience during dose escalation.

Prior to declaring the MTD (or MAD), any cohort previously established to be safe is expanded in order to obtain additional experience or to investigate dose levels intermediate to those defined in the protocol. Dose escalation rules (cohort size, DLT evaluation interval, cohort expansion criteria, etc.) apply to these expanded or additional cohorts. A maximum of 9 subjects are enrolled in any additional or expanded dose cohorts.

No within-subject dose escalations are permitted. If a dose level is found to exceed the MTD, subjects enrolled in that dose level are reduced to a lower dose.

4. Cohort Expansion

The purpose of cohort expansion is to gather additional safety, tolerability, preliminary efficacy, pharmacokinetic, and pharmacodynamic information regarding the combination of BMS-986016 and BMS-936558. The doses selected for Part C do not exceed the MTD (or MAD if no MTD is determined) in Part B, but may incorporate assessment of other data including toxicities and PK and pharmacodynamic data from Parts A and B. Doses include doses intermediate to those evaluated in Part B. Modeling is used to help inform the selection of the combination dose level to carry forward in Part C if a dose below the MTD is chosen. Six expansion cohorts are restricted to the tumor types listed below in Tables 3A and 3B. Continuous evaluation of toxicity events in the cohort expansions is performed throughout enrollment in the expansion cohorts. If, at any time, the aggregate rate of treatment-related toxicities meeting DLT criteria exceeds 33% across all subjects treated in the Part C cohort expansion, further enrollment is interrupted. Depending on the nature and grade of the toxicity and after assessing the risk:benefit ratio, a new dose(s) for all cohorts is initiated at a previously tested lower dose level or at a dose level intermediate to previously tested lower dose levels.

Upon determination of the MTD (or MAD if no MTD is determined) in Part A, a BMS-986016 monotherapy cohort is evaluated in cohort expansion. This expansion cohort is restricted to the tumor type(s) found to be responsive to BMS-986016 monotherapy. The dose selected for monotherapy expansion does not exceed the Part A MTD (or MAD if no MTD is determined) and incorporates assessment of

other data, including toxicities and PK and pharmacodynamic data from Part A. The dose selected is intermediate to those tested in Part A. Modeling is used to help inform the selection of the dose level to carry forward in Part C if a dose below the MTD is chosen.

Table 3A: Tumor Types Eligible For Part C — Cohort Expansion Combination Therapy

Tumor Type	Total Subjects
Melanoma: naive to ICMARs[a]	approximately 16
Melanoma: anti–PD-1 or anti–PD-L1 antibody as most recent therapy[b]	approximately 16
NSCLC[c]: naive to ICMARs[a]	approximately 16
NSCLC[c]: anti-PD-1 or anti–PD-L1 antibody as most recent therapy[b]	approximately 16
HPV[d]-positive head and neck cancer naive to ICMARs[a]	approximately 16
Gastric adenocarcinoma naive to ICMARs[a]	approximately 16
Total	approximately 96

a ICMARs: immune cell-modulating antibody regimens (such as, but not limited to, ipilimumab, tremelimumab, anti-PD-1, anti-PD-L1, anti-PD-L2, anti-KIR, anti-CD137, and/or anti-OX40 antibodies)

b Subjects with anti–PD-1 or anti–PD-L1 antibody as most recent therapy are nonresponsive subjects with progression within 16 weeks of starting therapy. Subjects must provide informed consent within 60 days after the last dose of anti-PD-1 or anti-PD-L1 antibody and should not have discontinued antibody therapy due to serious and/or life-threatening toxicity (*e.g.*, dose-limiting toxicity in prior study). Subjects with anti-PD-1 or anti-PD-L1 antibody as most recent therapy cannot have had prior exposure to any other ICMARs.

c NSCLC: non-small cell lung cancer

d HPV: human papilloma virus

Table 3B: Tumor Types Eligible For Part C — Cohort Expansion Combination Therapy

Tumor Type	Total Subjects
Melanoma: naive to ICMARs[e]	approximately 16
Melanoma: prior anti–CTLA-4 and anti-PD-1 or anti–PD-L1 antibody therapy[f]	approximately 16
NSCLC[g]: naive to ICMARs[a]	approximately 16
NSCLC[c]: anti-PD-1 or anti–PD-L1 antibody as most recent therapy[b]	approximately 16
Head and neck cancer naive to ICMARs[a]	approximately 16
Gastric adenocarcinoma naive to ICMARs[a]	approximately 16
Total	approximately 96

a ICMARs: immune cell-modulating antibody regimens (such as, but not limited to, anti-CTLA-4, and anti-PD-1 or anti-PD-L1, anti-PD-L2, anti-KIR, anti-CD137, and/or anti-OX40 antibodies)

b Melanoma subjects progressing while-on or after receiving anti-CTLA-4 and anti–PD-1 or anti–PD-L1 antibody therapies (in sequential or combination regimens), are eligible. Non-eligible melanoma subjects in this group include those with: 1) last does of anti-CTLA-4 antibody therapy received within 100 days of first dose of study medication; 2) prior exposure to ICMARs other than anti-CTLA-4, anti-PD-1 or anti-PD-L1 antibody therapy regimens; 3)discontinuation from anti-CTLA-4, anti-PD-1 or anti-PD-L1 antibody therapy due to serious and/or life-threatening toxicity (e.g., dose-limiting toxicity in prior exposure).

c NSCLC: non-small cell lung cancer

d NSCLC subjects whose disease progresses while-on or after therapy with anti-PD-1 or anti-PD-L1 antibody as most recent therapy. Subject should not have discontinued antibody therapy due to serious and/or life-threatening toxicity (e.g., dose-limiting toxicity in prior study). Subjects with anti-PD-1 or anti-PD-L1 antibody as most recent therapy cannot have had prior exposure to any other ICMARs.

5. Dose Limiting Toxicities

BMS-986016 has the potential to augment the frequency and severity of previously described adverse events associated with BMS-936558, or to produce new toxicities. For the purpose of guiding decisions regarding dose escalation in Part A and Part B, dose-limiting toxicity (DLT) is determined based on the incidence, intensity, and duration of adverse events that are related to study drug and that occur within 56 days (8 weeks) of initiation of study drug (*i.e.*, the DLT evaluation interval, through the completion of Cycle 1). The severity of adverse events is graded according to National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4.0. For the purpose of subject management, DLTs that occur at any time, whether during dose escalation (Part A and Part B) or cohort expansion (Part C) result in all study drug(s) being held, pending evaluation of the

event's relatedness to study drug. Subjects must meet criteria for re-treatment prior to re-initiation of study treatment.

Subjects who withdraw from the study during the DLT evaluation interval for reasons other than a DLT may be replaced at the same dose level. In the case that an infusion cannot be administered at a scheduled visit during the DLT evaluation interval, it must be administered as soon as possible. If the delay is between 1 and 7 days, the procedures at the original scheduled visit should be performed and subjects will be considered evaluable for DLT determination. If the delay is more than 7 days, the dose will be considered missed and will not be replaced. For the purpose of making decisions on dose escalation from a safety perspective, subjects will be considered evaluable if they have received 3 out of the 4 scheduled BMS-986016 doses in Part A (or 3 out of 4 schedules BMS-986016 and nivolumab doses in Part B) through the 8 week observation period, only if the one missed dose was secondary to progressive disease or non-medical reasons. Unevaluable subjects may be replaced at the same dose level.

Hepatic, non-hematologic, and hematologic DLTs are defined separately as outlined below.

Hepatic DLT

Any of the following drug-related events are considered a hepatic DLT:

- ALT or AST > 8 × ULN, regardless of duration, or
- ALT or AST > 5 × and ≤ 8 × ULN, that fails to return to ≤ Grade 1 within 2 weeks despite medical intervention, or
- Total bilirubin > 5 × ULN, or
- ALT or AST > 3 × ULN and concurrent total bilirubin > 2 × ULN

Non-Hematologic DLT

Any of the following drug-related events are considered a non-hematologic DLT:

- Grade 2 immune related-eye pain or reduction in visual acuity that requires systemic treatment, or
- Grade 2 eye pain or reduction in visual acuity that does not respond to topical therapy and that does not improve to Grade 1 within 2 weeks of initiation of topical therapy, or

- ≥ Grade 3 non-hepatic or non-hematologic toxicity with the exceptions noted below.

The following Grade 3 non-hematologic events are not considered DLTs:

- Grade 3 electrolyte abnormality that lasts < 72 hours, is not clinically complicated, and resolves spontaneously or responds to conventional medical intervention
- Grade 3 increase in amylase or lipase that persists for < 3 weeks and is not associated with clinical or radiographic evidence of pancreatitis
- Grade 3 nausea or vomiting that lasts < 48 hours, and resolves to ≤ Grade 1 either spontaneously or with conventional medical intervention
- Grade 3 fever that lasts < 72 hours, and is not associated with hemodynamic compromise (including hypotension, or clinical or laboratory evidence of end organ perfusion impairment)
- Grade 3 endocrinopathy that is well controlled by hormone replacement
- Grade 3 tumor flare (defined as pain, irritation, or rash that localizes to sites of known or suspected tumor)
- Grade 3 fatigue for less than 7 days

Hematologic DLT

Any of the following drug-related events are considered a hematologic DLT:

- Grade 4 febrile neutropenia of any duration
- Grade 4 neutropenia that lasts > 5 days
- Grade 4 thrombocytopenia
- Grade 4 anemia
- Grade 3 thrombocytopenia associated with clinically significant bleeding
- Grade 3 febrile neutropenia that lasts > 48 hours
- Grade 3 hemolysis

6. Inclusion Criteria

Signed Written Informed Consent

The subject must sign and date the IRB/IEC approved written informed consent form prior to the performance of any study-related procedures that are not considered part of standard of care.

Consent for tumor biopsy samples for Part A or Part B Dose Escalation: Subject must consent to allow the acquisition of existing formalin-fixed paraffin-embedded (FFPE) tumor tissue, either a block or unstained slides, for performance of correlative studies. If an archived sample is not available, subject must consent to allow a pre-treatment tumor biopsy. In either case, study personnel must ensure that the tissue block or slides physically exist prior to initiating therapy. Subjects unable to provide an archived tumor sample and who either do not consent to a pre-treatment tumor biopsy or do not have accessible lesions are not eligible. (However, subjects whose pre-treatment biopsy yields inadequate tissue quantity or quality are not ineligible on this basis alone.)

Consent for tumor biopsy samples for Part C Cohort Expansion: Subjects with melanoma or head and neck tumors: All subjects in the 2 melanoma cohorts and all subjects in the head and neck tumor cohort are required to undergo pre-treatment and on-treatment biopsies; therefore, subjects must have a lesion located such that the specimen can be obtained at acceptable clinical risk as judged by the Investigator. Biopsy sites for any subjects must be distinct from evaluable lesions. Subjects in the melanoma and head and neck cancer cohorts who do not meet these criteria are not eligible; however, subjects whose screening biopsy yields inadequate tissue quantity or quality are not be ineligible on this basis alone. Subjects in the remaining cohorts (NSCLC or gastric adenocarcinoma): Subject must consent to allow the acquisition of existing formalin-fixed paraffin-embedded (FFPE) tumor tissue, either a block or unstained slides, for performance of correlative studies. If an archived sample is not available, subject must consent to allow a pre-treatment tumor biopsy. In either case, study personnel must ensure that the tissue block or slides physically exist prior to initiating therapy. Subjects unable to provide an archived tumor sample and who either do not consent to a pre-treatment tumor biopsy or do not have accessible lesions are not eligible. (However, subjects whose pre-treatment biopsy yields inadequate tissue quantity or quality are not ineligible on this basis alone.) Biopsies cannot be collected in subjects with a single measureable lesion, even if accessible.

Target Population

a) Subjects must have histologic or cytologic confirmation of an incurable solid malignancy that is advanced (metastatic and/or unresectable):

i) Part A Dose Escalation: BMS-986016 monotherapy

(1) All solid tumor histologies are permitted except for subjects with primary CNS tumors

(2) Only subjects without prior exposure to immune cell-modulating antibody regimens (ICMARs) such as, but not limited to, CTLA-4, ipilimumab, tremelimumab, anti–PD-1, anti–PD-L1, anti–PD-L2, anti-KIR, anti-CD137, or anti-OX40 antibodies, are allowed;

(3) Subjects must have received, and then progressed or been intolerant to, at least one standard treatment regimen in the advanced or metastatic setting, if such a therapy exists.

ii) Part B Dose Escalation: BMS-986016 + BMS-936558

(1) All solid tumor histologies are permitted except for subjects with primary CNS tumors. Subjects with or without prior anti–PD-1 or anti–PD-L1 antibody therapy are eligible. Alternatively, all solid tumor histologies naive to ICMARs such as, but not limited to, anti-CTLA-4, anti-PD-1, anti-PD-L1, anti–PD-L2, anti-KIR, anti-CD137, or anti-OX40 antibodies, will be permitted except for subjects with primary CNS tumors.

(2) Subjects without prior anti–PD-1 or anti–PD-L1 antibody therapy cannot have had prior exposure to any other ICMARs such as, but not limited to, ipilimumab, tremelimumab, anti–PD-L2, anti-KIR, anti-CD137, or anti-OX40 antibodies. Alternatively, Subjects without prior anti–PD-1 or anti–PD-L1 antibody therapy cannot have had prior exposure to any other ICMARs such as, but not limited to, ipilimumab, tremelimumab, anti–PD-L2, anti-KIR, anti-CD137, or anti-OX40 antibodies.

(3) NSCLC subjects whose disease progresses while-on or after therapy with anti–PD-1 or anti–PD-L1 antibody as most recent therapy. Alternatively, subjects with prior anti–PD-1 or anti–PD-L1 antibody as most recent therapy:

(a) Disease is nonresponsive while on anti–PD-1 or anti–PD-L1 antibody therapy and presents with PD (per RECIST) within 16 weeks of starting therapy.

(b) Cannot have had therapy discontinued due to serious and/or life-threatening anti–PD-1 or anti–PD-L1 antibody-related toxicity (*e.g.*, dose-limiting toxicity in prior study).

(c) Must provide informed consent within 60 days after the last dose of anti–PD-1 or anti–PD-L1 antibody therapy.

(d) Cannot have had prior exposure to ICMARs such as, but not limited to, anti-CTLA-4 antibody therapy, ipilimumab, tremelimumab, anti–PD-L2, anti-KIR, anti-CD137, or anti-OX40 antibodies.

(4) Melanoma subjects whose disease is progressing while-on or after receiving anti-CTLA-4 and anti-PD-1 or anti-PD-L1 antibody therapies (a) Anti-CTLA-4 and anti-PD-1 or anti-PD-L1 antibody therapies could have been received in sequential or combination regimens (b) Last dose of anti-CTLA-4 antibody therapy must have been received ≥ 100 days of first dose of study medication (c) Cannot have had therapy discontinued due to serious and/or life-threatening antibody-related toxicity (e.g., dose-limiting toxicity in prior study) (d) Cannot have had prior exposure to any ICMARs other than anti-CTLA-4 and anti-PD-1 or anti-PD-L1 antibody therapy.

(5) Subjects must have received, and then progressed or been intolerant to at least one standard treatment regimen.

iii) Part C Cohort Expansion

(1) The following groups are enrolled:

(a) Melanoma – subjects naive to ICMARs

(b) Melanoma - subjects whose disease is nonresponsive while on anti–PD-1 or anti–PD-L1 antibody therapy as most recent therapy and presents with PD (per RECIST) within 16 weeks of starting therapy. Subject must provide informed consent within 60 days after last dose of anti–PD-1 or anti–PD-L1 antibody therapy and

should have not discontinued antibody therapy due to serious and/or life-threatening toxicity (*e.g.*, dose-limiting toxicity in prior study). These subjects cannot have had prior exposure to any other ICMARs such as, but not limited to, ipilimumab, tremelimumab, anti–PD-L2, anti-KIR, anti-CD137, or anti-OX40 antibodies. Alternatively, subjects whose disease is progressing while on or after receiving anti-CTLA-4 and anti-PD-1 or anti-PD-L1 antibody therapy (in sequential or combination regimens), are eligible. Last dose of anti-CTLA-4 antibody therapy must have been received ≥ 100 days of first dose of study medication. Subjects should have not discontinued antibody therapy due to serious and/or life-threatening toxicity (e.g., dose-limiting toxicity in prior study). These subjects cannot have had prior exposure to any ICMARs other than anti-CTLA-4 and PD-1 or anti-PD-L1 antibody therapy.

(c) Non-small cell lung cancer (NSCLC) – subjects naive to ICMARs

(d) NSCLC - subjects whose disease is nonresponsive while on anti–PD-1 or anti–PD-L1 antibody therapy as most recent therapy and presents with PD (per RECIST) within 16 weeks of starting therapy. Subject must provide informed consent within 60 days after last dose of anti–PD-1 or anti–PD-L1 antibody therapy and should have not discontinued antibody therapy due to serious and/or life-threatening toxicity (*e.g.*, dose-limiting toxicity in prior study). These subjects cannot have had prior exposure to any other ICMARs such as, but not limited to, ipilimumab, tremelimumab, anti–PD-L2, anti-KIR, anti-CD137, or anti-OX40 antibodies. Alternatively, NSCLC subjects whose disease progresses while-on or after therapy with anti-PD-1 or anti-PD-L1 antibody as most recent therapy. Subject should have not discontinued antibody therapy due to serious and/or life-threatening toxicity (e.g., dose-limiting toxicity in prior study). These subjects cannot have had prior exposure to any other ICMARs such as, but not limited to, anti-CTLA-4, anti-PD-L2, anti-KIR, anti-CD137, or anti-OX40 antibodies.

(e) HPV-associated head and neck tumors – subjects naive to ICMARs and with HPV positivity as defined by p16 immunohistochemistry (IHC)-positive and/or HPV-16 in situ hybridization (ISH)-positive

(i) Histology restricted to squamous cell carcinoma. Alternatively, subjects with advanced/metastatic head and neck tumors – naïve to ICMARs (i) Histology restricted to squamous cell carcinoma.

(f) Gastric adenocarcinoma subjects naive to ICMARs

(i) HER2(+) and HER2(-) subjects are allowed

b) Subjects must have received, and then progressed or been intolerant to, at least one standard treatment regimen in the advanced or metastatic setting, if such a therapy exists.

c) Subjects with any prior treatment regimens are eligible. The following are not considered separate lines of treatment: addition of a compound to an ongoing regimen, restarting the same regimen after a drug holiday, or switching from IV to oral therapy.

d) Presence of at least one lesion with measurable disease as defined by RECIST v1.1 criteria for response assessment. Subjects with lesions in a previously irradiated field as the sole site of measurable disease are permitted to enroll provided that the lesion(s) have demonstrated clear progression prior to the time of informed consent and can be measured accurately.

e) ECOG status of 0 or 1.

f) Life expectancy of $\geq$ 12 weeks at the time of informed consent.

g) Adequate organ function as defined by the following:

i) White blood cells (WBCs) $\geq$ 2000/μL (stable off any growth factor within 4 weeks of first study drug administration)

ii) Neutrophils $\geq$ 1500/μL (stable off any growth factor within 4 weeks of first study drug administration)

iii) Platelets $\geq 100 \times 10^3$/μL (transfusion to achieve this level is not permitted within 2 weeks of first study drug administration)

iv) Hemoglobin $\geq$ 8.5 g/dL (transfusion to achieve this level is not permitted within 2 weeks of first study drug administration)

v) Creatinine < 1.5 × ULN or creatinine clearance ≥ 40mL/min (Cockcroft-Gault formula)

vi) ALT and AST ≤ 3 × ULN

vii) Lipase and amylase < 1.5 × ULN

viii) Total bilirubin ≤ 1.5 × ULN (except subjects with Gilbert's Syndrome who must have normal direct bilirubin)

h) Normal thyroid function, or stable on hormone supplementation

i) Ability to comply with treatment, PK, and pharmacodynamic sample collection and required study follow-up.

j) Subject re-enrollment: This study permits the re-enrollment of a subject that has discontinued the study as a pre-treatment failure (*i.e.*, subject has not been randomized / has not been treated). If re-enrolled, the subject must be re-consented.

Age and Reproductive Status

a) Men and women, ages ≥ 18 years at the time of informed consent

b) Women of childbearing potential (WOCBP) must use methods of contraception. For a teratogenic study drug and/or when there is insufficient information to assess teratogenicity (preclinical studies have not been done), 2 forms of contraception are required. One method must be highly effective (failure rate of less than 1% when used consistently and correctly) and the second method may also be highly effective. The individual methods of contraception should be determined in consultation with the Investigator. WOCBP must follow instructions for birth control for a total of 24 weeks after the last dose of investigational drug (a period of 30 days plus the time required for the investigational drug to undergo 5 half-lives). Women of childbearing potential (WOCBP) are defined as any female who have experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) and is not postmenopausal. Menopause is defined as 12 months of amenorrhea in a woman over age 45 in the absence of other biological or physiological causes. In addition, females under the age of 55 must have a documented serum follicle-stimulating hormone (FSH) level > 40 mIU/mL to confirm menopause. Females treated with hormone replacement

therapy, (HRT) are likely to have artificially suppressed FSH levels and may require a washout period in order to obtain a physiologic FSH level. The duration of the washout period is a function of the type of HRT used. The duration of the washout period below are suggested guidelines and the investigators should use their judgement in checking serum FSH levels. If the serum FSH level is >40 mIU/ml at any time during the washout period, the woman can be considered postmenopausal.

1 week minimum for vaginal hormonal products, (rings, creams, gels)
4 week minimum for transdermal products
8 week minimum for oral products
Other parenteral products may require washout periods as long as 6 months.

c) Women must have a negative serum or urine pregnancy test (minimum sensitivity of urine pregnancy test of 25 IU/L of either total human chorionic gonadotropin (hCG) or the beta fraction) within 24 hours prior to the start of investigational product.

d) Women must not be breastfeeding.

e) Men who are sexually active with WOCBP must use methods of contraception. For a teratogenic study drug and/or when there is insufficient information to assess teratogenicity (preclinical studies have not been done), 2 forms of contraception are required. One method must be highly effective (failure rate of less than 1% when used consistently and correctly) and the second method may also be highly effective. Men who are sexually active with WOCBP must follow instructions for birth control for a total of 33 weeks after the last dose of investigational drug (a period of 90 days plus the time required for the investigational drug to undergo 5 half-lives).

Women who are not of childbearing potential (*i.e.*, who are postmenopausal or surgically sterile; and permanently azoospermic men (*e.g.*, bilateral orchiectomy) do not require contraception. Women of childbearing potential (WOCBP) are defined as females who have experienced menarche and who have not undergone surgical sterilization (hysterectomy or bilateraloophorectomy) or are not postmenopausal. Menopause is defined clinically as 6 months of amenorrhea in a woman over age 45 in the absence of other biological or physiological causes. In addition, women under the age of 55 must have a documented serum follicle-stimulatinghormone (FSH) level > 40 mIU/mL to confirm menopause.

7. Exclusion Criteria

Target Disease Exceptions

Subjects with known or suspected CNS metastases or with the CNS as the only site of active disease are excluded with the following exceptions:

i) Subjects with controlled brain metastases are allowed to enroll. Controlled brain metastases are defined as those with no radiographic progression for at least 4 weeks after radiation and/or surgical treatment at the time of consent. Subjects must have been off of steroids for at least 2 weeks prior to informed consent, and have no new or progressive neurological signs and symptoms.

ii) Subjects with signs or symptoms of brain metastases are not eligible unless brain metastases are ruled out by computed tomography (CT) or magnetic resonance imaging (MRI).

Participation in any prior clinical study with BMS-936558, including subjects in comparator arms, in which overall survival is listed as the primary or co-primary endpoint and which has not completed analysis based on the primary endpoint.

Medical History and Concurrent Diseases

Subjects with a prior malignancy are excluded, except adequately treated basal cell or squamous cell skin cancer, localized prostate cancer, carcinoma in situ of the cervix or carcinoma in situ of the bladder, or in situ ductal or lobular carcinoma of the breast. Subjects with other prior malignancies diagnosed more than 2 years previously (at the time of informed consent) who have received therapy with curative intent with no evidence of disease during the interval who are considered to present a low risk for recurrence are eligible.

Subjects with any active autoimmune disease or history of known or suspected autoimmune disease with the exception of subjects with isolated vitiligo, resolved childhood asthma/atopy, controlled hypoadrenalism or hypopituitarism, and euthyroid patients with a history of Grave's disease (subjects with controlled hyperthyroidism must be negative for thyroglobulin and thyroid peroxidase antibodies and thyroid-stimulating immunoglobulin prior to study drug administration).

A known or underlying medical condition that could make the administration of study drug hazardous to the subject or could adversely affect the ability of the subject to comply with or tolerate study.

Requirement for daily supplemental oxygen.

Uncontrolled or significant cardiovascular disease including, but not limited to, any of the following: Myocardial infarction or stroke/transient ischemic attack (TIA) within the 6 months prior to consent, uncontrolled angina within the 3 months prior to consent, any history of clinically significant arrhythmias (such as ventricular tachycardia, ventricular fibrillation, or torsades de pointes), QTc prolongation > 480 msec, history of other clinically significant heart disease (*i.e.*, cardiomyopathy, congestive heart failure with New York Heart Association [NYHA] functional classification III-IV, pericarditis, significant pericardial effusion).

Cardiovascular disease-related requirement for daily supplemental oxygen.

A confirmed history of encephalitis, meningitis, or uncontrolled seizures in the year prior to informed consent.

Positive blood screen for human immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome (AIDS).

History of any chronic hepatitis as evidenced by positive test for hepatitis A antibody (HepA IgM) (Note: history of resolved hepatitis A virus infection is not an exclusion criterion), positive test for hepatitis B surface antigen (HBsAg) and/or hepatitis B core antigen, positive test for qualitative hepatitis C viral load (by PCR).

Evidence of active infection that requires systemic antibacterial, antiviral, or antifungal therapy ≤ 7 days prior to initiation of study drug therapy.

Any other significant acute or chronic medical illness.

Subjects who are unable to undergo venipuncture and/or tolerate venous acces.

Any other sound medical, psychiatric, and/or social reason.

Any of the following procedures or medications:

Within 2 weeks prior to time of informed consent: systemic or topical corticosteroids at immunosuppressive doses (≥ 7.5 mg/day of prednisone or equivalent), palliative radiation and gamma knife radiosurgery in CNS, or medicinal herbal preparations.

Within 4 weeks prior to study drug administration: any investigational drug or placebo, any anticancer therapy (chemotherapy, biologics, therapeutic vaccines,

radiotherapy, or hormonal treatment), non-oncology vaccines containing live virus, allergen hyposensitization therapy, growth factors, *e.g.*, granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), erythropoietin, major surgery, or biphosphonates.

Within 10 weeks prior to study drug administration: receptor activator of nuclear factor kappa-B ligand (RANK-L) inhibitors.

Allergies and Adverse Drug Reaction

History of allergy to anti–PD-1 or anti–PD-L1 antibody therapy or to other monoclonal antibodies or related compounds or to any of their components (*e.g.*, history of severe hypersensitivity reactions to drugs formulated with polysorbate 80).

Other Exclusion Criteria

Prisoners or subjects who are involuntarily incarcerated.

Subjects who are compulsorily detained for treatment of either a psychiatric or physical (*e.g.*, infectious disease) illness.

Inability to comply with restrictions and prohibited activities and treatments.

8. Guidelines for Dose Modification

Intrasubject dose escalation of BMS-986016 or BMS-936558 is not permitted in this study. With the possible exception of subjects being treated at a dose level that is subsequently deemed to exceed the MTD, intrasubject dose reduction of BMS-986016 or BMS-936558 is not permitted.

In some cases, the natural history of select adverse events associated with immunotherapy can differ from and be more severe than adverse events caused by other therapeutic classes. Early recognition and management mitigates severe toxicity.

Additionally, management algorithms can assist with select toxicities. Toxicities for which management algorithms have been developed include:

- Pulmonary
- Gastrointestinal
- Hepatic
- Endocrine

- Renal
- Dermatologic
- Neurologic

Subjects who experience the following must have all study drug(s) held:

- DLTs (per definition, are related to study drug)
- Select drug-related adverse events and drug-related laboratory abnormalities:
  - ≥ Grade 1 pneumonitis
  - ≥ Grade 2 abnormality in AST, ALT, total bilirubin, amylase, or lipase
  - ≥ Grade 2 creatinine
  - ≥ Grade 2 diarrhea or colitis
  - ≥ Grade 2 neurological adverse event
- Adverse event, laboratory abnormality, or concurrent illness that, in the judgment of the Investigator, warrants delaying the dose of study drug.

Dose delays > 7 days are considered missed and is not be replaced.

9. Safety Assessments

Adverse events are assessed continuously during the study and for 135 days after the last treatment. Adverse events are evaluated according to the NCI CTCAE version 4.0. Adverse events are coded using the most current version of Medical Dictionary for Regulatory Activities (MedDRA) and reviewed for potential significance and importance.

10. Other Analyses

Various serologic tumor markers, gene mutation status, and additional analyses are required dependent upon the subject's tumor type as listed below in Table 4. With the exception of the serologic tumor markers, the assessments are not performed if the lab results from previous testing are available.

Table 4: Biomarkers by Tumor Type

Tumor Type	Study Part	Matrix	Lab Test	Assessment	Timepoint
Colorectal	Parts A, B	Blood	Serologic Tumor	CEA[a]	Multiple

Table 4: Biomarkers by Tumor Type

	ONLY		Marker		
	Parts A, B ONLY	Tumor Tissue	Gene Mutation Status	EGFR[b] K-RAS MSI[c]	Screening
Gastric	Parts A, B, C	Blood	Serologic Tumor Marker	CEA[a]	Multiple
	Parts A, B, C	Tumor Tissue	Gene Mutation Status	HER-2[d]	Screening
	Parts A, B, C	Tumor Tissue	Real Time qPCR[e]	EBV[f]	Screening
Germ Cell	Parts A, B ONLY	Blood	Serologic Tumor Marker	βhCG[g] AFP[h]	Multiple
Head and Neck	Part C ONLY	Tumor Tissue	IHC and/or ISH[i]	HPV[j]	Screening (Eligibility)
Melanoma	Parts A, B, C	Tumor Tissue	Gene Mutation Status	BRAF	Screening
NSCLC	Parts A, B, C	Tumor Tissue	Gene Mutation Status	ALK[k] K-RAS EGFR[b]	Screening
Ovarian	Parts A, B ONLY	Blood	Serologic Tumor Marker	CA125[l]	Multiple
Prostate	Parts A, B ONLY	Blood	Serologic Tumor Marker	PSA[m]	Multiple

a CEA: carcinoembryonic antigen
b EGFR: epidermal growth factor receptor
c MSI: microsatellite instability
d HER-2: human epidermal growth factor receptor 2 status via IHC and/or ISH
e Real time qPCR: real time quantitative polymerase chain reaction for BamH1-A Reading Frame-1(BARF1) gene
f EBV: Epstein-Barr virus
g βhCG: beta-human chorionic gonadotrophin
h AFP: alpha-fetoprotein
i IHC and/or ISH: p16 immunohistochemistry (IHC) and/or HPV-16 in situ hybridization (ISH)
j HPV: human papilloma virus
k ALK: anaplastic lymphoma kinase
l CA125: cancer antigen 125
m PSA: prostate specific antigen

Additional measures, including non-study-required laboratory tests, are performed as clinically indicated. Results of all laboratory tests required by this protocol are recorded.

11. Efficacy Assessments

Efficacy is evaluated in Parts A and B (dose escalation), as well as in Part C (cohort expansion). Changes in tumor measurements and tumor response at the time of each assessment are determined. The baseline assessment during the screening period requires CT or MRI scans of the chest, abdomen, and pelvis, and other anatomic regions as indicated by individual subject's tumor type and/or disease history. Subsequent timepoints require scans of the chest, abdomen, and pelvis, as well as other anatomic regions that were scanned at baseline based on the individual subject's tumor type and/or disease history. Scans of the brain are otherwise required as clinically indicated.

Analysis of response endpoints are performed according to immune-related response criteria, irRECIST that reflect the clinical experience with other T cell-directed immunotherapies in which objective and durable responses were observed in subjects following progression and without intervening alternative anticancer therapy (Wolchok JD, *et al.*, *Clin. Can. Res.* 2009;15(23):7412-7420). Individual subject's best overall response (BOR), duration of progression-free survival (PFS), and duration of response (DOR) is calculated as appropriate.

Tumor status are assessed at baseline, during treatment (every 8 weeks) for up to twelve 8-week cycles of therapy, and once during follow-up. CT and MRI scans re read and assessed locally per RECIST v1.1. All imaging scans are de-identified and archived in their native Digital Imaging and Communications in Medicine (DICOM) format as part of the subject's study file.

The efficacy assessments include the ORR (*e.g.*, PR + CR), DOR, and PFSR at landmark timepoints (*e.g.*, 24 weeks), based on assessment of tumor response using irRECIST and RECIST v1.1. Landmark 2-year overall survival (OS).

12. Pharmacokinetic Assessments

Serum samples for BMS-986016 pharmacokinetics and anti-drug antibody (ADA) assessments are collected for all subjects. Serum samples for BMS-936558 pharmacokinetics and ADA assessments re collected for all subjects enrolled in Part B and C. The serum samples are analyzed for BMS-986016 and BMS-936558 by a validated immunoassay. In addition, selected serum samples are analyzed by an

exploratory orthogonal method (*e.g.*, liquid chromatography [LC]-mass spectrometry [MS]/MS) that measures total BMS-986016 and/or BMS-936558.

13. Exploratory Biomarker Assessment

The pharmacodynamics of BMS-986016 treatment administered alone or in combination with BMS-936558 is assessed by quantifying biomarkers in peripheral blood and tumor tissue in the first 3 subjects enrolled at each dose level during the dose escalation (Parts A and B) and in subjects with melanoma and head and neck cancers during cohort expansion (Part C) phases of the study. Detailed schedules of pharmacodynamic evaluations are provided below in Tables 5-6. Details regarding the tumor tissues requirements for subjects in Parts A, B, and C of the study are provided below in Table 7.

Table 5A: Part A & B (Dose Escalation) — Biomarker Sampling Schedule (ONLY for First 3 Subjects in each Dose Level)

Collection Timing	Serum	PBMC		Tumor	Whole Blood	
Study Day	Soluble Biomarkers (Serum Biomarkers)	Immuno-phenotyping / Tetramer (Flow Cytometry/ PBMC)	Ex vivo Functional Assay (Cellular Assay)	Archival Tissue[a]	Gene Expression (Whole Blood mRNA)	SNP
Screening				X		
Cycle 1						
Day 1	X	X	X		X	X
Day 5[a]	X					
Day 8	X	X				
Day 15	X	X			X	
Day 29	X	X			X	
Day 43	X	X	X		X	
Cycle 2						
Day 29	X	X	X		X	
Upon Progression						
Upon Progression[b]	X	X	X	X	X	

a Day 5 visit can occur on Day 3 or Day 4
b Optional; collected upon confirmation of PD
NOTE: All samples are drawn pre-dose

Table 5B: Part A & B (Dose Escalation) — Biomarker Sampling Schedule (ONLY for First 3 Subjects in each Dose Level)

Collection Timing	Serum	PBMC		Tumor	Whole Blood	
Study Day	Soluble Biomarkers (Serum Biomarkers)	Immuno-phenotyping / Tetramer (Flow Cytometry/ PBMC)	Ex vivo Functional Assay (Cellular Assay)	Archival Tissue	Gene Expression (Whole Blood mRNA)	SNP
Screening				X		
Cycle 1						
Day 1	X	X	X		X	X
Day 5[b]	X					
Day 8	X	X				
Day 15	X	X			X	
Day 29	X	X			X	
Day 43	X	X	X		X	
Cycle 2						
Day 29	X	X	X		X	
Upon Progression						
Upon Progression[b]	X	X	X	X	X	
Upon Drug-related AE						
Upon occurrence of ≥ Grade 2 drug related pneumonitis or neurological AE	X	X	X			

a Day 5 visit can occur on Day 3 or Day 4. Day 8 visit can occur on Day 7 or Day 9.
b Optional; collected upon confirmation of PD
NOTE: All samples are drawn pre-dose

Table 6A: Part C (Cohort Expansion) — Biomarker Sampling Schedule (ONLY for Subjects with Melanoma and Head and Neck Cancer)

Collection Timing	Serum	PBMC		Tumor	Whole Blood	
Study Day	Soluble Biomarkers (Serum Biomarkers)	Immuno-phenotyping / Tetramer (Flow Cytometry/PBMC)	Ex vivo Functional Assay (Cellular Assay)	"Fresh" Tumor Biopsy	Gene Expression (Whole Blood mRNA)	SNP
Screening				X[a]		
Cycle 1						
Day 1	X[b]	X	X		X	X
Day 5[c]	X					
Day 8	X	X				
Day 15	X	X			X	
Day 29	X	X			X	
Day 43	X	X	X		X	
Day 50-56				X[d]		
Cycle 2						
Day 29	X	X	X		X	
Upon Progression						
Upon Progression[e]	X	X	X	X	X	

NOTE: All samples are drawn pre-dose

[a] Fresh tumor biopsy is mandatory for subjects with melanoma and head and neck cancer in Part C.

[b] Serum and plasma at Cycle 1 Day 1. Serum only at all other timepoints.

[c] Day 5 visit can occur on Day 3 or Day 4

[d] Fresh tumor biopsy is mandatory for subjects with melanoma and head and neck cancer in Part C. Biopsy is obtained at anytime during Cycle 1, Week 8 (Days 50-56) at the same time as diagnostic imaging.

[e] Optional; collected upon confirmation of PD

Table 6B: Part C (Cohort Expansion) — Biomarker Sampling Schedule (ONLY for Subjects with Melanoma and Head and Neck Cancer)

Collection Timing	Serum	Plasma	PBMC[b]		Tumor	Whole Blood	
Study Day	Soluble Biomarkers	(Serum Biomarkers)	Immuno-phenotyping / Tetramer (Flow Cytometry/ PBMC)	Ex vivo Functional Assay (Cellular Assay)	"Fresh" Tumor Biopsy	Gene Expression (Whole Blood mRNA)	SNP
Screening					X[a]		
Cycle 1							
Day 1	X	X	X	X		X	X
Day 5[c]	X						
Day 8[c]	X		X				
Day 15	X		X			X	
Day 29	X	X	X			X	
Day 36	X		X			X	
Day 43	X	X	X	X		X	
Day 50-56					X[d]		
Cycle 2							
Day 29	X		X	X		X	
Upon Progression							
Upon Progression[e]	X		X	X	X	X	
Upon Drug-related AE							
Upon occurrence of ≥ Grade 2 drug related pneumonitis or neurological AE	X		X	X			

a Fresh tumor biopsy is mandatory for subjects with melanoma and head and neck cancer Part C.

b PBMC samples only to be collected for subjects in the US, not required for subjects Ex-US.

c Day 5 visit can occur on Day 3 or Day 4. Day 8 visit can occur on Day 7 or Day 9.

d Fresh tumor biopsy is mandatory for subjects with melanoma and head and neck cancer in Part C. Biopsy can be obtained at anytime during Cycle 1, Week 8 (Days 50-56) at same time as diagnostic imaging.

e Optional; to be collected upon confirmation of PD.

Table 7: Tumor Tissue Requirements for Parts A, B, and C

Study Part	Part A and B (Dose Escalation)	Part C (Cohort Expansion)	
Subjects	ALL subjects in Part A or B	Subjects with melanoma or head and neck tumors ONLY	Subjects with NSCLC or gastric adenocarcinoma ONLY
Type of Specimen	Archived tumor tissue. If archived sample is not available, must obtain a "fresh" pre-treatment tumor biopsy	Mandatory "fresh" biopsies (pre- and on-treatment)	Archived tumor tissue. If archived sample is not available, must obtain a "fresh" pre-treatment tumor biopsy
Upon Progression	Optional "fresh" biopsy upon confirmation of PD	Optional "fresh" biopsy upon confirmation of PD	Optional "fresh" biopsy upon confirmation of PD

Soluble Biomarkers (Serum Biomarkers) — Parts A, B, and C

Pre-treatment and on-treatment serum levels of chemokines, cytokines, and tumor-associated soluble proteins is assessed by techniques that include, but are not limited to, ELISA or multiplex assays. Analytes include markers of inflammation, immune activation, host tumor growth factors, and tumor-derived proteins.

Antitumor Antibodies (Serum Biomarkers) — Parts A, B, and C

Treatment with BMS-986016 and BMS-936558 may result in the generation of novel, or an increase in existing, antibodies to tumor-associated antigens. An assessment of antibodies to a panel of > 8000 proteins is performed using pre-treatment and on-treatment serum in multiplex and ELISAs. These data are used to explore if antitumor antibodies are associated with clinical response and safety parameters, as well as inform pharmacodynamics of drug administration.

Immunophenotyping (Flow Cytometry/PBMC) — Parts A, B, and C

Peripheral blood mononuclear cells (PBMCs) are used to characterize and quantify the activation and regulatory status of myeloid and lymphoid cells by polychromatic flow cytometry. Subsets of cells characterized by immunophenotyping include naive, activated, and exhausted effector and memory T cell populations, regulatory T cells, and myeloid-derived suppressor cells.

Ex vivo Functional Assays (Cellular Assay) — Parts A, B, and C

To asssess whether BMS-986016 and BMS-936558 restores T cell activation and function, PBMCs are isolated and cryopreserved. The functional status of effector T cells, including, but not limited to, IFN-γ and granzyme B, is assessed by flow cytometric staining.

Peripheral Blood Gene Expression (Whole Blood mRNA) and Tumor Gene Expression — Parts A, B, and C

The expression level of genes related to response to BMS-986016 ± BMS-936558 is quantified by microarray and/or quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis in whole blood and tumor samples. Analysis includes, but is not necessarily be limited to, genes encoding BMS-986016–stimulated effector functions (perforin, granzyme B, and IFN-γ) and genes encoding T cell co-stimulatory receptors (PD-1, PD-L1, and CTLA-4).

Circulating Tumor DNA Analysis (Serum (plasma) Biomarkers) — Part C

The presence of cell-free DNA in circulating blood is a well-documented phenomenon. Fragments of DNA are shed into the blood stream from dividing cells during cell proliferation or cell death. In patients with cancer, a fraction of this DNA is tumor-derived and is termed circulating tumor DNA (ctDNA). Albeit small, fragments of DNA average between 180 to 200 bp and specific genomic regions can be amplified with PCR. Moreover, several studies have detected mutations in ctDNA that exactly correspond to mutations from the parent tumor. Using tissue and plasma from patients with known driver mutations in melanoma or head and neck cancer, BEAMing technology is utilized to count the frequency of mutations in circulation.

Single Nucleotide Polymorphism Analysis (SNP) — Parts A, B, and C

In order to identify potential polymorphisms associated with the safety and efficacy of BMS-986016 selected genes are evaluated for single nucleotide polymorphisms (SNPs). Genes of interest include, but are not limited to, PD-1, PD-L1, MHC class II, LAG-3, and CTLA-4.

Tumor Biopsy Analysis — Parts A and B

Tumor tissue is collected from all subjects in the dose escalation portion of the protocol. Immunohistochemistry is used to assess the number and composition of immune infiltrates to define the immune cell subsets present within FFPE tumor tissue before and potentially after exposure to BMS-986016 and BMS-936558. These IHC analyses include, but are not necessarily be limited to, the following markers: CD4, CD8, LAG-3, MHC II, PD-1, PD-L1, and PD-L2. Correlations between gene expression and IHC expression are made between assays performed if deemed to be informative.

Tumor-Based Biomarker Measures — Part C

Paired pre- and on-treatment tumor biopsies are mandatory for all subjects with melanoma or head and neck cancer who are enrolled in Part C (cohort expansion). Subjects for whom adequate paired pre- and on-treatment biopsies are not collected may be replaced.

Subjects have at least one lesion large enough to undergo repeated biopsies (pre- and on-treatment biopsies) via core needle (minimum size 18 gauge) or have at least 2 distinct lesions eligible for core needle or excisional biopsies. The expected core needle length should be greater than 5 mm. A punch biopsy is acceptable for cutaneous lesions. Fine needle aspirate biopsies are not accepted. At least two core biopsies are taken at each timepoint, but collection of additional cores is strongly encouraged if deemed clinically safe by the investigator. An assessment of biopsy quality by a pathologist is strongly encouraged at the time of the procedure. All biopsies collected must have a detailed pathology report submitted with the specimen.

Tumor biopsy specimens are obtained from consenting subjects prior to and during treatment with BMS-986016 and BMS-936558 to characterize immune cell populations and expression of selected tumor markers. Biopsy samples are used for the following assessments:

- *Characterization of TILs and tumor antigens.* Immunohistochemistry is used to assess the number and composition of immune infiltrates to define the immune cell subsets present within FFPE tumor tissue before and after exposure to BMS-986016 and nivolumab. These IHC analyses include, but are not necessarily limited to, the following markers: CD4, CD8, LAG-3, MHC II, PD-1, PD-L1, and PD-L2. Correlations between gene expression and IHC expression are made between assays performed if deemed to be informative.
- *Laser capture microdissection.* Isolation of tumor and/or TIL on FFPE sections is performed by laser capture microdissection (LCM) for high-throughput profiling of molecular events within the tumor microenvironment.
- *Characterization of T cell repertoire.* DNA sequencing is performed on pre- and post-treatment FFPE tumor tissue to assess the composition of the T cell repertoire. Low T cell receptor diversity may be a poor prognostic factor of overall survival in metastatic breast cancer patients. Currently, there is a poor understanding of T cell receptor diversity as a predictor factor of response to immunotherapy, given that the major mechanism of BMS-936558 and BMS-986016 is hypothesized to be the functional restoration of T cell antitumor immunity. Therefore, a characterization of the diversity of the T cell compartment in the periphery, and within the tumor, at baseline and on-treatment is performed by T cell receptor next-generation DNA sequencing. T cell repertoire analysis is also performed from DNA isolated from peripheral blood to compare the status of tumor and peripheral T cell repertoire pre and post treatment.
- *Gene expression profiling.* Tumor biopsies that are collected in RNAlater or similar reagent are examined for mRNA gene expression by Affymetrix gene array technology and/or quantitative real-time polymerase chain reaction (qPCR) to detect expression of selected immune-related genes.
- *In situ cytokine and negative regulator expression.* Tumors biopsies are quantitatively evaluated for RNA, including CD3, IFN-γ, LAG-3, and PD-1.

Subjects whose screening biopsy yields inadequate tissue quantity or quality are allowed to continue in the study. If on-treatment biopsy is not successful, subjects also continue on study. Such subjects are replaced in order to obtain 48 subjects with

adequate paired tumor biopsies. If subjects have a response to treatment, on-treatment biopsies might not be possible. In this case, subjects also continue on study.

The tumor tissue that is obtained from these biopsies is divided equally into FFPE and frozen samples, which can be used for histologic confirmation of melanoma, as well as for the assays listed above.

Biopsies are done with local anesthesia or conscious sedation. Institutional guidelines for the safe performance of biopsies are followed. Excisional biopsies are performed to obtain tumor biopsy samples. Invasive procedures that require general anesthesia are not performed to obtain a biopsy specimen. However, if a surgical procedure is performed for a clinical indication, excess tumor tissue is used for research purposes with the consent of the subject.

14. Immunogenicity Assessments

Serum samples collected at timepoints are analyzed by a validated immunogenicity assay. Selected serum samples are analyzed by an exploratory orthogonal method that measures anti-BMS-986016 or anti-BMS-936558. Potential results generated from any orthogonal method are intended as informational for technology exploration purposes and re not reported.

In addition, ad hoc serum samples designated for pharmacokinetic or biomarker assessments are used for immunogenicity analysis if required (*e.g.*, insufficient volume for complete immunogenicity assessment or to follow up on suspected immunogenicity related adverse event).

15. Adverse Events

An adverse event (AE) is defined as any new untoward medical occurrence or worsening of a preexisting medical condition in a clinical investigation subject administered an investigational (medicinal) product and that does not necessarily have a causal relationship with this treatment. An AE is therefore any unfavorable and unintended sign (such as an abnormal laboratory finding), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

The causal relationship to study drug is determined by a physician and used to assess all adverse events (AE). The casual relationship can be one of the following:

Related: There is a reasonable causal relationship between study drug administration and the AE.

Not related: There is not a reasonable causal relationship between study drug administration and the AE.

The term "reasonable causal relationship" means there is evidence to suggest a causal relationship.

Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or causes prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (*e.g.*, medical, surgical) to prevent one of the other serious outcomes listed in the definition above). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.) Potential drug induced liver injury (DILI) is also considered an important medical event.

Suspected transmission of an infectious agent (*e.g.*, pathogenic or nonpathogenic) via the study drug is an SAE. Although pregnancy, overdose, cancer, and potential drug induced liver injury (DILI) are not always serious by regulatory definition, these events must be handled as SAEs. Any component of a study endpoint that is considered related to study therapy (*e.g.*, death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported) is reported as SAE.

The following hospitalizations are not considered SAEs:

- a visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- elective surgery, planned prior to signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (*e.g.*, routine colonoscopy)
- medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases
- admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (*e.g.*, lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).

Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, are collected, including those thought to be associated with protocol-specified procedures. All SAEs are collected that occur during the screening period and within 135 days of discontinuation of dosing. If applicable, SAEs are collected that relate to any later protocol-specified procedure (*e.g.*, a follow-up skin biopsy). All SAEs are followed to resolution or stabilization.

Nonserious Adverse Events

A nonserious adverse event is an AE not classified as serious. The collection of nonserious AE information begins at initiation of study drug and continues for 135 days after discontinuation of dosing. Nonserious AEs are followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for nonserious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate. All identified nonserious AEs are recorded and described on the nonserious AE page of the CRF (paper or electronic).

Completion of supplemental CRFs are requested for AEs and/or laboratory abnormalities that are reported/identified during the course of the study.

16. Statistical Considerations

Sample Size Determination

Dose Escalation (Parts A and B): Sample size at each dose depends on observed toxicity and cannot be precisely determined. Part A and Part B have 3 to 9 subjects in each cohort.

Cohort Expansion (Part C): Cohort expansion allows for better estimation of the toxicity rate and provide better precision around preliminary estimates of efficacy. If ≤ 5 of 16 subjects (*i.e.*, ~30% of in a cohort) experience a toxicity, there is at least 90% confidence that the true toxicity rate is not greater than 50.4% (based on Clopper-Pearson exact binomial 1-sided 90% confidence interval). A sample size of 16 subjects per cohort also allows for estimation of the proportion of subjects with objective response (*i.e.*, CR + PR) within a cohort such that the maximum distance between the estimated rate and either limit of the exact 2-sided 95% Clopper-Pearson confidence interval is 27.4%.

Populations for Analyses

- *All Enrolled Subjects Analysis Set:* This analysis set contains all subjects (including screen failures) who signed an informed consent for the study.
- *All Treated Subjects-Analysis set:* This analysis set includes all subjects who receive either drug.
- *Response-Evaluable Subjects:* This analysis set includes all subjects who receive either study drug, have a baseline tumor assessment with measurable disease, and one of the following: (1) at least one evaluable on-treatment tumor assessment, (2) clinical progression, or (3) death prior to the first on-treatment tumor evaluation.
- *BMS-986016 Pharmacokinetic Analysis Set:* This analysis set includes all subjects who receive BMS-986016 and have at least one valid PK parameter to be included in statistical analyses of BMS-986016 PK data.
- *BMS-986016 Immunogenicity Analysis Set:* This analysis set includes all subjects who receive BMS-986016 and have at least one BMS-986016 immunogenicity sample available.

- *BMS-936558 Immunogenicity Analysis Set:* This analysis set includes all subjects who receive BMS-936558 and have at least one BMS-936558 immunogenicity sample available.
- *Pharmacodynamic Analysis Set:* This analysis set includes all treated subjects for whom pharmacodynamic measurements are available at baseline and at least one other timepoint.

Endpoints

The primary endpoint of this Phase 1 study is safety as measured by the rate of AEs, serious adverse events (SAEs), deaths, and laboratory abnormalities (*e.g.*, Grade 3 or higher per CTCAE v 4), assessed during treatment and for up to 135 days of follow-up. All subjects who receive at least one dose of BMS-986016 or BMS-936558 are analyzed for safety.

The PK of BMS-986016 administered both alone and in combination with BMS-936558 is assessed as a secondary objective using the following endpoints derived from serum concentration versus time data in Cycle 1 and Cycle 3:

Cmax	Maximum observed serum concentration
Tmax	Time of maximum observed serum concentration
Ctrough	Trough observed serum concentration
Ctau	Concentration at the end of a dosing interval (*e.g.*, concentration at 336 hours)
Css,avg	Average concentration over a dosing interval ([AUC(TAU)/tau]
AUC(TAU)	Area under the concentration-time curve in one dosing interval
CLT	Total body clearance
Vss	Volume of distribution at steady state
T-HALFeff AUC	Effective elimination half-life that explains the degree of AUC accumulation observed
T-HALFeff Cmax	Effective elimination half-life that explains the degree of Cmax accumulation observed
AI_AUC	Accumulation index; ration of AUC(TAU) at steady state to AUC(TAU) after the first dose
AI_Cmax	Cmax accumulation index; ratio of Cmax at steady state to Cmax after the first dose
AI_Ctau	Ctau accumulation index; ratio of Ctau at steady state to Ctau after the first dose
DF	Degree of fluctuation or fluctuation index ([Cmax - Ctau]/Css,avg)

Individual subject PK parameter values are derived by noncompartmental methods by a validated PK analysis program. Actual times are used for the analyses.

Efficacy

Efficacy is assessed as a secondary objective using the endpoints described below for irRECIST and RECIST v1.1. For the purposes of patient management, clinical decision making is based on RECIST. Statistical analysis and reporting are based on both criteria.

- *Best overall response (BOR)* is the best response designation recorded from the start of the study treatment until the last protocol specified tumor assessment (*e.g.*, 30 day follow-up visit) taking into account any requirement for confirmation, based on RECIST v1.1 or irRECIST criteria. CR or PR determinations included in the BOR assessment are confirmed by a consecutive second (confirmatory) evaluation meeting the criteria for response and performed at least 4 weeks after the criteria for response are first met.
- *Objective response rate (ORR)* is defined as the total number of subjects whose BOR is either CR or PR divided by the total number of subjects in the population of interest.
- *Duration of response (DOR)* computed only for subjects with a BOR of CR or PR is defined as the number of days between the date of first response and the subsequent date of objectively documented disease progression based on the criteria (RECIST v1.1 or irRECIST) or death, whichever occurs first. For those subjects who remain alive and have not progressed or received subsequent therapy, duration of response is censored on the date of last protocol specified tumor assessment. Subjects who receive subsequent therapy are censored at the start of subsequent therapy.
- *Progression-free survival (PFS)* is defined as the probability of a subject remaining progression-free and surviving. The probability is computed based on the number of days between the first dose of study drug and progressive disease (as defined by RECIST or irRECIST) or death. For those subjects who remain alive and have not progressed, PFS is censored on the date of the last protocol specified tumor assessment.

These endpoints are determined based on tumor measurements occurring every 8 weffeks during the Treatment Period (up to twelve 8-week cycles), and once during the Clinical Follow-up period (30 days), for a total of ~ 1.9 years.

Immunogenicity

At the sample level, individual samples are characterized as ADA-positive or ADA-negative. A subject is considered to have a positive sample at baseline if the last sample prior to the initiation of treatment is ADA-positive. For example, a post-baseline sample from a subject who is ADA-negative at baseline is considered ADA-positive if ADA is detected. A post-baseline sample from a subject who is ADA-positive at baseline is considered ADA-positive if there is a relevant increase in titer (magnitude of the increase in titer considered relevant may vary by drug and assay, and is delineated in the statistical analysis plan). At the subject level, relevant ADA endpoints may include:

- Proportion of subjects with an ADA-positive sample at baseline
- Proportion of ADA-positive subjects (on treatment and overall)
- Proportion of subjects who are persistently positive (*e.g.*, 2 or more sequential ADA-positive samples with an adequate elapse of time in-between)
- Proportion of subjects who have neutralizing antibodies detected in one or more samples

Centrally Read ECGs (Parts A and B)

In Part A and Part B, QTc is assessed by a central reader at follow-up visit 1, and on day 1 of Cycle 1 and Cycle 3 (pre-dose and 4 hour post-dose time points). These assessments are used to address the secondary objective of assessing the effect of BMS-986016 administered alone and in combination with BMS-936558 on QTc. ECGs assessed locally by the investigator are also collected at the start of each cycle.

Biomarker Endpoints

Biomarker endpoints from peripheral blood are generally measured at multiple timepoints, and evaluated as both predictive and pharmacodynamic markers in the context of the exploratory biomarker objectives. These may include measures such as

levels and change from baseline in levels of the following at each scheduled timepoint:

- Serum soluble factors
- The proportion of specific lymphocyte subsets/expression levels of T cell co-stimulatory markers assessed using flow cytometry
- Expression of genes encoding BMS-986016–stimulated effector functions (perforin, granzyme B, and IFN-γ) and genes encoding T cell co-stimulatory receptors (PD-1, PD-L1, and CTLA-4).
- Percent of subjects expressing single nucleotide polymorphisms linked to PD-1 genes (per SNP)
- Measures of the quantity and diversity of antibodies observed to tumor-associated antigens (Part C only)

Biomarker endpoints from tumor biopsies are explored predominantly in an effort to identify baseline markers predictive of efficacy, since they are only measured at baseline for most subjects. For the subset of subjects who have both pre-treatment and on-treatment biopsies, pharmacodynamic associations are explored. Endpoints may include measures, such as pre-treatment levels and change in levels observed on-treatment of:

- Functional status of lymphocytes measured as the percent of CD8+ T-cells positive for IFN-γ and granzyme B expression, and the geometric mean intensity (log-scale) of CD8+ cells that are positive for IFN-γ and granzyme B expression (via ex vivo functional assay)
- Expression of genes encoding BMS-986016-stimulated effector functions (perforin, granzyme B, and IFN-γ) and genes encoding T cell co-stimulatory receptors (PD-1, PD-L1, and CTLA-4)
- IHC assessment of the presence/absence and intensity (measured using a discrete scale: such as 0, 1, 2, 3, 4) of expression of LAG-3, MHC class II, PD-1, PD-L1, and PD-L2.

Appropriate functional transformation of these exploratory measures are applied as necessary.

Pharmacokinetics

BMS-936558 concentration-time data at scheduled trough (Ctrough) and end-of-infusion timepoints is evaluated as an exploratory endpoint. Measurements are collected on treatment (up to 12 cycles) and for up to 135 days during the post-treatment follow-up.

PK parameters for BMS-986016 are calculated using noncompartmental analyses. Summary statistics are tabulated for the PK parameters of BMS-986016 by dose and study day/cycle. To describe the association of these parameters with dose of BMS-986016, scatter plots of Cmax and AUC(TAU) versus dose are provided for each day/cycle measured. Dose proportionality of BMS-986016 when administered alone or co-administered with BMS-936558 is also assessed based on a power model. Trough concentrations of BMS-986016 re plotted versus study day and cycle. BMS-936558 end-of-infusion and trough (Ctrough) concentrations are tabulated using summary statistics.

SEQUENCE SUMMARY

SEQ ID NO:	SEQUENCE
1	Heavy Chain Amino Acid Sequence Anti-LAG-3 mAb (BMS-986016) (variable region underlined; constant region bold) QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYWNWIRQPPGKGLEWIGE INHRGSTNSNPSLKSRVTLSLDTSKNQFSLKLRSVTAADTAVYYCAFGYS DYEYNWFDPWGQGTLVTVSS**ASTKGPSVFPLAPCSRSTSESTAALGCLVK** **DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT** **YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDT** **LMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY** **RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT** **LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS** **DGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK***
2	Light Chain Amino Acid Sequence Anti-LAG-3 mAb (BMS-986016) (variable region underlined; constant region bold) EIVLTQSPATLSLSPGERATLSCRASQSISSYLAWYQQKPGQAPRLLIYD ASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPLTFGQ GTNLEIK**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV** **DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG** **LSSPVTKSFNRGEC***
3	Heavy Chain Variable Region (VH) Amino Acid Sequence Anti-LAG-3 mAb (BMS-986016) QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYWNWIRQPPGKGLEWI GEINHRGSTNSNPSLKSRVTLSLDTSKNQFSLKLRSVTAADTAVYYCAFG YSDYEYNWFDPWGQGTLVTVSS
4	Heavy Chain Variable Region (VH) Nucleotide Sequence Anti-LAG-3 mAb (BMS-986016) caggtgcagctacagcagtggggcgcaggactgttgaagccttcggagaccct gtccctcacctgcgctgtctatggtgggtccttcagtgattactactggaact ggatccgccagcccccagggaaggggctggagtggattggggaaatcaatcat cgtggaagcaccaactccaacccgtccctcaagagtcgagtcaccctatcact agacacgtccaagaaccagttctccctgaagctgaggtctgtgaccgccgcgg acacggctgtgtattactgtgcgtttggatatagtgactacgagtacaactgg ttcgacccctggggccagggaaccctggtcaccgtctcctca
5	Light Chain Variable Region (VL) Amino Acid Sequence Anti-LAG-3 mAb (BMS-986016) EIVLTQSPATLSLSPGERATLSCRASQSISSYLAWYQQKPGQAPRLLIYD

SEQ ID NO:	SEQUENCE
	ASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPLTFGQ GTNLEIK
6	Light Chain Variable Region (VL) Nucleotide Sequence Anti-LAG-3 mAb (BMS-986016) gaaattgtgttgacacagtctccagccaccctgtctttgtctccaggggaaag agccaccctctcctgcagggccagtcagagtattagcagctacttagcctggt accaacagaaacctggccaggctcccaggctcctcatctatgatgcatccaac agggccactggcatcccagccaggttcagtggcagtgggtctgggacagactt cactctcaccatcagcagcctagagcctgaagattttgcagtttattactgtc agcagcgtagcaactggcctctcacttttggccaggggaccaacctggagatc aaa
7	Heavy Chain CDR1 Amino Acid Sequence Anti-LAG-3 mAb (BMS-986016) DYYWN
8	Heavy Chain CDR2 Amino Acid Sequence Anti-LAG-3 mAb (BMS-986016) EINHRGSTNSNPSLKS
9	Heavy Chain CDR3 Amino Acid Sequence Anti-LAG-3 mAb (BMS-986016) GYSDYEYNWFDP
10	Light Chain CDR1 Amino Acid Sequence Anti-LAG-3 mAb (BMS-986016) RASQSISSYLA
11	Light Chain CDR2 Amino Acid Sequence Anti-LAG-3 mAb (BMS-986016) DASNRAT
12	Light Chain CDR3 Amino Acid Sequence Anti-LAG-3 mAb (BMS-986016) QQRSNWPLT
13	Human LAG-3 Amino Acid Sequence

SEQ ID NO:	SEQUENCE
	MWEAQFLGLLFLQPLWVAPVKPLQPGAEVPVVWAQEGAPAQLPCSPTIPLQD LSLLRRAGVTWQHQPDSGPPAAAPGHPLAPGPHPAAPSSWGPRPRRYTVLSVG PGGLRSGRLPLQPRVQLDERGRQRGDFSLWLRPARRADAGEYRAAVHLRDR ALSCRLRLRLGQASMTASPPGSLRASDWVILNCSFSRPDRPASVHWFRNRGQ GRVPVRESPHHHLAESFLFLPQVSPMDSGPWGCILTYRDGFNVSIMYNLTVLG LEPPTPLTVYAGAGSRVGLPCRLPAGVGTRSFLTAKWTPPGGGPDLLVTGDN GDFTLRLEDVSQAQAGTYTCHIHLQEQQLNATVTLAIITVTPKSFGSPGSLGKL LCEVTPVSGQERFVWSSLDTPSQRSFSGPWLEAQEAQLLSQPWQCQLYQGERL LGAAVYFTELSSPGAQRSGRAPGALPAGHLLLFLTLGVLSLLLLVTGAFGFHLW RRQWRPRRFSALEQGIHPPQAQSKIEELEQEPEPEPEPEPEPEPEPEPEQL*
14	LAG-3 Epitope PGHPLAPG
15	LAG-3 Epitope HPAAPSSW
16	LAG-3 Epitope PAAPSSWG
17	Heavy Chain Amino Acid Sequence Anti-PD-1 mAb (BMS936558; 5C4 in WO 2006/121168) (variable region underlined; constant region bold) QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGL EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDT AVYYCATNDDYWGQGTLVTVSS**ASTKGPSVFPLAPCSRSTSESTAA** **LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT** **VPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLG** **GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGV** **EVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKG** **LPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPS** **DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNV** **FSCSVMHEALHNHYTQKSLSLSLGK**
18	Light Chain Amino Acid Sequence Anti-PD-1 mAb (BMS936558; 5C4 in WO 2006/121168) (variable region underlined; constant region bold) EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLI YDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQSSNWPR TFGQGTKVEIK**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA** **KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK** **VYACEVTHQGLSSPVTKSFNRGEC**
19	Heavy Chain Variable Region (VH) Amino Acid Sequence

SEQ ID NO:	SEQUENCE
	Anti-PD-1 mAb (BMS936558; 5C4 in WO 2006/121168) (SEQ ID NO:4 from WO 2006/121168) QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAV IWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCATND DYWGQGTLVTVSS
20	Heavy Chain Variable Region (VH) Nucleotide Sequence Anti-PD-1 mAb (BMS936558; 5C4 in WO 2006/121168) (SEQ ID NO:60 from WO 2006/121168) cag gtg cag ctg gtg gag tct ggg gga ggc gtg gtc cag cct ggg agg tcc ctg aga ctc gac tgt aaa gcg tct gga atc acc ttc agt aac tct ggc atg cac tgg gtc cgc cag gct cca ggc aag ggg ctg gag tgg gtg gca gtt att tgg tat gat gga agt aaa aga tac tat gca gac tcc gtg aag ggc cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg ttt ctg caa atg aac agc ctg aga gcc gag gac acg gct gtg tat tac tgt gcg aca aac gac gac tac tgg ggc cag gga acc ctg gtc acc gtc tcc tca
21	Light Chain Variable Region (VL) Amino Acid Sequence Anti-PD-1 mAb (BMS936558; 5C4 in WO 2006/121168) (SEQ ID NO:11 from WO 2006/121168) EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYD ASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQSSNWPRTFGQ GTKVEIK
22	Light Chain Variable Region (VL) Nucleotide Sequence Anti-PD-1 mAb (BMS936558; 5C4 in WO 2006/121168) (SEQ ID NO:67 from WO 2006/121168) gaa att gtg ttg aca cag tct cca gcc acc ctg tct ttg tct cca ggg gaa aga gcc acc ctc tcc tgc agg gcc agt cag agt gtt agt agt tac tta gcc tgg tac caa cag aaa cct ggc cag gct ccc agg ctc ctc atc tat gat gca tcc aac agg gcc act ggc atc cca gcc agg ttc agt ggc agt ggg tct ggg aca gac ttc act ctc acc atc agc agc cta gag cct gaa gat ttt gca gtt tat tac tgt cag cag agt agc aac tgg cct cgg acg ttc ggc caa ggg acc aag gtg gaa atc aaa
23	Heavy Chain CDR1 Amino Acid Sequence Anti-PD-1 mAb (BMS936558; 5C4 in WO 2006/121168) (SEQ ID NO:18 from WO 2006/121168) NSGMH
24	Heavy Chain CDR2 Amino Acid Sequence

SEQ ID NO:	SEQUENCE
	Anti-PD-1 mAb (BMS936558; 5C4 in WO 2006/121168) (SEQ ID NO:25 from WO 2006/121168) VIWYDGSKRYYADSVKG
25	Heavy Chain CDR3 Amino Acid Sequence Anti-PD-1 mAb (BMS936558; 5C4 in WO 2006/121168) (SEQ ID NO:32 from WO 2006/121168) NDDY
26	Light Chain CDR1 Amino Acid Sequence Anti-PD-1 mAb (BMS936558; 5C4 in WO 2006/121168) (SEQ ID NO:39 from WO 2006/121168) RASQSVSSYLA
27	Light Chain CDR2 Amino Acid Sequence Anti-PD-1 mAb (BMS936558; 5C4 in WO 2006/121168) (SEQ ID NO:46 from WO 2006/121168) DASNRAT
28	Light Chain CDR3 Amino Acid Sequence Anti-PD-1 mAb (BMS936558; 5C4 in WO 2006/121168) (SEQ ID NO:53 from WO 2006/121168) QQSSNWPRT
29	Complete PD-1 sequence (GenBank Accession No.: U64863) agtttccctt ccgctcacct ccgcctgagc agtggagaag gcggcactct ggtggggctg ctccaggcat gcagatccca caggcgccct ggccagtcgt ctgggcggtg ctacaactgg gctggcggcc aggatggttc ttagactccc cagacaggcc ctggaacccc cccaccttct tcccagccct gctcgtggtg accgaagggg acaacgccac cttcacctgc agcttctcca acacatcgga gagcttcgtg ctaaactggt accgcatgag ccccagcaac cagacggaca agctggccgc cttccccgag gaccgcagcc agcccggcca ggactgccgc ttccgtgtca cacaactgcc caacgggcgt gacttccaca tgagcgtggt cagggcccgg cgcaatgaca gcggcaccta cctctgtggg gccatctccc tggcccccaa ggcgcagatc aaagagagcc tgcgggcaga gctcagggtg acagagagaa gggcagaagt gcccacagcc caccccagcc cctcacccag gccagccggc cagttccaaa ccctggtggt tggtgtcgtg ggcggcctgc tgggcagcct ggtgctgcta gtctgggtcc tggccgtcat ctgctcccgg gccgcacgag ggacaatagg agccaggcgc accggccagc ccctgaagga ggacccctca gccgtgcctg tgttctctgt ggactatggg gagctggatt tccagtggcg agagaagacc ccggagcccc ccgtgccctg tgtccctgag cagacggagt atgccaccat tgtctttcct agcggaatgg gcacctcatc

SEQ ID NO:	SEQUENCE
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	Heavy Chain Nucleotide Sequence Anti-LAG-3 mAb (BMS-986016) caggtgcagctacagcagtggggcgcaggactgttgaagccttcggagaccctgtccct cacctgcgctgtctatggtgggtccttcagtgattactactggaactggatccgccag cccccagggaaggggctggagtggattggggaaatcaatcatcgtggaagcaccaact ccaacccgtccctcaagagtcgagtcaccctatcactagacacgtccaagaaccagtt ctccctgaagctgaggtctgtgaccgccgcggacacggctgtgtattactgtgcgtttg gatatagtgactacgagtacaactggttcgacccctggggccagggaaccctggtcacc gtctcctcagctagcaccaagggcccatccgtcttccccctggcgccctgctccaggagc acctccgagagcacagccgccctgggctgcctggtcaaggactacttccccgaaccggtg acggtgtcgtggaactcaggcgccctgaccagcggcgtgcacaccttcccggctgtccta cagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggc acgaagacctacacctgcaacgtagatcacaagcccagcaacaccaaggtggacaagaga gttgagtccaaatatggtccccatgcccaccatgcccagcacctgagttcctgggggga ccatcagtcttcctgttcccccaaaacccaaggacactctcatgatctcccggacccct gaggtcacgtgcgtggtggtggacgtgagccaggaagaccccgaggtccagttcaactgg tacgtggatggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagttcaa cagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaacggcaa ggagtacaagtgcaaggtctccaacaaaggcctcccgtcctccatcgagaaaaccatctc caaagccaaagggcagccccgagagccacaggtgtacaccctgcccccatcccaggagga

SEQ ID NO:	SEQUENCE
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	Light Chain Nucleotide Sequence Anti-LAG-3 mAb (BMS-986016) gaaattgtgttgacacagtctccagccaccctgtctttgtctccaggggaaagagccacc ctctcctgcagggccagtcagagtattagcagctacttagcctggtaccaacagaaacct ggccaggctcccaggctcctcatctatgatgcatccaacagggccactggcatcccag ccaggttcagtggcagtgggtctgggacagacttcactctcaccatcagcagcctagagc ctgaagattttgcagtttattactgtcagcagcgtagcaactggcctctcacttttggcc aggggaccaacctggagatcaaacgtacggtggctgcaccatctgtcttcatcttcccgc catctgatgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttct atcccagagaggccaaagtacagtggaaggtggataacgccctccaatcgggtaactccc aggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctga cgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagg gcctgagctcgcccgtcacaaagagcttcaacaggggagagtgttag

CLAIMS

What is claimed is:

1. A method of treating a solid tumor in a human patient, the method comprising administering to the patient an effective amount of each of:

(a) an anti-LAG-3 antibody comprising CDR1, CDR2 and CDR3 domains of the heavy chain variable region having the sequence set forth in SEQ ID NO:3, and CDR1, CDR2 and CDR3 domains of the light chain variable region having the sequence set forth in SEQ ID NO:5,

(b) an anti-PD-1 antibody comprising CDR1, CDR2 and CDR3 domains of the heavy chain variable region having the sequence set forth in SEQ ID NO:19, and CDR1, CDR2 and CDR3 domains of the light chain variable region having the sequence set forth in SEQ ID NO:21,

wherein the method comprises at least one administration cycle, wherein the cycle is a period of eight weeks, wherein for each of the at least one cycles, four doses of the anti-LAG-3 antibody are administered at a dose of 3, 20, 80, or 240 mg and four doses of the anti-PD-1 antibody are administered at a dose of 80 or 240 mg.

2. The method of claim 1, wherein the anti-LAG-3 antibody and anti-PD-1 antibody are administered at the following doses:

(a) 3 mg anti-LAG-3 antibody and 80 mg of anti-PD-1 antibody;
(b) 3 mg anti-LAG-3 antibody and 240 mg of anti-PD-1 antibody;
(c) 20 mg anti-LAG-3 antibody and 240 mg of anti-PD-1 antibody;
(d) 80 mg anti-LAG-3 antibody and 240 mg of anti-PD-1 antibody; or
(e) 240 mg anti-LAG-3 antibody and 240 mg of anti-PD-1 antibody.

3. The method of claim 1, wherein the anti-PD-1 and anti-LAG-3 antibodies are formulated for intravenous administration.

4. The method of claim 1, wherein the anti-PD-1 and anti-LAG-3 antibodies are formulated together.

5. The method of claim 1, wherein the anti-PD-1 and anti-LAG-3 antibodies are formulated separately.

6. The method of claim 1, wherein the treatment consists of up to 12 cycles.

7. The method of claim 1, wherein anti-PD-1 antibody is administered on Days 1, 15, 29, and 43 of each cycle.

8. The method of claim 1, wherein anti-LAG-3 antibody is administered on Days 1, 15, 29, and 43 of each cycle.

9. The method of claim 1, wherein the anti-PD-1 antibody is administered prior to administration of the anti-LAG-3 antibody.

10. The method of claim 9, wherein the anti-LAG-3 antibody is administered within about 30 minutes prior to administration of the anti-PD-1 antibody.

11. The method of claim 1, wherein the anti-PD-1 antibody is administered after administration of the anti-LAG-3 antibody.

12. The method of claim 1, wherein the anti-PD-1 antibody is administered concurrently with the anti-LAG-3 antibody.

13. The method of claim 1, wherein the treatment produces at least one therapeutic effect chosen from a reduction in size of a tumor, reduction in number of metastasic lesions over time, complete response, partial response, and stable disease.

14. The method of claim 1, wherein the solid tumor is chosen from melanoma, non-small cell lung cancer (NSCLC), human papilloma virus (HPV)-related tumor, and gastric adenocarcinoma.

15. The method of claim 1, wherein the anti-LAG-3 antibody comprises

(a) a heavy chain variable region CDR1 comprising the sequence set forth in SEQ ID NO:7;

(b) a heavy chain variable region CDR2 comprising the sequence set forth in SEQ ID NO:8;

(c) a heavy chain variable region CDR3 comprising the sequence set forth in SEQ ID NO:9;

(d) a light chain variable region CDR1 comprising the sequence set forth in SEQ ID NO:10;

(e) a light chain variable region CDR2 comprising the sequence set forth in SEQ ID NO:11; and

(f) a light chain variable region CDR3 comprising the sequence set forth in SEQ ID NO:12.

16. The method of claim 1, wherein the anti-LAG-3 antibody comprises heavy and light chain variable regions comprising the sequences set forth in SEQ ID NOs:3 and 5, respectively.

17. The method of claim 1, wherein the anti-LAG-3 antibody comprises heavy and light chains comprising the sequences set forth in SEQ ID NOs:1 and 2, respectively.

18. The method of claim 1, wherein the anti-PD-1 antibody comprises

(a) a heavy chain variable region CDR1 comprising the sequence set forth in SEQ ID NO:23;

(b) a heavy chain variable region CDR2 comprising the sequence set forth in SEQ ID NO:24;

(c) a heavy chain variable region CDR3 comprising the sequence set forth in SEQ ID NO:25;

(d) a light chain variable region CDR1 comprising the sequence set forth in SEQ ID NO:26;

(e) a light chain variable region CDR2 comprising the sequence set forth in SEQ ID NO:27; and

(f) a light chain variable region CDR3 comprising the sequence set forth in SEQ ID NO:28.

19. The method of claim 1, wherein the anti-PD-1 antibody comprises heavy and light chain variable regions comprising the sequences set forth in SEQ ID NOs:19 and 21, respectively.

20. The method of claim 1, wherein the anti-PD-1 antibody comprises heavy and light chains comprising the sequences as set forth in SEQ ID NOs:17 and 18, respectively.

21. A kit for treating a solid tumor in a human patient, the kit comprising:

(a) a dose of an anti-LAG-3 antibody comprising CDR1, CDR2 and CDR3 domains of the heavy chain variable region having the sequence set forth in SEQ ID NO:3, and CDR1, CDR2 and CDR3 domains of the light chain variable region having the sequence set forth in SEQ ID NO:5;

(b) a dose of an anti-PD-1 antibody comprising CDR1, CDR2 and CDR3 domains of the heavy chain variable region having the sequence set forth in SEQ ID NO:19, and CDR1, CDR2 and CDR3 domains of the light chain variable region having the sequence set forth in SEQ ID NO:21; and

(c) instructions for using the anti-LAG-3 antibody and anti-PD-1 antibody in the method of claim 1.

22. An anti-LAG-3 antibody comprising CDR1, CDR2 and CDR3 domains of the heavy chain variable region having the sequence set forth in SEQ ID NO:3, and CDR1, CDR2 and CDR3 domains of the light chain variable region having the sequence set forth in SEQ ID NO:5, for co-administration with an anti-PD-1 antibody comprising CDR1, CDR2 and CDR3 domains of the heavy chain variable region having the sequence set forth in SEQ ID NO:19, and CDR1, CDR2 and CDR3 domains of the light chain variable region having the sequence set forth in SEQ ID NO:21, in at least one cycle, wherein for each cycle four doses of the anti-LAG-3 antibody are administered at a dose of 3, 20, 80, or 240 mg and four doses of the anti-PD-1 antibody are administered at a dose of 80 or 240 mg.

23. An anti-LAG-3 antibody comprising CDR1, CDR2 and CDR3 domains of the heavy chain variable region having the sequence set forth in SEQ ID NO:3, and CDR1, CDR2 and CDR3 domains of the light chain variable region having the sequence set forth in SEQ ID NO:5, for co-administration with an anti-PD-1 antibody comprising CDR1, CDR2 and CDR3 domains of the heavy chain variable region having the sequence set forth in SEQ ID NO:19, and CDR1, CDR2 and CDR3 domains of the light chain variable region having the sequence

set forth in SEQ ID NO:21, in at least one cycle, wherein for each cycle four doses of the anti-LAG-3 antibody are administered at a dose of 3, 20, 80, or 240 mg/kg and four doses of the anti-PD-1 antibody are administered at a dose of 80 or 240 mg/kg.

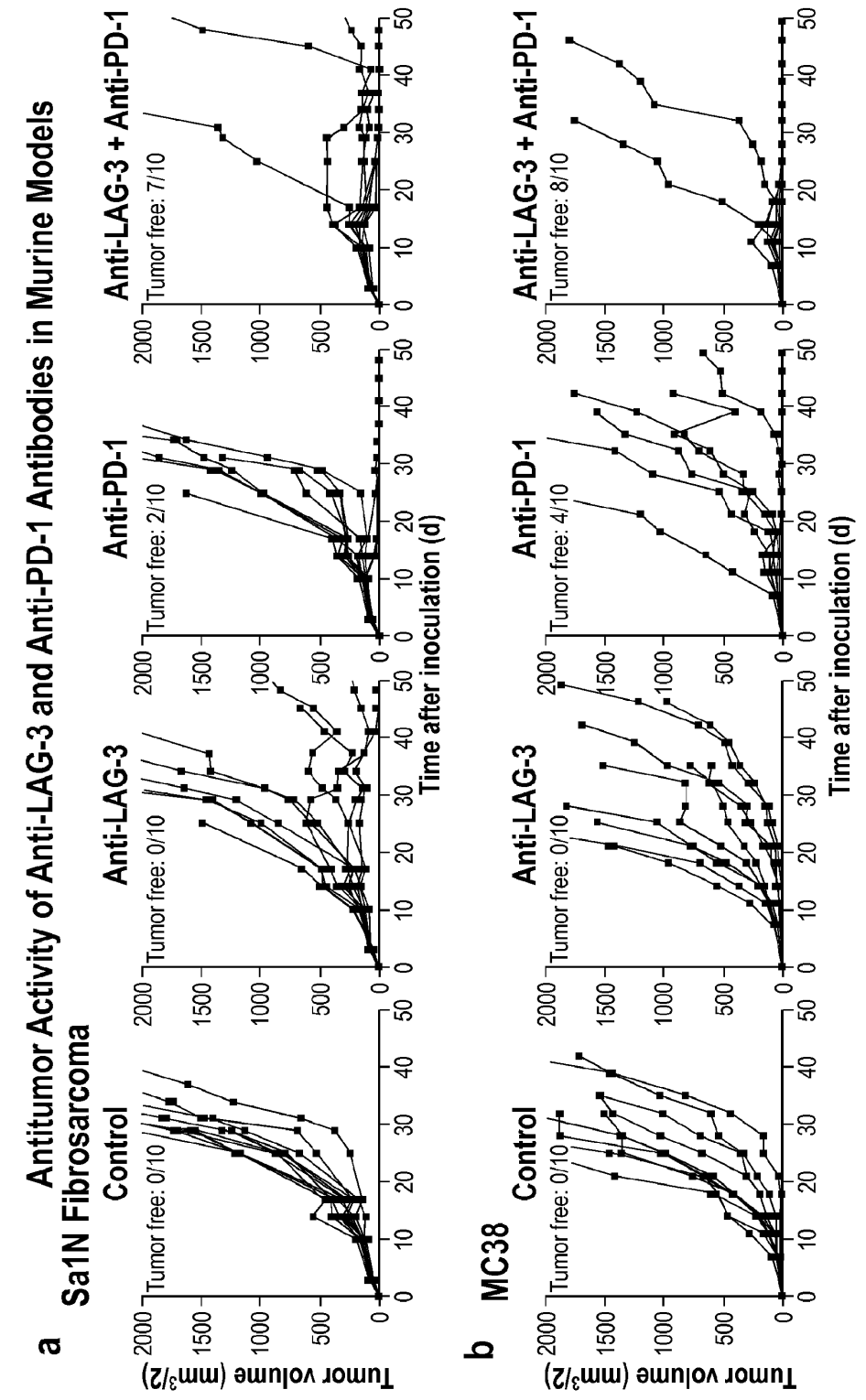
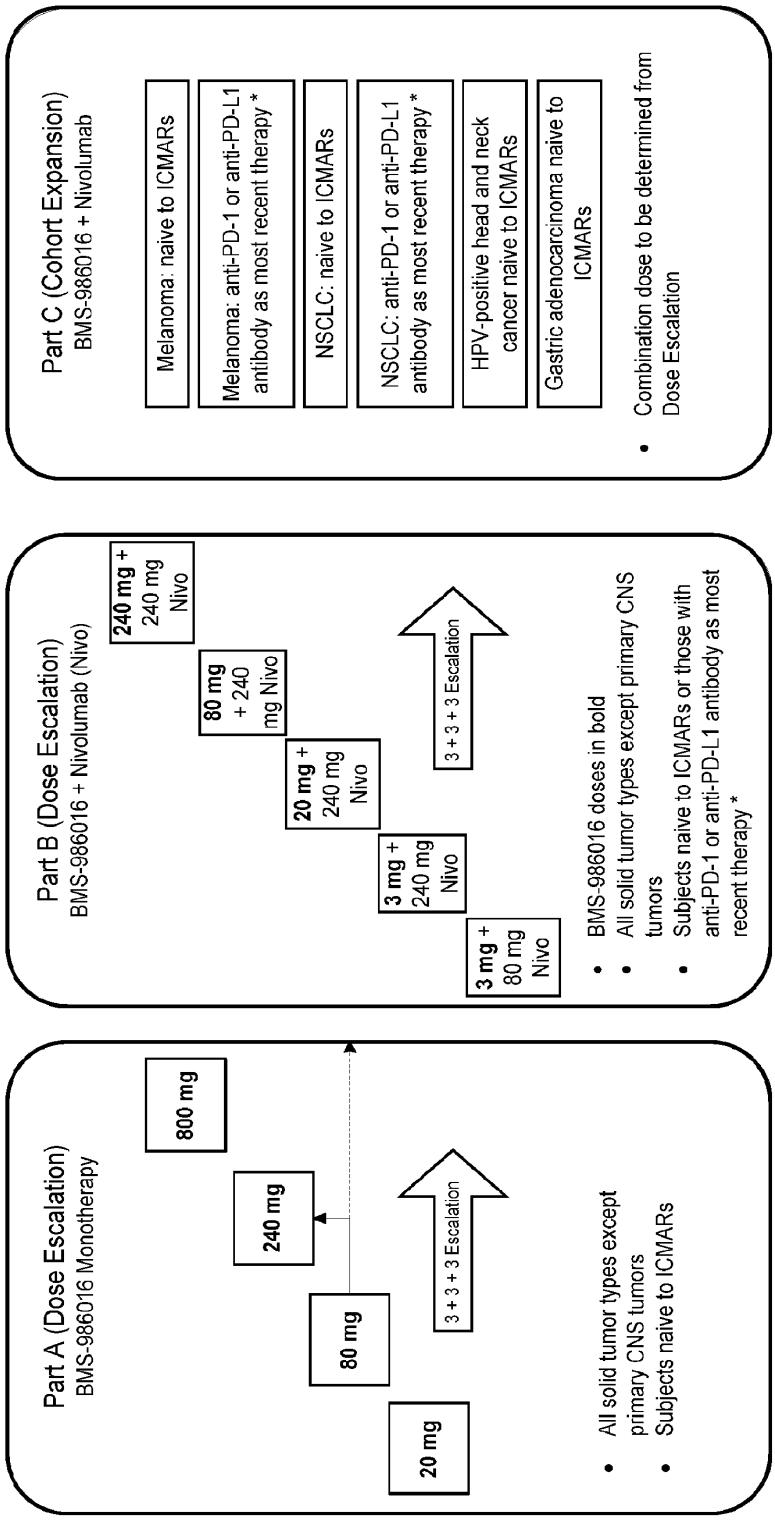

a
Antitumor Activity of Anti-LAG-3 and Anti-PD-1 Antibodies in Murine Models
Sa1N Fibrosarcoma
Control
Anti-LAG-3
Anti-PD-1
Anti-LAG-3 + Anti-PD-1
Tumor free: 0/10
Tumor free: 0/10
Tumor free: 2/10
Tumor free: 7/10
b
MC38
Control
Anti-LAG-3
Anti-PD-1
Anti-LAG-3 + Anti-PD-1
Tumor free: 0/10
Tumor free: 0/10
Tumor free: 4/10
Tumor free: 8/10
Tumor volume (mm3/2)
Time after inoculation (d)

Fig. 1



ICMARs = immune cell-modulating antibody regimens (such as, but not limited to, ipilimumab, tremelimumab, anti–PD-1, anti–PD-L1, anti–PD-L2, anti-KIR, anti-CD137, and/or anti-OX40 antibodies)

Fig. 2A

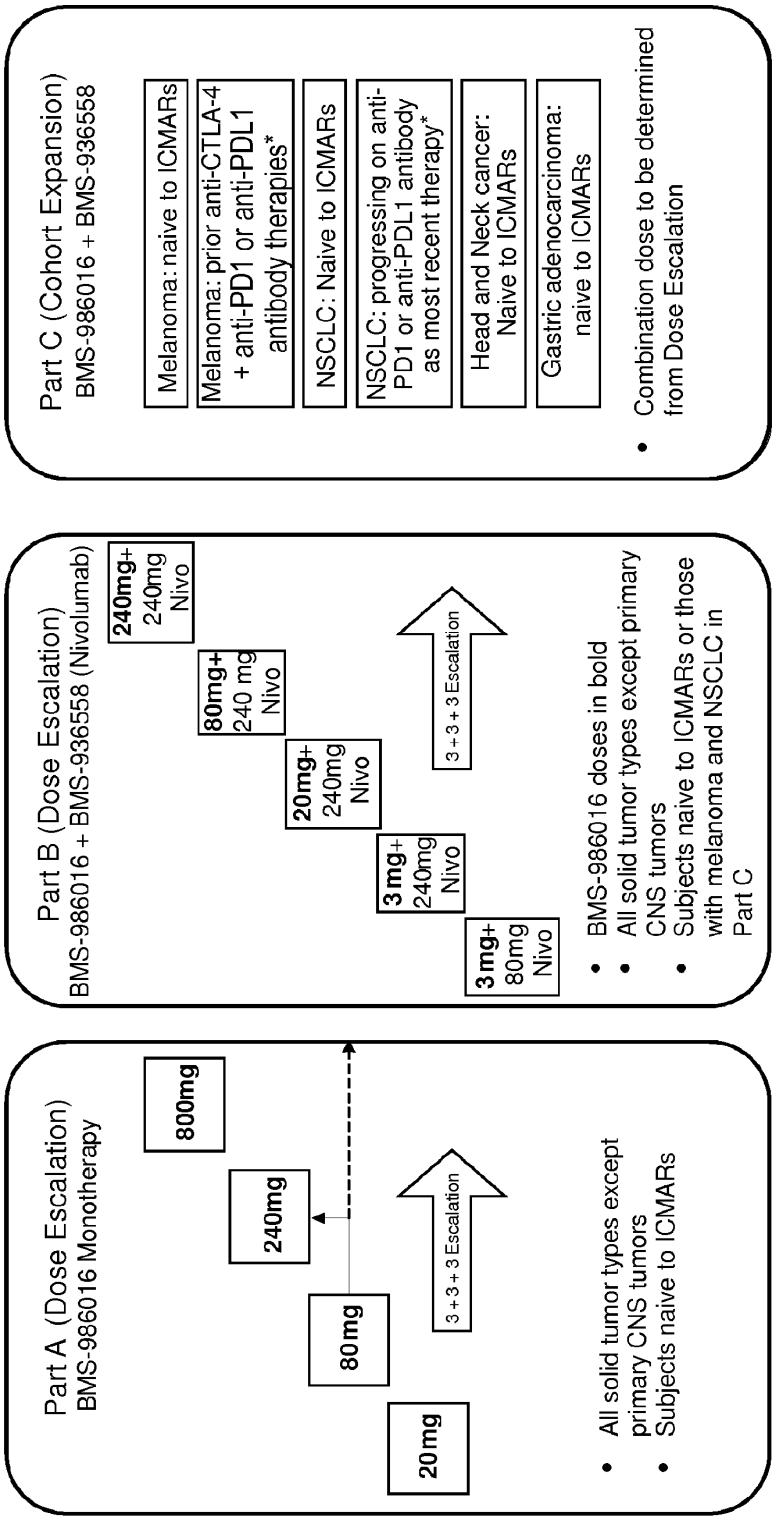
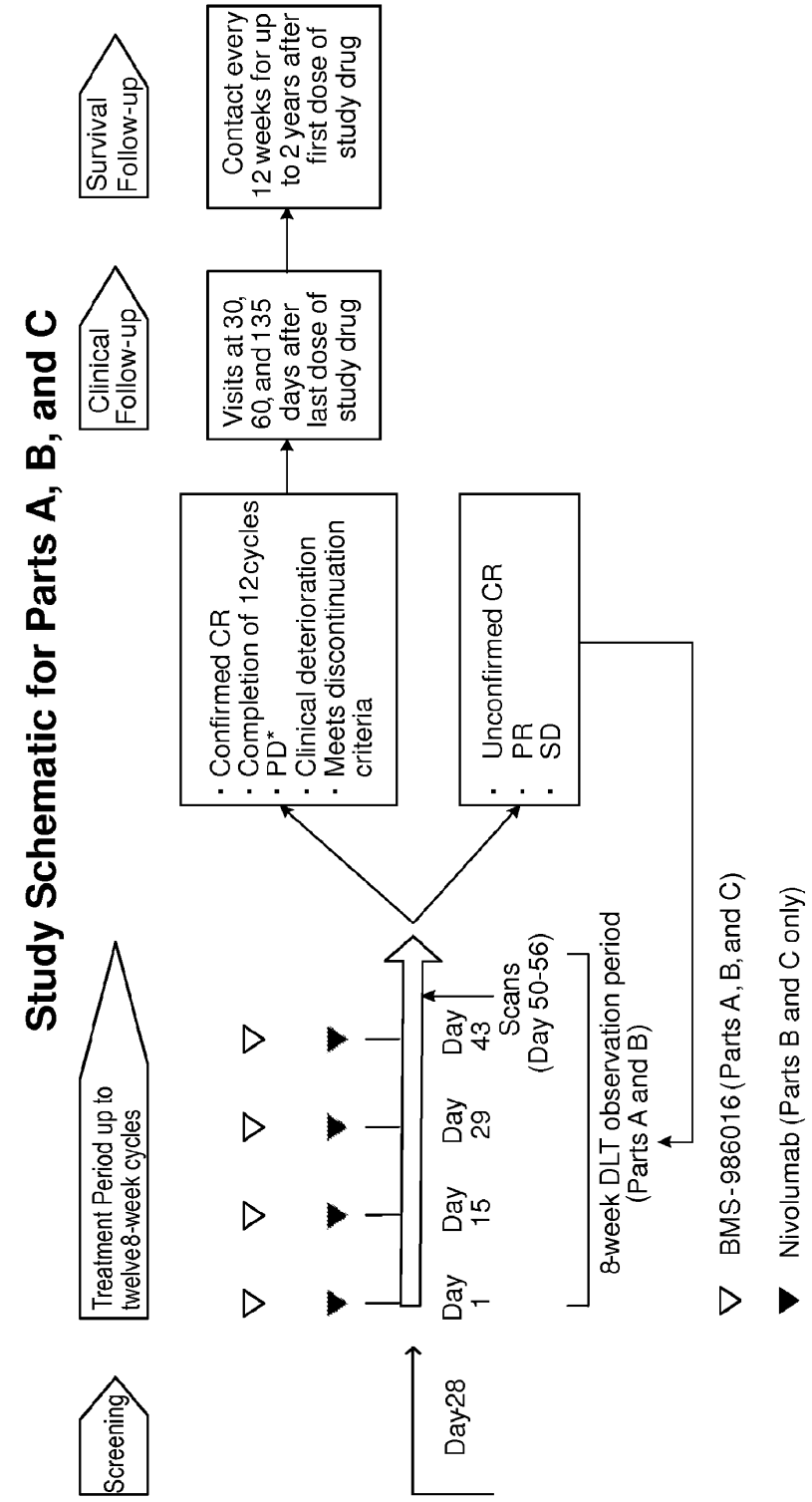

Study Schematic
Part A (Dose Escalation)
BMS-986016 Monotherapy
20 mg
80 mg
240mg
800mg
3 + 3 + 3 Escalation
All solid tumor types except primary CNS tumors
Subjects naive to ICMARs
Part B (Dose Escalation)
BMS-986016 + BMS-936558 (Nivolumab)
3 mg+ 80mg Nivo
3 mg+ 240mg Nivo
20mg+ 240mg Nivo
80mg+ 240 mg Nivo
240mg+ 240mg Nivo
3 + 3 + 3 Escalation
BMS-986016 doses in bold
All solid tumor types except primary CNS tumors
Subjects naive to ICMARs or those with melanoma and NSCLC in Part C
Part C (Cohort Expansion)
BMS-986016 + BMS-936558
Melanoma: naive to ICMARs
Melanoma: prior anti-CTLA-4 + anti-PD1 or anti-PDL1 antibody therapies*
NSCLC: Naive to ICMARs
NSCLC: progressing on anti-PD1 or anti-PDL1 antibody as most recent therapy*
Head and Neck cancer: Naive to ICMARs
Gastric adenocarcinoma: naive to ICMARs
Combination dose to be determined from Dose Escalation
ICMARs = immune cell-modulating antibody regimens (such as, but not limited to, ipilimumab, tremelimumab, anti–PD-1, anti–PD-L1, anti–PD-L2, anti-KIR, anti-CD137, and/or anti-OX40 antibodies)

Fig. 2B



Diagnostic imaging to be performed every 12 weeks until progression in subjects who discontinue due to CR and in subjects with PR at the end of Cycle 12.
For treatment visits where both BMS-986016 and nivolumab are administered, nivolumab will be administered first, followed by BMS-986016 within 30 minutes after completion of the nivolumab infusion.

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/056277

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61K39/395 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/019570 A2 (MEDAREX INC [US]; THUDIUM KENT B [US]; KORMAN ALAN J [US]; LEBLANC HEI) 18 February 2010 (2010-02-18)	1-15, 18-21
Y	sequences 37,43 example 6 pages 66,53 pages 40,41	16,17

X,P	WO 2014/008218 A1 (SQUIBB BRISTOL MYERS CO [US]) 9 January 2014 (2014-01-09)	22,23
Y,P	sequences 12,37	16,17
L	pages 58, 61	22,23

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 January 2015	04/02/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bumb, Peter

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/056277

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GODING STEPHEN R ET AL: "Combination of adoptive cell transfer, anti-PD-L1 and anti-LAG-3 antibodies for the treatment of recurrent tumors Better with more", ONCOIMMUNOLOGY, vol. 2, no. 8, August 2013 (2013-08), XP002734389, the whole document	1-21
A	TURNIS M E ET AL: "Combinatorial immunotherapy: PD-1 may not be LAG-ing behind any more", ONCOIMMUNOLOGY, vol. 1, no. 7, 2012, pages 1172-1174, XP002734390, LANDES BIOSCIENCE USA ISSN: 2162-4011 page 1172 - page 1173	1-21
A	S.-R. WOO ET AL: "Immune Inhibitory Molecules LAG-3 and PD-1 Synergistically Regulate T-cell Function to Promote Tumoral Immune Escape", CANCER RESEARCH, vol. 72, no. 4, 20 December 2011 (2011-12-20), pages 917-927, XP055151722, ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-11-1620 page 924, column center page 925, column center	1-21
T	Bristol-Myers Squibb: "View of NCT01968109 on 2014_06_20", ClinicalTrials.gov Website , 20 June 2014 (2014-06-20), XP002734391, Retrieved from the Internet: URL:https://clinicaltrials.gov/archive/NCT01968109/2014_06_20 [retrieved on 2015-01-13] the whole document	1-21
T	Bristol-Myers Squibb: "View of NCT02061761 on 2014_08_28", ClinicalTrials.gov Website , 28 August 2014 (2014-08-28), XP002734392, Retrieved from the Internet: URL:https://clinicaltrials.gov/archive/NCT02061761/2014_08_28 [retrieved on 2015-01-13] the whole document	1-21

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/056277

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	Agrawal, S., Fen Y., Kollia G., Saeger S., Ullmann., McDonald D., Gupta A. K., Roy A., Masson E.: "Clinical pharmacokinetics (PK) of BMS-936558, a fully human anti-PD-1 monoclonal antibody.", 2012 ASCO Annual Meeting Website , XP002734393, Retrieved from the Internet: URL:http://meetinglibrary.asco.org/content/98623-114 [retrieved on 2015-01-13] the whole document -----	1-21

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

Information on patent family members

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010019570 A2	18-02-2010	AR 072999 A1	06-10-2010
		AU 2009282134 A1	18-02-2010
		AU 2014221286 A1	02-10-2014
		CA 2734335 A1	18-02-2010
		CL 2013002062 A1	10-01-2014
		CN 102176921 A	07-09-2011
		CN 103923213 A	16-07-2014
		CO 6351751 A2	20-12-2011
		EA 201100340 A1	30-08-2011
		EP 2320940 A2	18-05-2011
		JP 5647981 B2	07-01-2015
		JP 2012500006 A	05-01-2012
		KR 20110050507 A	13-05-2011
		NZ 590991 A	30-11-2012
		NZ 602780 A	30-04-2014
		PE 03062011 A1	21-05-2011
		PE 16582014 A1	08-11-2014
		TW 201019958 A	01-06-2010
		US 2011150892 A1	23-06-2011
		WO 2010019570 A2	18-02-2010
WO 2014008218 A1	09-01-2014	TW 201406784 A	16-02-2014
		US 2014093511 A1	03-04-2014
		UY 34887 A	31-12-2013
		WO 2014008218 A1	09-01-2014

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(71) Applicant: **BRISTOL-MYERS SQUIBB COMPANY** [US/US]; Route 206 and Province Line Road, Princeton, New Jersey 08543 (US).

(72) Inventor: **YANG, Arvin**; Route 206 and Province Line Road, Princeton, New Jersey 08543 (US).

(74) Agents: **KIM, Ji-Eun** et al.; Sterne, Kessler, Goldstein & Fox P.L.L.C., 1100 New York Avenue, NW, Washington, District of Columbia 20005 (US).

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(54) **Title**: TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY

(57) **Abstract**: The invention provides a method of treating a melanoma comprising (i) identifying a patient having a PD-L1-negative melanoma and (ii) administering to the patient a combination of an anti-PD-1 antibody or an antigen-binding portion thereof and an anti-CTLA-4 antibody or an antigen-binding portion thereof. The methods of the invention can extend progression-free survival for over 8 months and/or reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration.

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TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY

[0001] Throughout this application, various publications are referenced in parentheses by author name and date, or by Patent No. or Patent Publication No. The disclosures of these publications are hereby incorporated in their entireties by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein. However, the citation of a reference herein should not be construed as an acknowledgement that such reference is prior art to the present invention.

FIELD OF THE INVENTION

[0002] This invention relates to a method of treating PD-L1-negative melanoma comprising administering a combination of an anti-PD-1 antibody and an anti-CTLA-4 antibody.

BACKGROUND OF THE INVENTION

[0003] Human cancers harbor numerous genetic and epigenetic alterations, generating neoantigens potentially recognizable by the immune system (Sjoblom *et al.* (2006) *Science* 314:268-74). The adaptive immune system, comprised of T and B lymphocytes, has powerful anti-cancer potential, with a broad capacity and exquisite specificity to respond to diverse tumor antigens. Further, the immune system demonstrates considerable plasticity and a memory component. The successful harnessing of all these attributes of the adaptive immune system would make immunotherapy unique among all cancer treatment modalities.

[0004] Recently, several immune checkpoint pathway inhibitors have begun to provide new immunotherapeutic approaches for treating cancer, including the development of an antibody (Ab), ipilimumab (YERVOY®), that binds to and inhibits Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) for the treatment of patients with advanced melanoma and the development of antibodies such as nivolumab and pembrolizumab (formerly lambrolizumab; USAN Council Statement, (2013) Pembrolizumab: Statement on a

nonproprietary name adopted by the USAN Council (ZZ-165), November 27, 2013) that bind specifically to the Programmed Death-1 (PD-1) receptor and block the inhibitory PD-1/PD-1 ligand pathway.

[0005] The promise of the emerging field of personalized medicine is that advances in pharmacogenomics will increasingly be used to tailor therapeutics to defined sub-populations, and ultimately, individual patients in order to enhance efficacy and minimize adverse effects. Recent successes include, for example, the development of imatinib mesylate (GLEEVEC®), a protein tyrosine kinase inhibitor that inhibits the bcr-abl tyrosine kinase, to treat Philadelphia chromosome-positive chronic myelogenous leukemia (CML); crizotinib (XALKORI®) to treat the 5% of patients with late-stage non-small cell lung cancers who express a mutant anaplastic lymphoma kinase (ALK) gene; and vemurafenib (ZELBORAF®), an inhibitor of mutated B-RAF protein (V600E-BRAF) which is expressed in around half of melanoma tumors. However, unlike the clinical development of small molecule agents that target discrete activating mutations found in select cancer populations, a particular challenge in cancer immunotherapy has been the identification of mechanism-based predictive biomarkers to enable patient selection and guide on-treatment management.

SUMMARY OF THE INVENTION

[0006] The present disclosure provides a method for treating a melanoma comprising (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4. The present disclosure also provides a method for treating a melanoma comprising administering to a patient afflicted with a melanoma tumor (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration.

[0007] The present disclosure further provides a method for extending a progression-free survival period for over 8 months in a patient afflicted with a melanoma tumor comprising administering to the patient (a) an anti-PD-1 antibody or an antigen-binding

portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the patient demonstrates progression-free survival for over 8 months. In certain embodiments, the progression-free survival of the patient is extended after the administration for over about 11 months, about 12 months, about 13 months, about 14 months, about 15 months, about 16 months, about 17 months, about 18 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, or about 10 years. In one particular embodiment, the progression-free survival of the patient is extended for over 11 months.

[0008] The present disclosure also provides a method for reducing a tumor size at least by 10% in a patient afflicted with a melanoma tumor comprising administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration.

[0009] The present disclosure also provides a method for increasing an objective response rate to be higher than 40% in a patient population, each of whom is afflicted with a melanoma tumor, in a cancer treatment comprising administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein each patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the objective response rate is higher than 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%.

[0010] The present disclosure also provides a method for selecting a patient suitable for an anti-PD-1 antibody and anti-CTLA-4 antibody combination therapy comprising (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) instructing a healthcare provider to administer to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4

antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

[0011] In certain embodiments, the methods disclosed herein further comprise identifying the patient as having a melanoma tumor that does not express PD-L1 prior to the administration. In some embodiments, the patient is characterized by (i) extended progression-free survival for over 8 months, (ii) tumor size reduction at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration, or (iii) both.

[0012] In some embodiments, the methods disclosed herein further comprise measuring a PD-L1 expression on the melanoma tumor. In certain embodiments, the measuring further comprises assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface. In one particular embodiment, the presence of PD-L1 is determined using an automated IHC assay.

[0013] The present disclosure further provides a kit for treating a patient afflicted with a melanoma tumor, the kit comprising (a) a dosage ranging from 0.1 to 10 mg/kg body weight of an anti-PD-1 antibody or an antigen-binding portion thereof; (b) a dosage ranging from 0.1 to 10 mg/kg body weight of an anti-CTLA-4 antibody or an antigen-binding portion thereof; and (c) instructions for using the anti-PD-1 antibody or the antigen-binding portion thereof and the anti-CTLA-4 antibody or the antigen-binding portion thereof in any of the methods disclosed herein.

BRIEF DESCRIPTION OF THE FIGURES

[0014] Figure 1 shows a patient flow diagram of the randomized, double-blind, multicenter, phase 3 trial.

[0015] Figures 2A-C show progression-free survival data in the intention-to-treat population (Figure 2A), in patients with positive PD-L1 status (Figure 2B), and Negative PD-L1 Status (Figure 2C). Each graph shows the progression free survival for patients treated with nivolumab alone (solid line), ipilimumab alone (dashed line), or the combination of nivolumab and ipilimumab (dotted line) in months (Figures 2A-C). The number at risk in months for each of nivolumab, nivolumab plus ipilimumab, and ipilimumab is shown below each x-axis (Figures 2A-C). PD-L1 expression status is based on verified PD-L1 assay data (Figures 2B-C).

[0016] Figures 3A-B show subgroup analyses of progression-free survival among patients treated with nivolumab alone compared to ipilimumab alone (Figure 3A) and patients treated with nivolumab plus ipilimumab compared to ipilimumab alone (Figure 3B).

[0017] Figures 4A-C show the tumor burden change in target lesions in patients treated with nivolumab alone (Figure 4A), nivolumab plus ipilimumab (Figure 4B), and ipilimumab alone (Figure 4C). In each graph, the y-axis shows the best reduction from baseline in target lesions (%) and the x-axis represents each patient (Figures 4A-C).

DETAILED DESCRIPTION OF THE INVENTION

[0018] The present invention relates to identifying an optimal strategy to treat a patient having a PD-L1-negative melanoma. The present invention shows that in a patient having a PD-L1-negative melanoma tumor, a combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody provides a better response (e.g., progression-free survival) than a monotherapy of either an anti-PD-1 antibody or an anti-CTLA-4 antibody. Furthermore, the combination therapy when given to a population of patients can increase the objective response rate compared to the monotherapy.

Definitions

[0019] In order that the present disclosure may be more readily understood, certain terms are first defined. As used in this application, except as otherwise expressly provided herein, each of the following terms shall have the meaning set forth below. Additional definitions are set forth throughout the application.

[0020] The term "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0021] It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0022] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0023] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. The headings provided herein are not limitations of the various aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0024] "Administering" refers to the physical introduction of a composition comprising a therapeutic agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. Routes of administration for the formulations disclosed herein include intravenous, intramuscular, subcutaneous, intraperitoneal, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as *in vivo* electroporation. In some embodiments, the formulation is administered via a non-parenteral route, in some embodiments, orally. Other non-parenteral routes include a topical, epidermal or mucosal route of administration, for example, intranasally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

[0025] An "adverse event" (AE) as used herein is any unfavorable and generally unintended or undesirable sign (including an abnormal laboratory finding), symptom, or disease associated with the use of a medical treatment. For example, an adverse event may be associated with activation of the immune system or expansion of immune system cells (*e.g.*, T cells) in response to a treatment. A medical treatment may have one or more associated AEs and each AE may have the same or different level of severity. Reference to methods capable of "altering adverse events" means a treatment regime that decreases the incidence and/or severity of one or more AEs associated with the use of a different treatment regime.

[0026] An "antibody" (Ab) shall include, without limitation, a glycoprotein immunoglobulin which binds specifically to an antigen and comprises at least two heavy (H) chains and two light (L) chains interconnected by disulfide bonds, or an antigen-binding portion thereof. Each H chain comprises a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region comprises three constant domains, C_{H1}, C_{H2} and C_{H3}. Each light chain comprises a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprises one constant domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L comprises three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system.

[0027] An immunoglobulin may derive from any of the commonly known isotypes, including but not limited to IgA, secretory IgA, IgG and IgM. IgG subclasses are also well known to those in the art and include but are not limited to human IgG1, IgG2, IgG3 and IgG4. "Isotype" refers to the antibody class or subclass (*e.g.*, IgM or IgG1) that is encoded by the heavy chain constant region genes. The term "antibody" includes, by way of example, both naturally occurring and non-naturally occurring antibodies; monoclonal

and polyclonal antibodies; chimeric and humanized antibodies; human or nonhuman antibodies; wholly synthetic antibodies; and single chain antibodies. A nonhuman antibody may be humanized by recombinant methods to reduce its immunogenicity in man. Where not expressly stated, and unless the context indicates otherwise, the term "antibody" also includes an antigen-binding fragment or an antigen-binding portion of any of the aforementioned immunoglobulins, and includes a monovalent and a divalent fragment or portion, and a single chain antibody.

[0028] The term "monoclonal antibody" ("mAb") refers to a non-naturally occurring preparation of antibody molecules of single molecular composition, *i.e.*, antibody molecules whose primary sequences are essentially identical, and which exhibits a single binding specificity and affinity for a particular epitope. A mAb is an example of an isolated antibody. MAbs may be produced by hybridoma, recombinant, transgenic or other techniques known to those skilled in the art.

[0029] A "human" antibody (HuMAb) refers to an antibody having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region is also derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody," as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. The terms "human" antibodies and "fully human" antibodies and are used synonymously.

[0030] A "humanized antibody" refers to an antibody in which some, most or all of the amino acids outside the CDR domains of a non-human antibody are replaced with corresponding amino acids derived from human immunoglobulins. In one embodiment of a humanized form of an antibody, some, most or all of the amino acids outside the CDR domains have been replaced with amino acids from human immunoglobulins, whereas some, most or all amino acids within one or more CDR regions are unchanged. Small additions, deletions, insertions, substitutions or modifications of amino acids are permissible as long as they do not abrogate the ability of the antibody to bind to a

particular antigen. A "humanized" antibody retains an antigenic specificity similar to that of the original antibody.

[0031] A "chimeric antibody" refers to an antibody in which the variable regions are derived from one species and the constant regions are derived from another species, such as an antibody in which the variable regions are derived from a mouse antibody and the constant regions are derived from a human antibody.

[0032] An "anti-antigen" antibody refers to an antibody that binds specifically to the antigen. For example, an anti-PD-1 antibody binds specifically to PD-1 and an anti-CTLA-4 antibody binds specifically to CTLA-4.

[0033] An "antigen-binding portion" of an antibody (also called an "antigen-binding fragment") refers to one or more fragments of an antibody that retain the ability to bind specifically to the antigen bound by the whole antibody.

[0034] A "cancer" refers a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth results in the formation of malignant tumors that invade neighboring tissues and may also metastasize to distant parts of the body through the lymphatic system or bloodstream. A "cancer" or "cancer tissue" can include a tumor.

[0035] "Cytotoxic T-Lymphocyte Antigen-4" (CTLA-4) refers to an immunoinhibitory receptor belonging to the CD28 family. CTLA-4 is expressed exclusively on T cells *in vivo*, and binds to two ligands, CD80 and CD86 (also called B7-1 and B7-2, respectively). The term "CTLA-4" as used herein includes human CTLA-4 (hCTLA-4), variants, isoforms, and species homologs of hCTLA-4, and analogs having at least one common epitope with hCTLA-4. The complete hCTLA-4 sequence can be found under GenBank Accession No. AAB59385.

[0036] The term “progression-free survival,” which can be abbreviated as PFS, as used herein refers to the length of time during and after the treatment of a solid tumor (*i.e.*, melanoma) that a patient lives with the disease but it does not get worse.

[0037] "Dosing interval," as used herein, means the amount of time that elapses between multiple doses of a formulation disclosed herein being administered to a subject. Dosing interval can thus be indicated as ranges.

[0038] The term "dosing frequency" as used herein refers to the frequency of administering doses of a formulation disclosed herein in a given time. Dosing frequency

can be indicated as the number of doses per a given time, *e.g.*, once a week or once in two weeks.

[0039] The use of the term "fixed dose" with regard to a composition of the invention means that two or more different antibodies in a single composition are present in the composition in particular (fixed) ratios with each other. In some embodiments, the fixed dose is based on the weight (*e.g.*, mg) of the antibodies. In certain embodiments, the fixed dose is based on the concentration (*e.g.*, mg/ml) of the antibodies. In some embodiments, the ratio is at least about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9, about 1:10, about 1:15, about 1:20, about 1:30, about 1:40, about 1:50, about 1:60, about 1:70, about 1:80, about 1:90, about 1:100, about 1:120, about 1:140, about 1:160, about 1:180, about 1:200, about 200:1, about 180:1, about 160:1, about 140:1, about 120:1, about 100:1, about 90:1, about 80:1, about 70:1, about 60:1, about 50:1, about 40:1, about 30:1, about 20:1, about 15:1, about 10:1, about 9:1, about 8:1, about 7:1, about 6:1, about 5:1, about 4:1, about 3:1, or about 2:1 mg first antibody to mg second antibody. For example, the 3:1 ratio of a first antibody and a second antibody can mean that a vial can contain about 240 mg of the first antibody and 80 mg of the second antibody or about 3 mg/ml of the first antibody and 1 mg/ml of the second antibody.

[0040] The use of the term "flat dose" with regard to the composition of the invention means a dose that is administered to a patient without regard for the weight or body surface area (BSA) of the patient. The flat dose is therefore not provided as a mg/kg dose, but rather as an absolute amount of the agent (*e.g.,* the anti-CTLA-4 antibody and/or anti-PD-1 antibody). For example, a 60 kg person and a 100 kg person would receive the same dose of the composition (*e.g.*, 240 mg of an anti-PD-1 antibody and 80 mg of an anti-CTLA-4 antibody in a single fixed dosing formulation vial containing both 240 mg of an anti-PD-1 antibody and 80 mg of an anti-CTLA-4 antibody (or two fixed dosing formulation vials containing 120 mg of an anti-PD-1 antibody and 40 mg of an anti-CTLA-4 antibody, etc.)).

[0041] The term "weight based dose" as referred to herein means that a dose that is administered to a patient is calculated based on the weight of the patient. For example, when a patient with 60 kg body weight requires 3 mg/kg of an anti-PD-1 antibody in combination with 1 mg/kg of an anti-CTLA-4 antibody, one can draw the appropriate

amounts of the anti-PD-1 antibody (*i.e.*, 180 mg) and the anti-CTLA-4 antibody (*i.e.*, 60 mg) at once from a 3:1 ratio fixed dosing formulation of an anti-PD1 antibody and an anti-CTLA-4 antibody.

[0042] The term "anti-PD-1 antibody monotherapy" as used herein includes a therapy of an anti-PD-1 antibody without an anti-CTLA-4 antibody therapy. The anti-PD-1 antibody monotherapy comprises, consists essentially of, or consists of administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include administering an anti-CTLA-4 antibody. In one embodiment, the anti-PD-1 antibody monotherapy comprises administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include administering an anti-CTLA-4 antibody. In another embodiment, the anti-PD-1 antibody monotherapy comprises administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include administering an antibody specifically targeting a protein other than PD-1. In other embodiments, the anti-PD-1 antibody monotherapy comprises administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include administering another anti-cancer agent.

[0043] An "immune response" refers to the action of a cell of the immune system (for example, T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells and neutrophils) and soluble macromolecules produced by any of these cells or the liver (including antibodies, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from a vertebrate's body of invading pathogens, cells or tissues infected with pathogens, cancerous or other abnormal cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

[0044] "PD-L1 negative" or "PD-L1 expression negative," relating to cell surface PD-L1 expression, refers to the lack of a detectable amount of cell surface PD-L1. For cell surface expression assayed by IHC, e.g., with the mAb 28-8, a PD-L1 negative tumor or PD-L1 expression negative tumor means that less than 0.01% of cells express a detectable level of PD-L1. In some embodiments, a PD-L1 negative tumor or PD-L1 expression negative tumor means that zero (0) cells express a detectable level of PD-L1. In some embodiments, a PD-L1 negative or a PD-L1 expression negative tumor is any tumor other than a PD-L1 positive or a PD-L1 expression positive tumor.

[0045] The term "PD-L1 positive" or "PD-L1 expression positive," relating to cell surface PD-L1 expression, refers to the proportion of cells in a test tissue sample comprising tumor cells and tumor- infiltrating inflammatory cells above which the sample is scored as expressing cell surface PD-L1. For cell surface expression assayed by immunohistochemistry (IHC), e.g., with the mAb 28- 8, the PD-L1 positive tumor or PD-L1 expression positive tumor means that at least about 0.01%, at least about 0.5%, at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 30% of the total number of cells express PD-L1. PD-L1 positive tumor or PD-L1 expression positive tumor can also be expressed herein as tumor expressing PD-L1. In other embodiments, the PD-L1 positive tumor or PD-L1 expression positive tumor means that at least about 0.1% to at least about 20% of the total number of cells express PD-L1. In certain embodiments, the PD-L1 positive tumor or PD-L1 expression positive tumor means that at least about 0.1% to at least about 10% of the total number of cells express PD-L1. In some embodiments, the PD-L1 positive or PD-L1 expression positive tumor means that at least about 1% of the total number of cells express PD-L1 on the cell surface. In other embodiments, the PD-L1 positive or PD-L1 expression positive tumor means that at least about 5% of the total number of cells express PD-L1 on the cell surface. In one particular embodiment, PD-L1 positive or PD-L1 expression positive tumor means that at least about 1%, or in the range of 1- 5% of the total number of cells express PD-L1 on the cell surface.

[0046] "Programmed Death-1 (PD-1)" refers to an immunoinhibitory receptor belonging to the CD28 family. PD-1 is expressed predominantly on previously activated T cells *in vivo*, and binds to two ligands, PD-L1 and PD-L2. The term "PD-1" as used herein includes human PD-1 (hPD-1), variants, isoforms, and species homologs of hPD-1, and analogs having at least one common epitope with hPD-1. The complete hPD-1 sequence can be found under GenBank Accession No. U64863.

[0047] "Programmed Death Ligand-1 (PD-L1)" is one of two cell surface glycoprotein ligands for PD-1 (the other being PD-L2) that down-regulate T cell activation and cytokine secretion upon binding to PD-1. The term "PD-L1" as used herein includes human PD-L1 (hPD-L1), variants, isoforms, and species homologs of hPD-L1, and

analogs having at least one common epitope with hPD-L1. The complete hPD-L1 sequence can be found under GenBank Accession No. Q9NZQ7.

[0048] A "patient" as used herein includes any patient who is afflicted with a cancer (*e.g.*, melanoma). The terms "subject" and "patient" are used interchangeably herein.

[0049] A "therapeutically effective amount" or "therapeutically effective dosage" of a drug or therapeutic agent is any amount of the drug that, when used alone or in combination with another therapeutic agent, protects a subject against the onset of a disease or promotes disease regression evidenced by a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. The ability of a therapeutic agent to promote disease regression can be evaluated using a variety of methods known to the skilled practitioner, such as in human subjects during clinical trials, in animal model systems predictive of efficacy in humans, or by assaying the activity of the agent in *in vitro* assays.

[0050] "Treatment" or "therapy" of a subject refers to any type of intervention or process performed on, or the administration of an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, slowing down or preventing the onset, progression, development, severity or recurrence of a symptom, complication or condition, or biochemical indicia associated with a disease.

[0051] A "tumor-infiltrating inflammatory cell" is any type of cell that typically participates in an inflammatory response in a subject and which infiltrates tumor tissue. Such cells include tumor-infiltrating lymphocytes (TILs), macrophages, monocytes, eosinophils, histiocytes and dendritic cells.

[0052] The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the indefinite articles "a" or "an" should be understood to refer to "one or more" of any recited or enumerated component.

[0053] The terms "about" or "comprising essentially of" refer to a value or composition that is within an acceptable error range for the particular value or composition as determined by one of ordinary skill in the art, which will depend in part on how the value or composition is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" or "comprising essentially of" can mean within 1 or more

than 1 standard deviation per the practice in the art. Alternatively, "about" or "comprising essentially of" can mean a range of up to 10% or 20% (*i.e.*, ±10% or ±20%). For example, about 3mg can include any number between 2.7 mg and 3.3 mg (for 10%) or between 2.4 mg and 3.6 mg (for 20%). Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values or compositions are provided in the application and claims, unless otherwise stated, the meaning of "about" or "comprising essentially of" should be assumed to be within an acceptable error range for that particular value or composition.

[0054] The terms "about once a week," "once about every week," "once about every two weeks," or any other similar dosing interval terms as used herein means approximate number, and "about once a week" or "once about every week" can include every seven days ± two days, *i.e.*, every five days to every nine days. The dosing frequency of "once a week" thus can be every five days, every six days, every seven days, every eight days, or every nine days. "Once about every two weeks" can include every fourteen days ± three days, *i.e.*, every eleven days to every seventeen days. Similar approximations apply, for example, to once about every three weeks, once about every four weeks, once about every five weeks, once about every six weeks and once about every twelve weeks. In some embodiments, a dosing interval of once about every six weeks or once about every twelve weeks means that the first dose can be administered any day in the first week, and then the next dose can be administered any day in the sixth or twelfth week, respectively. In other embodiments, a dosing interval of once about every six weeks or once about every twelve weeks means that the first dose is administered on a particular day of the first week (e.g., Monday) and then the next dose is administered on the same day of the sixth or twelfth weeks (i.e., Monday), respectively.

[0055] As described herein, any concentration range, percentage range, ratio range or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one-tenth and one-hundredth of an integer), unless otherwise indicated.

[0056] Various aspects of the invention are described in further detail in the following subsections.

Methods of the Invention

[0057] The present invention is directed to a method for treating a PD-L1-negative melanoma in a subject in need thereof. The present invention shows that for PD-L1 negative tumors, a combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody is more suitable than a monotherapy of either an anti-PD-1 antibody or an anti-CTLA-4 antibody.

[0058] Not bound to any theory, the present invention identifies that in patients having a PD-L1-negative tumor progression-free survival and overall response rate are higher following treatment with a combination therapy with an anti-PD-1 antibody and an anti-CTLA-4 antibody than following treatment with either an anti-PD-1 antibody or an anti-CTLA-4 antibody alone. Therefore, in order to increase the response of a patient having a PD-L1-negative tumor, the present invention provides identifying a patient suitable for the combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody.

[0059] In one embodiment, the invention includes a method of treating a PD-L1 negative melanoma comprising administering to a patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration. In another embodiment, the invention includes a method for treating a melanoma in a patient in need thereof comprising: (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

[0060] In certain embodiments, the invention includes a method for extending a progression-free survival period for over 9 months in a patient afflicted with a PD-L1 negative melanoma tumor comprising administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the patient demonstrates progression-free survival for over 9 months. In other embodiments, the

invention provides a method for extending a progression-free survival period for over 9 months in a patient afflicted with a melanoma tumor comprising: (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient demonstrates progression-free survival for over 9 months. According to the invention, the progression-free survival of the patient can be extended, after the administration, over about 10 months, over about 11 months, over about 12 months, over about 13 months, about 14 months, about 15 months, about 16 months, about 17 months, about 18 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, or about 10 years. In a particular embodiment, the progression-free survival of the patient is extended for over 10 months.

[0061] In still other embodiments, the invention is directed to a method for reducing a tumor size at least by 10% in a patient afflicted with a PD-L1 negative melanoma tumor comprising administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration. In yet other embodiments, the method comprises (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration. The tumor size, after the administration, can be reduced at least about 60%, 70%, 80%, 90% or 100%. The tumor can be completely eliminated from the patient's body after the administration.

[0062] The invention can also include a method of preventing a relapse and/or induce a remission to a patient having a PD-L1 negative melanoma tumor comprising

administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration. In some embodiments, the method of the invention comprises (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

[0063] In certain embodiments, the invention includes a method for increasing an objective response rate to be higher than 40% in a patient population, wherein each patient of the patient population is afflicted with a melanoma tumor, in a cancer treatment comprising administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein each patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the objective response rate is higher than 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%. The methods can further comprise identifying each patient as having a PD-L1-negative melanoma tumor prior to the administration. In other embodiments, each patient in the methods can further be characterized by (i) extended progression-free survival for over 11 months, (ii) tumor size reduction at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration, or (iii) both. In some embodiments, the patient population can be at least 100 patients having a PD-L1-negative melanoma tumor. In some embodiments, the patient population can be at least 200, 300, 400, 500, 600, 700, 800, 900, or 1000 patients having a PD-L1-negative melanoma tumor.

[0064] In further embodiments, the invention provides a method for selecting a suitable cancer therapy course in a patient having a PD-L1 negative melanoma tumor comprising: (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) instructing a healthcare provider to administer to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4

antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4. The method further comprises administering the antibodies to the patient.

[0065] The methods of the invention as a result of the combination therapy can treat the melanoma tumor, reduce the tumor size, prevent growth of the tumor, eliminate the tumor from the patient, prevent a relapse of a tumor, induce a remission in a patient, or any combination thereof. In certain embodiments, the combination therapy induces a complete response. In other embodiments, the combination therapy induces a partial response.

Melanoma

[0066] Melanoma (MEL) is a malignant tumor of melanocytes, the melanin-producing cells found predominantly in skin. Though less common than other skin cancers, it is the most dangerous of skin cancers if not diagnosed early and causes the majority (75%) of skin cancer deaths. The incidence of MEL is increasing worldwide in Caucasian populations, especially where peoples with low amounts of skin pigmentation receive excessive ultraviolet light exposure from the sun. In Europe, the incidence rate is < 10-20 per 100,000 population; in the USA 20-30 per 100,000; and in Australia, where the highest incidence is observed, 50-60 per 100,000 (Garbe *et al.*, *Eur. J. Cancer. 48(15)*:2375-90 (2012)). MEL accounts for about 5% of all new cases of cancer in the United States (U.S.), and the incidence continues to rise by almost 3% per year. This translates to an estimated 76,690 new cases in the U.S. in 2013 with 9,480 associated deaths (Siegel *et al.*, *CA Cancer J. Clin. 63(1)*:11-30 (2013)).

[0067] For in situ (stage 0) or early-stage MEL (Stages I-II), surgical excision is the primary treatment. In general, the prognosis is excellent for patients with localized disease and tumors 1.0 mm or less in thickness, with 5-year survival rates of more than 90% (NCCN GUIDELINES®, 2013- Melanoma). Where surgical excision is not feasible for in situ melanoma due to comorbidity or cosmetically sensitive tumor location, topical imiquimod (INN) and radiotherapy are emerging as treatments, especially for *lentigo maligna*. Chemotherapeutic agents for treating MEL include dacarbazine, temozolomide and imatinib for melanoma with a c-KIT mutation, high-dose interleukin-2, and paclitaxel with or without carboplatin. However, these treatments have modest success, with response rates below 20% in first-line (1L) and second-line (2L) settings.

[0068] For patients with localized melanomas more than 1.0 mm in thickness, survival rates range from 50-90%. The likelihood of regional nodal involvement increases with increasing tumor thickness. With Stage III MEL (clinically positive nodes and/or in-transit disease), 5-year survival rates range from 20-70%. By far the most lethal is Stage IV MEL where long-term survival in patients with distant metastatic melanoma is less than 10% (NCCN GUIDELINES®, 2013 - melanoma).

[0069] The types of melanoma that can be treated with the present methods include, but are not limited to, lentigo maligna, lentigo maligna melanoma, superficial spreading melanoma, acral lentiginous melanoma, nucosal melanoma, nodular melanoma, polypoid melanoma, desmoplastic melanoma, amelanotic melanoma, soft-tissue melanoma, melanoma with small nevus-like cells, melanoma with features of a Spitz nevus, or uveal melanoma. The stages of melanoma that can be treated with the present methods include, but are not limited to, (i) Stage I / II (invasive melanoma): T1a characterized by less than 1.0 mm primary tumor thickness, without ulceration, and mitosis $< 1/mm^2$; T1b characterized by less than 1.0 mm primary tumor thickness, with ulceration or mitoses $\geq 1/mm^2$; T2a characterized by 1.01–2.0 mm primary tumor thickness, without ulceration; (ii) Stage II (high risk melanoma): T2b characterized by 1.01–2.0 mm primary tumor thickness, with ulceration; T3a characterized by 2.01–4.0 mm primary tumor thickness, without ulceration; T3b characterized by 2.01–4.0 mm primary tumor thickness, with ulceration; T4a characterized by greater than 4.0 mm primary tumor thickness, without ulceration; or T4b characterized by greater than 4.0 mm primary tumor thickness, with ulceration; (iii) Stage III (regional metastasis): N1 characterized by single positive lymph node; N2 characterized by two to three positive lymph nodes or regional skin/in-transit metastasis; or N3 characterized by four positive lymph nodes or one lymph node and regional skin/in-transit metastases; and (iv) Stage IV (distant metastasis): M1a characterized by distant skin metastasis, normal LDH; M1b characterized by Lung metastasis, normal LDH; or M1c characterized by other distant metastasis or any distant metastasis with elevated LDH. PD-L1-negative tumors that are treatable by the present methods can lack PD-L1 expression on the surface of tumor cells and/or tumor infiltrating inflammatory cells.

Measurement of PD-L1 expression

[0070] In certain embodiments, identifying a patient suitable for a combination therapy for the present methods includes measuring or assessing a PD-L1 expression on the surface of the melanoma tumor cells or tumor infiltrating inflammatory cells. The phrases "tumors expressing PD-L1," "PD-L1 expressing tumor," "PD-L1 positive tumor," and "PD-L1 expression positive tumor" are used interchangeably herein. The meaning of the phrases is provided elsewhere herein. The methods of measuring or assessing the PD-L1 expression can be achieved by any methods applicable.

[0071] In order to assess the PD-L1 expression, in one embodiment, a test tissue sample is obtained from the patient who is in need of the therapy. In another embodiment, the assessment of PD-L1 expression can be achieved without obtaining a test tissue sample. In some embodiments, selecting a suitable patient includes (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and/or tumor-infiltrating inflammatory cells; and (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the surface of the cells based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is lower than a predetermined threshold level. A test tissue sample can be considered to be PD-L1-negative if less than about 5% of the cells in the test tissue express PD-L1 on the cell surface. In other embodiments, a test tissue sample is considered to be PD-L1-negative if less than about 4%, less than about 3%, less than about 2%, less than about 1%, less than about 0.5%, less than about 0.1%, less than about 0.01% or 0% of the cells in the test tissue express PD-L1 on the cell surface. In one particular example, the test tissue sample can be considered to be PD-L1-negative if less than about 5% of the cells in the test tissue express PD-L1 on the cell surface.

[0072] In any of the methods comprising the measurement of PD-L1 expression in a test tissue sample, however, it should be understood that the step comprising the provision of a test tissue sample obtained from a patient is an optional step. That is, in certain embodiments the method includes this step, and in other embodiments, this step is not included in the method. It should also be understood that in certain embodiments the "measuring" or "assessing" step to identify, or determine the number or proportion of, cells in the test tissue sample that express PD-L1 on the cell surface is performed by a transformative method of assaying for PD-L1 expression, for example by performing a

reverse transcriptase-polymerase chain reaction (RT-PCR) assay or an IHC assay. In certain other embodiments, no transformative step is involved and PD-L1 expression is assessed by, for example, reviewing a report of test results from a laboratory. In certain embodiments, the steps of the methods up to, and including, assessing PD-L1 expression provides an intermediate result that may be provided to a physician or other healthcare provider for use in selecting a suitable candidate for the combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody. In certain embodiments, the steps that provide the intermediate result is performed by a medical practitioner or someone acting under the direction of a medical practitioner. In other embodiments, these steps are performed by an independent laboratory or by an independent person such as a laboratory technician.

[0073] In certain embodiments of any of the present methods, the proportion of cells that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 RNA. In further embodiments, the presence of PD-L1 RNA is determined by RT-PCR, *in situ* hybridization or RNase protection. In other embodiments, the proportion of cells that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 polypeptide. In further embodiments, the presence of PD-L1 polypeptide is determined by immunohistochemistry (IHC), enzyme-linked immunosorbent assay (ELISA), *in vivo* imaging, or flow cytometry. In some embodiments, PD-L1 expression is assayed by IHC. In other embodiments of all of these methods, cell surface expression of PD-L1 is assayed using, *e.g.*, IHC or *in vivo* imaging.

[0074] Imaging techniques have provided important tools in cancer research and treatment. Recent developments in molecular imaging systems, including positron emission tomography (PET), single-photon emission computed tomography (SPECT), fluorescence reflectance imaging (FRI), fluorescence-mediated tomography (FMT), bioluminescence imaging (BLI), laser-scanning confocal microscopy (LSCM) and multiphoton microscopy (MPM), will likely herald even greater use of these techniques in cancer research. Some of these molecular imaging systems allow clinicians to not only see where a tumor is located in the body, but also to visualize the expression and activity of specific molecules, cells, and biological processes that influence tumor behavior and/or responsiveness to therapeutic drugs (Condeelis and Weissleder, *Cold Spring Harb. Perspect. Biol. 2(12)*:a003848 (2010)). Antibody specificity, coupled with the sensitivity

and resolution of PET, makes immunoPET imaging particularly attractive for monitoring and assaying expression of antigens in tissue samples (McCabe and Wu, *Cancer Biother. Radiopharm. 25(3)*:253-61 (2010); Olafsen *et al.*, *Protein Eng. Des. Sel. 23(4)*:243-9 (2010)). In certain embodiments of any of the present methods, PD-L1 expression is assayed by immunoPET imaging. In certain embodiments of any of the present methods, the proportion of cells in a test tissue sample that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 polypeptide on the surface of cells in the test tissue sample. In certain embodiments, the test tissue sample is a FFPE tissue sample. In other embodiments, the presence of PD-L1 polypeptide is determined by IHC assay. In further embodiments, the IHC assay is performed using an automated process. In some embodiments, the IHC assay is performed using an anti-PD-L1 mAb to bind to the PD-L1 polypeptide, if present.

Assaying Cell-Surface PD-L1 Expression by Automated IHC

[0075] In one embodiment of the present methods, an automated IHC method is used to assay the expression of PD-L1 on the surface of cells in FFPE tissue specimens. This disclosure provides methods for detecting the presence of human PD-L1 antigen in a test tissue sample, or quantifying the level of human PD-L1 antigen or the proportion of cells in the sample that express the antigen, which methods comprise contacting the test sample, and a negative control sample, with a mAb that specifically binds to human PD-L1, under conditions that allow for formation of a complex between the antibody or portion thereof and human PD-L1. In certain embodiments, the test and control tissue samples are FFPE samples. The formation of a complex can then be detected, wherein a difference in complex formation between the test sample and the negative control sample is indicative of the presence of human PD-L1 antigen in the sample. Various methods are used to quantify PD-L1 expression.

[0076] In a particular embodiment, the automated IHC method comprises: (a) deparaffinizing and rehydrating mounted tissue sections in an autostainer; (b) retrieving antigen using a decloaking chamber and pH 6 buffer, heated to 110°C for 10 min; (c) setting up reagents on an autostainer; and (d) running the autostainer to include steps of neutralizing endogenous peroxidase in the tissue specimen; blocking non-specific protein-binding sites on the slides; incubating the slides with primary Ab; incubating with a

postprimary blocking agent; incubating with NovoLink Polymer; adding a chromogen substrate and developing; and counterstaining with hematoxylin.

[0077] For assessing PD-L1 expression in tumor tissue samples, a pathologist examines the number of membrane PD-L1$^+$ tumor cells in each field under a microscope and mentally estimates the percentage of cells that are positive, then averages them to come to the final percentage. The different staining intensities are defined as 0/negative, 1+/weak, 2+/moderate, and 3+/strong. Typically, percentage values are first assigned to the 0 and 3+ buckets, and then the intermediate 1+ and 2+ intensities are considered. For highly heterogeneous tissues, the specimen is divided into zones, and each zone is scored separately and then combined into a single set of percentage values. The percentages of negative and positive cells for the different staining intensities are determined from each area and a median value is given to each zone. A final percentage value is given to the tissue for each staining intensity category: negative, 1+, 2+, and 3+. The sum of all staining intensities needs to be 100%.

[0078] Staining is also assessed in tumor-infiltrating inflammatory cells such as macrophages and lymphocytes. In most cases macrophages serve as an internal positive control since staining is observed in a large proportion of macrophages. While not required to stain with 3+ intensity, an absence of staining of macrophages should be taken into account to rule out any technical failure. Macrophages and lymphocytes are assessed for plasma membrane staining and only recorded for all samples as being positive or negative for each cell category. Staining is also characterized according to an outside/inside tumor immune cell designation. "Inside" means the immune cell is within the tumor tissue and/or on the boundaries of the tumor region without being physically intercalated among the tumor cells. "Outside" means that there is no physical association with the tumor, the immune cells being found in the periphery associated with connective or any associated adjacent tissue.

[0079] In certain embodiments of these scoring methods, the samples are scored by two pathologists operating independently, and the scores are subsequently consolidated. In certain other embodiments, the identification of positive and negative cells is scored using appropriate software.

[0080] A histoscore is used as a more quantitative measure of the IHC data. The histoscore is calculated as follows:

Histoscore = [(% tumor x 1 (low intensity)) + (% tumor x 2 (medium intensity)) + (% tumor x 3 (high intensity)]

[0081] To determine the histoscore, the pathologist estimates the percentage of stained cells in each intensity category within a specimen. Because expression of most biomarkers is heterogeneous the histoscore is a truer representation of the overall expression. The final histoscore range is 0 (no expression) to 300 (maximum expression). A test sample can be designated as PD-L1-negative even though some level of PD-L1 expression is detected. For example, a designation of PD-L1-negative can be assigned to a specimen having a final histoscore of about 15 or less, of about 10 or less, about 9 or less, about 8 or less, about 7 or less, about 6 or less, about 5 or less, about 4 or less, about 3 or less, about 2 or less, about 1 or less, or of 0.

[0082] An alternative means of quantifying PD-L1 expression in a test tissue sample IHC is to determine the adjusted inflammation score (AIS) score defined as the density of inflammation multiplied by the percent PD-L1 expression by tumor-infiltrating inflammatory cells (Taube *et al.*, *Sci. Transl. Med. 4(127)*:127ra37 (2012)).

Anti-PD-1 antibodies

[0083] PD-1 is a key immune checkpoint receptor expressed by activated T and B cells and mediates immunosuppression. PD-1 is a member of the CD28 family of receptors, which includes CD28, CTLA-4, ICOS, PD-1, and BTLA. Two cell surface glycoprotein ligands for PD-1 have been identified, Programmed Death Ligand-1 (PD-L1) and Programmed Death Ligand-2 (PD-L2), that are expressed on antigen-presenting cells as well as many human cancers and have been shown to down regulate T cell activation and cytokine secretion upon binding to PD-1. Inhibition of the PD-1/PD-L1 interaction mediates potent antitumor activity in preclinical models.

[0084] HuMAbs that bind specifically to PD-1 with high affinity have been disclosed in U.S. Patent Nos. 8,008,449 and 8,779,105. Other anti-PD-1 mAbs have been described in, for example, U.S. Patent Nos. 6,808,710, 7,488,802, 8,168,757 and 8,354,509, and PCT Publication No. WO 2012/145493. Each of the anti-PD-1 HuMAbs disclosed in U.S. Patent No. 8,008,449 has been demonstrated to exhibit one or more of the following characteristics: (a) binds to human PD-1 with a K_D of 1 x 10^{-7} M or less, as determined by surface plasmon resonance using a Biacore biosensor system; (b) does not substantially bind to human CD28, CTLA-4 or ICOS; (c) increases T-cell proliferation in a Mixed

Lymphocyte Reaction (MLR) assay; (d) increases interferon-γ production in an MLR assay; (e) increases IL-2 secretion in an MLR assay; (f) binds to human PD-1 and cynomolgus monkey PD-1; (g) inhibits the binding of PD-L1 and/or PD-L2 to PD-1; (h) stimulates antigen-specific memory responses; (i) stimulates antibody responses; and (j) inhibits tumor cell growth *in vivo*. Anti-PD-1 antibodies useful for the present invention include mAbs that bind specifically to human PD-1 and exhibit at least one, at least two, at least three, at least four, or at least five, of the preceding characteristics.

[0085] In one embodiment, the anti-PD-1 antibody is nivolumab. Nivolumab (also known as "OPDIVO®"; formerly designated 5C4, BMS-936558, MDX-1106, or ONO-4538) is a fully human IgG4 (S228P) PD-1 immune checkpoint inhibitor antibody that selectively prevents interaction with PD-1 ligands (PD-L1 and PD-L2), thereby blocking the down-regulation of antitumor T-cell functions (U.S. Patent No. 8,008,449; Wang *et al.*, *Cancer Immunol Res.* 2(9):846-56 (2014)). In another embodiment, the anti-PD-1 antibody or fragment thereof cross-competes with nivolumab. In other embodiments, the anti-PD-1 antibody or fragment thereof binds to the same epitope as nivolumab. In certain embodiments, the anti-PD-1 antibody has the same CDRs as nivolumab.

[0086] In another embodiment, the anti-PD-1 antibody or fragment thereof cross-competes with pembrolizumab. In some embodiments, the anti-PD-1 antibody or fragment thereof binds to the same epitope as pembrolizumab. In certain embodiments, the anti-PD-1 antibody has the same CDRs as pembrolizumab. In another embodiment, the anti-PD-1 antibody is pembrolizumab. Pembrolizumab (also known as "KEYTRUDA®", lambrolizumab, and MK-3475) is a humanized monoclonal IgG4 antibody directed against human cell surface receptor PD-1 (programmed death-1 or programmed cell death-1). Pembrolizumab is described, for example, in U.S. Patent Nos. 8,354,509 and 8,900,587; *see also* http://www.cancer.gov/drugdictionary?cdrid=695789 (last accessed: December 14, 2014). Pembrolizumab has been approved by the FDA for the treatment of relapsed or refractory melanoma.

[0087] In other embodiments, the anti-PD-1 antibody or fragment thereof cross-competes with MEDI0608. In still other embodiments, the anti-PD-1 antibody or fragment thereof binds to the same epitope as MEDI0608. In certain embodiments, the anti-PD-1 antibody has the same CDRs as MEDI0608. In other embodiments, the anti-PD-1 antibody is MEDI0608 (formerly AMP-514), which is a monoclonal antibody. MEDI0608 is

described, for example, in US Pat. No. 8,609,089B2 or in http://www.cancer.gov/drugdictionary?cdrid=756047 (last accessed December 14, 2014).

[0088] In certain embodiments, the first antibody is an anti-PD-1 antagonist. One example of the anti-PD-1 antagonist is AMP-224, which is a B7-DC Fc fusion protein. AMP-224 is discussed in U.S. Publ. No. 2013/0017199 or in http://www.cancer.gov/publications/dictionaries/cancer-drug?cdrid=700595 (last accessed July 8, 2015).

[0089] In other embodiments, the anti-PD-1 antibody or fragment thereof cross-competes with BGB-A317. In some embodiments, the anti-PD-1 antibody or fragment thereof binds the same epitope as BGB-A317. In certain embodiments, the anti-PD-1 antibody has the same CDRs as BGB-A317. In certain embodiments, the anti-PD-1 antibody is BGB-A317, which is a humanized monoclonal antibody. BGB-A317 is described in U.S. Publ. No. 2015/0079109.

[0090] In some embodiments, the antibody is Pidilizumab (CT-011), which is an antibody previously reported to bind to PD-1 but which is believed to bind to a different target. Pidilizumab is described in US Pat. No. 8,686,119 B2 or WO 2013/014668 A1.

[0091] Anti-PD-1 antibodies useful for the disclosed compositions also include isolated antibodies that bind specifically to human PD-1 and cross-compete for binding to human PD-1 with nivolumab (*see, e.g.*, U.S. Patent Nos. 8,008,449 and 8,779,105; WO 2013/173223). The ability of antibodies to cross-compete for binding to an antigen indicates that these antibodies bind to the same epitope region of the antigen and sterically hinder the binding of other cross-competing antibodies to that particular epitope region. These cross-competing antibodies are expected to have functional properties very similar to those of nivolumab by virtue of their binding to the same epitope region of PD-1. Cross-competing antibodies can be readily identified based on their ability to cross-compete with nivolumab in standard PD-1 binding assays such as Biacore analysis, ELISA assays or flow cytometry (*see, e.g.*, WO 2013/173223).

[0092] In certain embodiments, the antibodies that cross-compete for binding to human PD-1 with, or bind to the same epitope region of human PD-1 as, nivolumab are mAbs. For administration to human subjects, these cross-competing antibodies can be chimeric antibodies, or humanized or human antibodies. Such chimeric, humanized or human mAbs can be prepared and isolated by methods well known in the art.

[0093] Anti-PD-1 antibodies useful for the compositions of the disclosed invention also include antigen-binding portions of the above antibodies. It has been amply demonstrated that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_{H1} domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_{H1} domains; and (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody.

[0094] Anti-PD-1 antibodies suitable for use in the disclosed compositions are antibodies that bind to PD-1 with high specificity and affinity, block the binding of PD-L1 and or PD-L2, and inhibit the immunosuppressive effect of the PD-1 signaling pathway. In any of the compositions or methods disclosed herein, an anti-PD-1 "antibody" includes an antigen-binding portion or fragment that binds to the PD-1 receptor and exhibits the functional properties similar to those of whole antibodies in inhibiting ligand binding and upregulating the immune system. In certain embodiments, the anti-PD-1 antibody or antigen-binding portion thereof cross-competes with nivolumab for binding to human PD-1. In other embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is a chimeric, humanized or human monoclonal antibody or a portion thereof. In certain embodiments, the antibody is a humanized antibody. In other embodiments, the antibody is a human antibody. Antibodies of an IgG1, IgG2, IgG3 or IgG4 isotype can be used.

[0095] In certain embodiments, the anti-PD-1 antibody or antigen-binding portion thereof comprises a heavy chain constant region which is of a human IgG1 or IgG4 isotype. In certain other embodiments, the sequence of the IgG4 heavy chain constant region of the anti-PD-1 antibody or antigen-binding portion thereof contains an S228P mutation which replaces a serine residue in the hinge region with the proline residue normally found at the corresponding position in IgG1 isotype antibodies. This mutation, which is present in nivolumab, prevents Fab arm exchange with endogenous IgG4 antibodies, while retaining the low affinity for activating Fc receptors associated with wild-type IgG4 antibodies (Wang *et al.*, *Cancer Immunol Res.* 2(9):846-56 (2014)). In yet other embodiments, the antibody comprises a light chain constant region which is a human kappa or lambda constant region. In other embodiments, the anti-PD-1 antibody or antigen-binding portion

thereof is a mAb or an antigen-binding portion thereof. In certain embodiments of any of the therapeutic methods described herein comprising administration of an anti-PD-1 antibody, the anti-PD-1 antibody is nivolumab. In other embodiments, the anti-PD-1 antibody is pembrolizumab. In other embodiments, the anti-PD-1 antibody is chosen from the human antibodies 17D8, 2D3, 4H1, 4A11, 7D3 and 5F4 described in U.S. Patent No. 8,008,449. In still other embodiments, the anti-PD-1 antibody is MEDI0608 (formerly AMP-514), AMP-224, or BGB-A317.

[0096] Because anti-PD-1 and anti-PD-L1 target the same signaling pathway and have been shown in clinical trials to exhibit similar levels of efficacy in a variety of cancers, including RCC (*see* Brahmer *et al.* (2012) *N Engl J Med* 366:2455-65; Topalian *et al.* (2012a) *N Engl J Med* 366:2443-54; WO 2013/173223), an anti-PD-L1 antibody may be substituted for the anti-PD-1 Ab in any of the therapeutic methods disclosed herein. In certain embodiments, the anti-PD-L1 antibody is BMS-936559 (formerly 12A4 or MDX-1105) (*see, e.g.*, U.S. Patent No. 7,943,743; WO 2013/173223). In other embodiments, the anti-PD-L1 antibody is MPDL3280A (also known as RG7446) (*see, e.g.*, Herbst et al. (2013) *J Clin Oncol* 31(suppl):3000. Abstract; U.S. Patent No. 8,217,149) or MEDI4736 (Khleif (2013) In: Proceedings from the European Cancer Congress 2013; September 27-October 1, 2013; Amsterdam, The Netherlands. Abstract 802). In certain embodiments, the antibodies that cross-compete for binding to human PD-L1 with, or bind to the same epitope region of human PD-L1 as the above-references PD-L1 antibodies are mAbs. For administration to human subjects, these cross-competing antibodies can be chimeric antibodies, or can be humanized or human antibodies. Such chimeric, humanized or human mAbs can be prepared and isolated by methods well known in the art.

Anti-PD-L1 Antibodies

[0097] In certain embodiments, the present application encompasses use of an anti-PD-L1 antibody in lieu of anti-PD-1 antibody. In one embodiment, the anti-PD-L1 antibody inhibits the binding of PD-L1 receptor, i.e., PD-1, with its ligand PD-L1.

[0098] Anti-PD-L1 antibodies useful for the invention include antibodies engineered starting from antibodies having one or more of the V_H and/or V_L sequences disclosed herein, which engineered antibodies can have altered properties from the starting antibodies. An anti-PD-L1 antibody can be engineered by a variety of modifications as described above for the engineering of modified anti-PD-1 antibodies of the invention.

[0099] Anti-PD-L1 antibodies of the invention also include isolated antibodies selected for their ability to bind to PD-L1 in formalin-fixed, paraffin-embedded (FFPE) tissue specimens. The use of FFPE samples is essential for the long-term follow-up analysis of the correlation between PD-L1 expression in tumors and disease prognosis or progression. The use of different antibodies to stain PD-L1 in frozen versus FFPE tissues, and the ability of certain antibodies to distinguish membranous and/or cytoplasmic forms of PD-Ll, may account for some of the disparate data reported in the literature correlating PD-Ll expression with disease prognosis (Hamanishi *et al.*, *Proc. Natl. Acad. Sci. USA 104(9)*:3360-3365 (2007); Gadiot *et al.*, *Cancer 117(10)*:2192-2201 (2011)). This disclosure provides several rabbit mAbs that bind with high affinity specifically to membranous human PD-Ll in FFPE tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells.

[0100] In some embodiments, an anti-PD-L1 antibody useful for the present methods includes mAb 28-8 set forth in SEQ ID NOs. 1 and 2, respectively. The sequences of the heavy and light chain CDR domains of mAb 28-8, as delineated using the Kabat system, are set forth in SEQ ID NOs. 3-8. In other embodiments, an anti-PD-L1 antibody useful for the invention comprises mAbs 28-1, 28-12, 29-8 and 20-12 or an antigen-binding portion thereof, for example, including Fab, $F(ab')_2$ Fd, Fv, and scFv, di-scFv or bi-scFv, and scFv-Fc fragments, diabodies, triabodies, tetrabodies, and isolated CDRs.

Anti-CTLA-4 antibodies

[0101] Anti-CTLA-4 antibodies of the instant invention bind to human CTLA-4 so as to disrupt the interaction of CTLA-4 with a human B7 receptor. Because the interaction of CTLA-4 with B7 transduces a signal leading to inactivation of T-cells bearing the CTLA-4 receptor, disruption of the interaction effectively induces, enhances or prolongs the activation of such T cells, thereby inducing, enhancing or prolonging an immune response.

[0102] HuMAbs that bind specifically to CTLA-4 with high affinity have been disclosed in U.S. Patent Nos. 6,984,720 and 7,605,238. Other anti-PD-1 mAbs have been described in, for example, U.S. Patent Nos. 5,977,318, 6,051,227, 6,682,736, and 7,034,121. The anti-PD-1 HuMAbs disclosed in U.S. Patent No. Nos. 6,984,720 and 7,605,238 have been demonstrated to exhibit one or more of the following characteristics: (a) binds specifically to human CTLA-4 with a binding affinity reflected by an equilibrium association constant

(K_a) of at least about 10^7 M^{-1}, or about 10^9 M^{-1}, or about 10^{10} M^{-1} to 10^{11} M^{-1} or higher, as determined by Biacore analysis; (b) a kinetic association constant (k_a) of at least about 10^3, about 10^4, or about 10^5 m^{-1} s^{-1}; (c) a kinetic disassociation constant (k_d) of at least about 10^3, about 10^4, or about 10^5 m^{-1} s^{-1}; and (d) inhibits the binding of CTLA-4 to B7-1 (CD80) and B7-2 (CD86). Anti-CTLA-4 antibodies useful for the present invention include mAbs that bind specifically to human CTLA-4 and exhibit at least one, at least two, or at least three of the preceding characteristics.

[0103] An exemplary clinical anti-CTLA-4 antibody is the human mAb 10D1 (now known as ipilimumab and marketed as YERVOY®) as disclosed in U.S. Patent No. 6,984,720. Ipilimumab is an anti-CTLA-4 antibody for use in the methods disclosed herein. Ipilimumab is a fully human, IgG1 monoclonal antibody that blocks the binding of CTLA-4 to its B7 ligands, thereby stimulating T cell activation and improving overall survival (OS) in patients with advanced melanoma.

[0104] Another anti-CTLA-4 antibody useful for the present methods is tremelimumab (also known as CP-675,206). Tremelimumab is human IgG2 monoclonal anti-CTLA-4 antibody. Tremelimumab is described in WO/2012/122444, U.S. Publ. No. 2012/263677, or WO Publ. No. 2007/113648 A2.

[0105] Anti-CTLA-4 antibodies useful for the disclosed composition also include isolated antibodies that bind specifically to human CTLA-4 and cross-compete for binding to human CTLA-4 with ipilimumab or tremelimumab or bind to the same epitope region of human CTLA-4 as ipilimumab or tremelimumab. In certain embodiments, the antibodies that cross-compete for binding to human CTLA-4 with, or bind to the same epitope region of human CTLA-4 as does ipilimumab or tremelimumab, are antibodies comprising a heavy chain of the human IgG1 isotype. For administration to human subjects, these cross-competing antibodies are chimeric antibodies, or humanized or human antibodies. Useful anti-CTLA-4 antibodies also include antigen-binding portions of the above antibodies such as Fab, $F(ab')_2$, Fd or Fv fragments.

Dosages

[0106] The combination of an anti-PD-1 antibody and an anti-CTLA-4 antibody can be administered to a suitable patient in therapeutically effective amounts. For example, each antibody can be administered at a dosage ranging from at least about 0.1 to at least about 20.0 mg/kg body weight. In certain embodiments, each of the anti-PD-1 and anti-CTLA-4

antibodies is individually administered at a dosage of at least about 0.1, at least about 0.3, at least about 0.5, at least about 1, at least about 3, at least about 5, at least about 10 or at least about 20 mg/kg, *e.g.*, at least about 1 to at least about 10 mg/kg, *e.g.*, at least about 1 to at least about 3 mg/kg, *e.g.*, at least about 3 mg/kg, *e.g.*, at least about 1 mg/kg. Each of the anti-PD-1 antibody and anti-CTLA-4 antibody can be administered at a dosing frequency of at least about once every week, at least about once every 2 weeks, at least about once every 3 weeks, or at least about once every 4 weeks, or at least about once a month, for up to 6 to up to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In some embodiments, the anti-PD-1 antibody is administered at a dosage of about 1 or about 3 mg/kg. In certain embodiments, the sequenced regimen comprises administering the anti-PD-1 antibody to the subject at a dosing frequency of once about every week, once about every 2 weeks, once about every 3 weeks, or once about every 4 weeks, or once a month for 6 to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In other embodiments, the anti-PD-1 is administered at a dosage of about 1 mg/kg at a dosing frequency of once about every 3 weeks for up to 48 doses. In some embodiments, the anti-CTLA-4 antibody is administered at a dosage of about 1 or about 3 mg/kg. In certain embodiments, the sequenced regimen comprises administering the anti-CTLA-4 antibody to the subject at a dosing frequency of once about every week, at least about once every 2 weeks, at least about once every 3 weeks, or at least about once every 4 weeks, or about once a month for 6 to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In other embodiments, the anti-CTLA-4 antibody is administered at a dosage of about 3 mg/kg at a dosing frequency of once about every 3 weeks for up to 48 doses.

[0107] In other embodiments, the anti-PD-1 and anti-CTLA-4 antibodies are administered at the following dosages: (a) 0.1 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (b) 0.3 mg/kg anti-PD-1 antibody and 3 mg/kg of anti- CTLA-4 antibody; (c) 1 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (d) 3 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (e) 5 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (f) 10 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (g) 0.1 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; (h) 0.3 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4

antibody; (i) 1 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; (j) 3 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; (k) 5 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; or (l) 10 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody. In a particular embodiment, the methods include administration of 1 mg/kg of an anti-PD-1 antibody and 3 mg/kg of an anti-CTLA-4 antibody.

[0108] In some embodiments, the dosage of each of the anti-PD-1 and anti-CTLA-4 antibodies is kept constant during the induction dosing schedule and the maintenance dosing schedule. In certain embodiments, a regimen comprises: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of at least once about every 2 weeks, at least once about every 3 weeks, or at least once about every 4 weeks, or at least about once a month, for at least 2, 4, 6, 8 or 10 doses, followed by administration of the anti-PD-1 antibody alone at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 12 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of at least about once every 8 weeks, once about every 12 weeks, or once about every 16 weeks, or at least once a quarter, for at least 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In another embodiment, the dosing schedule comprises administering about 3 mg/kg of an anti-PD-1 antibody every 2 weeks, or about 1 mg/kg of an anti-PD-1 antibody every 3 weeks plus about 3 mg/kg of an anti-CTLA-4 antibody every 3 weeks for 4 doses, followed by about 3 mg/kg of an anti-PD-1 antibody every 2 weeks for cycle 3 and beyond, or 3 mg/kg of an anti-CTLA-4 antibody every 3 weeks for 4 doses. In one particular embodiment, the dosing schedule comprises administering about 1 mg/kg of an anti-PD-1 antibody followed by an anti-CTLA-4 antibody (*e.g.*, about 3 mg/kg anti-CTLA-4 antibody) on the same day every 3 weeks for 4 doses, followed by about 3 mg/kg of an anti-PD-1 antibody every 2 weeks.

[0109] In certain embodiments of this method, the maintenance dosing schedule comprises combined administration of up to 4, 6, 8, 10, 12 or 16 doses of the anti-PD-1 and anti-CTLA-4 antibodies. In other embodiments, the concurrent regimen comprises: (i) an induction dosing schedule comprising combined administration of the anti-PD-1

and anti- CTLA-4 antibodies at a dosing frequency of once about every 2 weeks, at least once about every 3 weeks, or at least once about every 4 weeks, or about once a month, for 2, 4, 6 or 8 doses, followed by administration of the anti-PD-1 antibody alone at a dosing frequency of once about every 2 weeks, at least once about every 3 weeks, or at least once about every 4 weeks, or about once a month, for 2, 4, 6, 8 or 12 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of once every once about every 8, once about every 12 or once about every 16 weeks, or once a quarter, for 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs.

[0110] In a particular embodiment, the anti-PD-1 and anti-CTLA-4 antibodies are administered at dosages of about 1 mg/kg anti-PD-1 antibody every three weeks and about 3 mg/kg of anti-CTLA-4 antibody every three weeks. In another embodiment, the patient is administered at a dose of about 1 mg/kg of an anti-PD-1 antibody, followed by an anti-CTLA-4 antibody on the same day, every 3 weeks for 4 doses, then 3 mg/kg of an anti-PD-1 antibody every 2 weeks.

[0111] In certain embodiments of the present methods, the anti-PD-1 and anti-CTLA-4 antibodies are formulated for parenteral administration, *e.g.*, intravenous administration. In certain other embodiments, when the anti-PD-1 and anti-CTLA-4 antibodies are administered in combination, they are administered within about 30 minutes of each other. Either antibody may be administered first, that is, in certain embodiments, the anti-PD-1 antibody is administered before the anti-CTLA-4 antibody, whereas in other embodiments, the anti-CTLA-4 antibody is administered before the anti-PD-1 antibody. Typically, each antibody is administered intravenously over a period of about 60 minutes. In further embodiments, the anti-PD-1 and anti-CTLA-4 antibodies are administered concurrently, either admixed as a single composition in a pharmaceutically acceptable formulation for concurrent administration, or concurrently as separate compositions with each antibody in a pharmaceutically acceptable formulation.

[0112] In some embodiments, the anti-PD-1 antibody and the anti-CTLA-4 antibody are formulated in a single composition. The ratio of the amount of the anti-PD-1 antibody and the amount of the anti-CTLA-4 antibody in the single composition can be 10:1 to 1:10. In other embodiments, the ratio of the amount of the anti-PD-1 antibody and the amount of

the anti-CTLA-4 antibody in the single composition is 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, or 1:5. In a particular embodiment, the ratio of the amount of the anti-PD-1 antibody and the amount of the anti-CTLA-4 antibody in the single composition is 1:3.

[0113] In some embodiments, the composition is administered at a flat dose regardless of the weight of the patient. For example, each of the anti-PD-1 antibody with the anti-CTLA-4 antibody may be administered at a flat dose of 20, 50, 75, 80, 160, 200, 240, 300, 360, 400, 480, 500, 750 or 1500 mg, without regard to the patient's weight. In some embodiments the composition is administered at a weight-based dose at any dose disclosed herein. In some embodiments, the amount of the anti-PD-1 antibody and the amount of the anti-CTLA-4 antibody administered to the patient at a single dose are identical.

[0114] In certain embodiments of the present methods, the therapeutically effective dosage of the anti-PD-1 antibody or antigen-binding portion thereof is a flat dosing (not a bodyweight based dosing) and comprises about 60 mg, about 80 mg, about 100 mg, about 120 mg, about 140 mg, about 160 mg, about 180 mg, about 200 mg, about 220 mg, about 240 mg, about 260 mg, about 280 mg, or about 300 mg. In other embodiments, the therapeutically effective dosage of the anti-PD-1 antibody or antigen-binding portion thereof comprises about 320 mg, 360mg, 400 mg, 420mg, 480 mg, 500mg, 540 mg, 550mg, 600mg, 620 mg, 650mg, 680mg, 700 mg, 720mg, 780 mg, 800mg, 840mg, or 900mg.. In some embodiments, the dose of the anti-PD-1 antibody in the composition is between about 60 mg and about 300 mg, between about 60 mg and about 100 mg, between about 100 mg and about 200 mg, or between about 200 mg and about 300 mg. In some embodiments, the amount of the anti-PD-1 antibody in the composition is at least about 80 mg, about 160 mg, or about 240mg. In some embodiments, the dose of the anti-PD-1 antibody in the composition is at least about 240 mg or at least about 80 mg.

[0115] In other embodiments, the dosages are given every week, every two weeks, every three weeks, every four weeks, every five weeks, every six weeks, every seven weeks, every eight weeks, every nine weeks, or every 10 weeks.

[0116] In some embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of about 240 mg. In embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of about 360 mg. In embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is administered

at a dose of about 480 mg. In one embodiment, 360mg of the anti-PD-1 antibody or antigen binding fragment is administered once every 3 weeks. In another embodiment, 480mg of the anti-PD-1 antibody or antigen binding fragment is administered once every 4 weeks.

[0117] In other embodiments, the therapeutically effective dosage of the anti-CTLA-4 antibody or antigen-binding portion thereof is a flat dosing (not a bodyweight based dosing) and comprises about 60 mg, about 80 mg, about 100 mg, about 120 mg, about 140 mg, about 160 mg, about 180 mg, about 200 mg, about 220 mg, about 240 mg, about 260 mg, about 280 mg, or about 300 mg. In other embodiments, the therapeutically effective dosage of the anti-CTLA-4 antibody or antigen-binding portion thereof comprises about 320 mg, 360mg, 400 mg, 420mg, 480 mg, 500mg, 540 mg, 550mg, 600mg, 620 mg, 650mg, 680mg, 700 mg, 720mg, 780 mg, 800mg, 840mg, or 900mg. In some embodiments, the dose of the anti-CTLA-4 antibody in the composition is between about 60 mg and about 300 mg, between about 60 mg and about 100 mg, between about 100 mg and about 200 mg, or between about 200 mg and about 300 mg. In some embodiments, the amount of the anti-CTLA-4 antibody in the composition is at least about 80 mg, about 160 mg, or about 240 mg. In some embodiments, the dose of the anti-CTLA-4 antibody in the composition is at least about 240 mg or at least about 80 mg.

[0118] In some embodiments, a flat dose of an anti-PD-1 antibody is 80 mg, and a flat dose of an anti-CTLA-4 antibody is 240 mg.

[0119] In some embodiments, the anti-PD-1 antibody is administered at a subtherapeutic dose, *i.e.*, a dose of the therapeutic agent that is significantly lower than the usual or FDA-approved dose when administered as monotherapy for the treatment of the cancer. The quantity of the second antibody in the composition is calculated based on the desired ratio.

[0120] In some embodiments, the composition is administered by intravenous infusion about once per week, about once every 2 weeks, about once every 3 weeks, or about once a month. In certain embodiments, the composition is administered once every 3 weeks. In some embodiments, the infusion occurs over at least about 10 minutes, about 20 minutes, about 30 minutes, about 45 minutes, about 60 minutes, about 90 minutes, about 2 hours, about 3 hours, about 4 hours or about 5 hours.

[0121] Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention can be flat or varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being unduly toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts. A composition of the present invention can be administered via one or more routes of administration using one or more of a variety of methods well known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

Kits

[0122] Also within the scope of the present invention are kits, including pharmaceutical kits, comprising a combination of an anti-PD-1 antibody and an anti-CTLA-4 antibody, for therapeutic uses, and diagnostic kits comprising an anti-PD-Ll antibody for assaying membranous PD-L1 expression as a biomarker for screening patients for the combination therapy or for predicting the efficacy of the combination therapy. Kits typically include a label indicating the intended use of the contents of the kit and instructions for use. The term "label" includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit. In certain embodiments of a pharmaceutical kit, the anti-PD-1 antibody is co-packaged with an anti-CTLA-4 antibody in unit dosage form. In certain embodiments of a diagnostic kit, the anti-PD-Ll antibody is co-packaged with an anti-PD-1 antibody and an anti-CTLA-4 antibody for performing an assay to detect and/or quantify PD-L1 expression.

[0123] In certain embodiments, the pharmaceutical kit comprises the anti-human PD-1 HuMAb, nivolumab. In other embodiments, the pharmaceutical kit comprises the anti-human PD-L1 HuMAb, BMS-936559. In yet other embodiments, the pharmaceutical kit comprises the anti-human CTLA-4 HuMAb, ipilimumab. In certain embodiments, the

diagnostic kit comprises the rabbit anti-human PD-L1 mAb, 28-8, comprising the V_H and V_L regions whose amino acid sequences are set forth in SEQ ID NOs. 1 and 2, respectively. In other embodiments, the diagnostic kit comprises the murine anti-human PD-L1 mAb, 5H1 (Dong *et al.*, *Nature Med. 8(8)*:793-800 (2002)).

[0124] In some embodiments, the invention provides a kit for treating a patient afflicted with a melanoma tumor, the kit comprising:

(a) a dosage ranging from at least about 0.1 to at least about 10 mg/kg body weight of an anti-PD-1 antibody or an antigen-binding portion thereof;

(b) a dosage ranging from at least about 0.1 to at least about 10 mg/kg body weight of an anti-CTLA-4 antibody or an antigen-binding portion thereof; and

(c) instructions for using the anti-PD-1 antibody or the antigen-binding portion thereof and the anti-CTLA-4 antibody or the antigen-binding portion thereof in the methods disclosed herein.

[0125] The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of all references cited throughout this application are expressly incorporated herein by reference.

EXAMPLES

EXAMPLE 1

[0126] A randomized, double-blind, multicenter, phase 3 trial was conducted to evaluate the safety and efficacy of nivolumab alone or nivolumab combined with ipilimumab in comparison with ipilimumab alone in previously untreated metastatic melanoma.

Patients

[0127] Eligible patients had histologically confirmed stage III (unresectable) or stage IV melanoma, and no prior systemic treatment for unresectable or metastatic melanoma. Other eligibility criteria included an age of at least 18 years, an Eastern Cooperative Oncology Group (ECOG) performance status score of 0 (indicating no symptoms) or 1 (indicating mild symptoms), measurable disease by computed tomography or magnetic resonance imaging per RECIST v1.1, availability of tissue collected from metastatic or unresectable tumors for the assessment of PD-L1 status, and known BRAF V600 mutation status (or consent to BRAF V600 mutation testing per local standards). Key exclusion criteria were presence of active brain metastases, ocular melanoma, or

autoimmune disease, and any prior treatment with an anti-PD-1, anti-PD-L1, anti-PD-L2, or anti-CTLA-4 antibody. Patients who required systemic corticosteroid treatment or other immunosuppressive medications within 14 days of study drug administration were excluded.

Study Design and Treatment

[0128] In the double-blind, phase 3 study, enrolled patients were randomly assigned in a 1:1:1 ratio to receive 3 mg of nivolumab per kilogram of body weight every 2 weeks (plus ipilimumab-matched placebo), or 1 mg of nivolumab per kilogram every 3 weeks plus 3 mg of ipilimumab per kilogram every 3 weeks for 4 doses (plus nivolumab-matched placebo), followed by 3 mg of nivolumab per kilogram every 2 weeks for cycle 3 and beyond, or 3 mg of ipilimumab per kilogram every 3 weeks for 4 doses (plus nivolumab-matched placebo).

[0129] Both nivolumab and ipilimumab were administered by intravenous infusion. Randomization was stratified according to tumor PD-L1 status (positive vs. negative or indeterminate), BRAF mutation status (V600 mutation positive vs. wild-type), and American Joint Committee on Cancer metastasis stage (M0, M1a, or M1b vs. M1c). Treatment continued until RECIST v1.1-defined disease progression, unacceptable toxicity, or withdrawal of consent. Patients could be treated beyond progression provided they had a clinical benefit without clinical deterioration, and did not have substantial adverse effects, as assessed by the investigator.

[0130] Progression-free survival and overall survival were co-primary end points. Secondary end points included objective response rate, tumor PD-L1 expression as a predictive biomarker for progression-free and overall survival, and health-related quality of life. Exploratory end points include duration of objective response and safety/tolerability of study drug therapy.

Assessments

[0131] Patients were assessed for tumor response, according to RECIST v1.1 at 12 weeks after randomization and continuing every 6 weeks for 49 weeks, and then every 12 weeks until progression or treatment discontinuation, whichever occurred later. Progression-free survival was defined as the time between the date of randomization and the first date of documented progression or death, whichever occurred first. Patients treated beyond

progression were considered to have progressive disease at the time of the initial progression event, as assessed by the investigator, regardless of subsequent tumor responses. Expression of PD-L1 on the surface of the tumor cells was assessed in a central laboratory by immunohistochemistry in formalin-fixed, paraffin-embedded tumor specimens using a rabbit monoclonal anti-human PD-L1 antibody (clone 28-8) and an analytically validated automated assay developed by Dako (Carpinteria, CA). PD-L1 positivity was defined as at least 5% of tumor cells showing cell surface PD-L1 staining of any intensity in a section containing at least 100 tumor cells that could be evaluated. Indeterminate status was attributed to samples for which tumor cell–surface expression could not be discerned because of melanin content or strong cytoplasmic staining.

[0132] Any patient who received at least one dose of study drug in each of the 3 treatment groups was included in the assessment of safety. The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.18. Safety assessments were made continuously during the treatment phase, and up to 100 days after the last dose of study drug.

Statistical Analysis

[0133] A study sample size of approximately 915 patients, randomized to the 3 treatment arms in a 1:1:1 ratio, was planned. For the comparison of progression-free survival, the number of events projected to be observed at a follow-up of at least 9 months provided approximately 83% power to detect an average hazard ratio of 0.71 with a type I error of 0.005 (two-sided). Progression-free survival was compared between nivolumab plus ipilimumab and ipilimumab alone, and between nivolumab alone and ipilimumab alone with the use of a two-sided log-rank test stratified according to PD-L1 status, BRAF mutation status, and metastasis stage (as described above). The study was not designed for a formal statistical comparison between the nivolumab alone and nivolumab plus ipilimumab groups. Hazard ratios and corresponding two-sided 99.5% confidence intervals (CIs) were estimated using a Cox proportional hazards model, with treatment group as a single covariate, stratified by the above factors. Progression-free survival curves, medians with 95% CIs, and progression-free survival rates at 6, 12, and 18 months with 95% CIs were estimated using Kaplan-Meier methodology. Overall survival will be analyzed when all patients have a minimum follow-up of 22 months.

Results

Patients and Treatment

[0134] From July 2013 through March 2014, a total of 1296 patients were enrolled at 137 centers in Australia, Europe, Israel, New Zealand, and North America. A total of 945 patients underwent randomization: 316 patients were assigned to the nivolumab group, 314 to the nivolumab plus ipilimumab group, and 315 to the ipilimumab group (Figure 1). Baseline characteristics were balanced across the three groups. A total of 58.0% had stage M1c disease, 36.1% had an elevated lactate dehydrogenase level, 31.5% had a BRAF mutation, and 73.5% had negative PD-L1 status (Table 1).

Table 1. Baseline Characteristics of the Patients.

Characteristic	Nivolumab alone (N=316)	Nivolumab plus Ipilimumab (N=314)	Ipilimumab alone (N=315)	Total (N=945)
Age — yr				
Mean	58.7	59.3	60.8	59.6
Range	25–90	18–88	18–89	18–90
Age groups — no. (%)				
<65 yr	198 (62.7)	185 (58.9)	182 (57.8)	565 (59.8)
≥ 65, < 75 yr	79 (25.0)	94 (29.9)	89 (28.3)	262 (27.7)
≥ 75 yr	39 (12.3)	35 (11.1)	44 (14.0)	118 (12.5)
Sex — no. (%)				
Male	202 (63.9)	206 (65.6)	202 (64.1)	610 (64.6)
Female	114 (36.1)	108 (34.4)	113 (35.9)	335 (35.4)
ECOG performance status — no. (%)				
0	238 (75.3)	230 (73.2)	224 (71.1)	692 (73.2)
1	77 (24.4)	83 (26.4)	91 (28.9)	251 (26.6)
2	1 (0.3)	0	0	1 (0.1)
Not reported	0	1 (0.3)	0	1 (0.1)
M stage — no. (%)				
M1c	184 (58.2)	181 (57.6)	183 (58.1)	548 (58.0)
M0, M1a, or M1b	132 (41.8)	133 (42.4)	132 (41.9)	397 (42.0)
Lactate dehydrogenase — no. (%)*				
≤ ULN	196 (62.0)	199 (63.4)	194 (61.6)	589 (62.3)
>ULN	112 (35.4)	114 (36.3)	115 (36.5)	341 (36.1)
≤2x ULN	271 (85.8)	276 (87.9)	279 (88.6)	826 (87.4)

Characteristic	Nivolumab alone (N=316)	Nivolumab plus Ipilimumab (N=314)	Ipilimumab alone (N=315)	Total (N=945)
>2x ULN	37 (11.7)	37 (11.8)	30 (9.5)	104 (11.0)
Unknown	8 (2.5)	1 (0.3)	6 (1.9)	15 (1.6)
Brain metastases at baseline — no. (%)				
Yes	8 (2.5)	11 (3.5)	15 (4.8)	34 (3.6)
No	308 (37.5)	303 (96.5)	300 (95.2)	911 (96.4)
PD-L1 status — no. (%)				
Positive	80 (27.8)	68 (24.5)	75 (27.1)	223(26.4)
Negative	208 (72.2)	210 (75.5)	202 (72.9)	620 (73.5)
BRAF status — no. (%)				
Mutation	100 (31.6)	101 (32.2)	97 (30.8)	298 (31.5)
No mutation	216 (68.4)	213 (67.8)	218 (69.2)	647 (68.5)

* ULN denotes upper limit of normal.

[0135] All randomized patients had been followed for a minimum of 9 months at the time of database lock (February 17, 2015); 117 of 313 patients (37.4%) in the nivolumab group, 93 of 313 patients (29.7%) in the nivolumab plus ipilimumab group, and 50 of 311 patients (16.1%) in the ipilimumab group were continuing study treatment (Table 2). The most frequent reason for discontinuation was disease progression in the nivolumab and ipilimumab monotherapy groups (154 of 313 patients [49.2%] and 202 of 311 patients [65.0%], respectively), versus study drug toxicity in the nivolumab plus ipilimumab group (120 of 313 patients [38.3%]). The number of patients who had died was 85 (27.2%) in the nivolumab group, 86 (27.5%) in the nivolumab plus ipilimumab group, and 114 (36.7%) in the ipilimumab group.

[0136] The median number of doses in patients who received nivolumab alone or ipilimumab alone was 15 (range 1-38) and 4 (1-4), respectively. In the combination group, the median number of doses was 4 (range 1–39) for nivolumab and 4 (range 1–4) for ipilimumab; 147 of 313 patients (47%) received four or more doses of nivolumab monotherapy after combination treatment.

Efficacy

[0137] The median progression-free survival was 6.5 months (95% confidence interval [CI], 4.3 to 9.5) in the nivolumab group, 11.5 months (95% CI, 8.9 to 16.5) in the nivolumab plus ipilimumab group, and 2.9 months (95% CI, 2.8 to 3.4) in the ipilimumab group (Figure 2A). A significant improvement in progression-free survival was observed in the nivolumab plus ipilimumab group as compared with the ipilimumab group (hazard ratio, 0.42; 95% CI, 0.31 to 0.57; P<0.0001) (Figure 2A). A significant improvement in progression-free survival was also observed in the nivolumab group as compared with the ipilimumab group (hazard ratio, 0.57; 95% CI, 0.43 to 0.76; P<0.00001) (Figure 2A). The hazard ratio for the comparison between nivolumab plus ipilimumab and nivolumab groups was 0.74 (95% CI, 0.60 to 0.92).

[0138] Analyses of progression-free survival among prespecified patient subgroups showed a consistent improvement with nivolumab or nivolumab plus ipilimumab as compared with ipilimumab, including subgroups defined by PD-L1 status, BRAF mutation status, and metastasis stage (Figure 3). In the combination group, median PFS was 11.7 months (95% CI, 8.0 to not reached) among patients with a BRAF mutation and was 11.2 months (95% CI, 8.3 to not reached) in patients with wild-type BRAF (Figure

3B). For patients with a positive PD-L1 tumor status, median progression-free survival in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups was 14.0 months (95% CI, 9.1 to not reached), 14.0 months (95% CI, 9.7 to not reached), and 3.9 months (95% CI, 2.8 to 4.2), respectively (Figure 2B). For patients with a negative PD-L1 tumor status, median progression-free survival in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups was 5.3 months (95% CI, 2.8 to 7.1 months), 11.2 months (95% CI, 8.0 to not reached) and 2.8 months (95% CI, 2.8 to 3.1), respectively (Fig. 2C).

[0139] Investigator-assessed objective response rates were 43.7% (95% CI, 38.1 to 49.3%), 57.6% (95% CI, 52.0 to 63.2), and 19.0% (95% CI, 14.9 to 23.8) in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively (Table 3). The percentage of patients with a complete response was higher in the nivolumab plus ipilimumab group than in either the nivolumab or ipilimumab alone groups (11.5% vs. 8.9% and 2.2%) (Table 3). Time to objective response was similar in each group (Table 3), and the median duration of response was not reached in any of the groups.

[0140] Median reduction in the sum of the longest diameters of tumor target lesions was -34.5% (interquartile range: -75.4 to 15.4), -51.1% (-75.8 to -10.2), and 5.8% (-28.0 to 33.3) in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively (Figure 4). Among patients with PD-L1-positive tumors, the objective response rates were 57.5% (95% CI, 45.9 to 68.5), 72.1% (95% CI, 59.9 to 82.3), and 21.3% (95% CI, 12.7 to 32.3) for the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively; in patients with PD-L1-negative tumors, the objective response rates were 41.3% (95% CI, 34.6 to 48.4), 54.8% (95% CI, 47.8 to 61.6), and 17.8% (95% CI, 12.8 to 23.8) (Table 4).

Table 2. Patient Disposition.*

	Nivolumab alone (N=313)	Nivolumab plus Ipilimumab (N=313)	Ipilimumab alone (N=311)
Patients in the treatment period — no. (%)			
Continuing	117 (37.4)	93 (29.7)	50 (16.1)
Not continuing	196 (62.6)	220 (70.3)	261 (83.9)
Reason for not continuing the treatment — no. (%)			
Disease progression	154 (49.2)	69 (22.0)	202 (65.0)
Study drug toxicity	27 (8.6)	120 (38.3)	47 (15.1)
Adverse event unrelated to study drug	5 (1.6)	12 (3.8)	4 (1.3)
Patient request to discontinue treatment	5 (1.6)	5 (1.6)	4 (1.3)
Death	5 (1.6)	5 (1.6)	4 (1.3)
Maximum clinical benefit	2 (0.6)	2 (0.6)	0
Poor/non-compliance	1 (0.3)	1 (0.3)	1 (0.3)
Patient withdrew consent	0	3 (1.0)	0
Lost to follow-up	1 (0.3)	0	0
Patient no longer meets study criteria	0	1 (0.3)	0
Other	0	3 (1.0)	2 (0.6)
Patients in the study — no. (%)			
Continuing	223 (71.2)	221 (70.6)	189 (60.8)
Died	85 (27.2)	86 (27.5)	114 (36.7)
Not continuing	5 (1.6)	6 (1.9)	8 (2.6)

***At the time of database lock on February 17, 2015**.

Table 3. Response to Treatment.

Response	Nivolumab alone (N=316)	Nivolumab plus Ipilimumab (N=314)	Ipilimumab alone (N=315)
Best overall response — no. (%)[†]			
Complete response	28 (8.9)	36 (11.5)	7 (2.2)
Partial response	110 (34.8)	145 (46.2)	53 (16.8)
Stable disease	34 (10.8)	41 (13.1)	69 (21.9)
Progressive disease	119 (37.7)	71 (22.6)	154 (48.9)
Could not be determined	25 (7.9)	21 (6.7)	32 (10.2)
Objective response[‡]			
No. of patients (% [95% CI])	138 (43.7 [38.1–49.3])	181 (57.6 [52.0–63.2])	60 (19.0 [14.9–23.8])
Estimated odds ratio (95% CI)[§]	3.40 (2.02–5.72)	6.11 (3.59–10.38)	---
Two-sided P value	<0.00001	<0.00001	---
Time to objective response — mo			
No. of responders	138	181	60
Median	2.78	2.76	2.79
Range	2.3–12.5	1.1–11.6	2.5–12.4

[†] ***Best overall response were assessed by the investigators with the use of RECIST v1.1.***

[‡] ***Data include patients with a complete response and those with a partial response.***

[§] ***Relative to ipilimumab alone.***

Table 4. Objective Response Rate by PD-L1 Status.

PD-L1 Positive			
	Nivolumab alone (N=80)	**Nivolumab plus Ipilimumab (N=68)**	**Ipilimumab alone (N=75)**
Objective response†			
No. of patients (% [95% CI])	46 (57.5 [45.9–68.5])	49 (72.1 [59.9–82.3])	16 (21.3 [12.7–32.3])
Estimated odds ratio (95% CI)§	5.03 (2.44–10.37)	10.41 (4.63–23.40)	---
PD-L1 Negative			
	Nivolumab alone (N=208)	**Nivolumab plus Ipilimumab (N=210)**	**Ipilimumab alone (N=202)**
Objective response†			
No. of patients (% [95% CI])	86 (41.3 [34.6–48.4])	115 (54.8 [47.8–61.6])	36 (17.8 [12.8–23.8])
Estimated odds ratio (95% CI)§	3.25 (2.05–5.13)	5.90 (3.71–9.38)	---

† ***Best overall response was assessed by the investigators with the use of RECIST v1.1.***

§ Relative to ipilimumab alone.

Adverse Events

[0141] Treatment-related adverse events of any grade occurred in 82.1%, 95.5%, and 86.2% of patients in the nivolumab, nivolumab plus ipilimumab groups, and ipilimumab groups, respectively (Table 5). The most common adverse events in the nivolumab group were fatigue (in 34.2% of patients), rash (in 21.7%), and diarrhea (in 19.2%). In the nivolumab plus ipilimumab and ipilimumab groups, diarrhea (in 44.1% and 33.1% of patients, respectively), fatigue (in 35.1% and 28.0%), and pruritus (in 33.2% and 35.4%) were most common (Table 5). The incidence of treatment-related adverse events of grade 3 or 4 was also higher in the nivolumab plus ipilimumab group than in either the nivolumab or ipilimumab groups (55.0% vs. 16.3% and 27.3%), with diarrhea being the most common (2.2% , 9.3%, and 6.1% in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively) (Table 5). Treatment-related adverse events of any grade leading to discontinuation occurred in 7.7%, 36.4%, and 14.8% of patients in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively, with the most common being diarrhea (in 1.9%, 8.3%, and 4.5%, respectively) and colitis (in 0.6%, 8.3%, and 7.7%, respectively) (Table 5). One death due to study-drug toxicity was reported in the nivolumab group (neutropenia) and one in the ipilimumab group (cardiac arrest), although such adverse events have not been associated with these drugs in prior studies. No treatment-related deaths were reported in the combination group.

[0142] The most frequent grade 3 or 4 treatment-related select adverse events were diarrhea (2.2%, 9.3%, and 6.1% of patients in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively), colitis (in 0.6%, 7.7%, and 8.7%, respectively), increased alanine aminotransferase (in 1.3%, 8.3%, and 1.6%, respectively), and increased aspartate aminotransferase (in 1.0%, 6.1%, and 0.6%, respectively) (Table 6). With the use of immune modulatory agents, resolution rates for grade 3 or 4 select adverse events were generally similar across treatment groups, and were between 85-100% across organ categories in the nivolumab plus ipilimumab group. As observed in prior studies, most endocrine events in all treatment groups did not resolve (Table 7).

Table 5. Adverse Events (Safety Population).*

Event	Nivolumab alone (N=313)		Nivolumab plus Ipilimumab (N=313)		Ipilimumab alone (N=311)	
	Total	Grade 3 or 4	Total	Grade 3 or 4	Total	Grade 3 or 4
	no. of patients with event (%)					
Any adverse event	311 (99.4)	136 (43.5)	312 (99.7)	215 (68.7)	308 (99.0)	173 (55.6)
Treatment-related adverse event†	257 (82.1)	51 (16.3)	299 (95.5)	172 (55.0)	268 (86.2)	85 (27.3)
Diarrhea	60 (19.2)	7 (2.2)	138 (44.1)	29 (9.3)	103 (33.1)	19 (6.1)
Fatigue	107 (34.2)	4 (1.3)	110 (35.1)	13 (4.2)	87 (28.0)	3 (1.0)
Pruritus	59 (18.8)	0	104 (33.2)	6 (1.9)	110 (35.4)	1 (0.3)
Rash	81 (25.9)	2 (0.6)	126 (40.3)	15 (4.8)	102 (32.8)	6 (1.9)
Nausea	41 (13.1)	0	81 (25.9)	7 (2.2)	50 (16.1)	2 (0.6)
Pyrexia	18 (5.8)	0	58 (18.5)	2 (0.6)	21 (6.8)	1 (0.3)
Decreased appetite	34 (10.9)	0	56 (17.9)	4 (1.3)	39 (12.5)	1 (0.3)
Increase in alanine aminotransferase	12 (3.8)	4 (1.3)	55 (17.6)	26 (8.3)	12 (3.9)	5 (1.6)
Vomiting	20 (6.4)	1 (0.3)	48 (15.3)	8 (2.6)	23 (7.4)	1 (0.3)
Increase in aspartate aminotransferase	12 (3.8)	3 (1.0)	48 (15.3)	19 (6.1)	11 (3.5)	2 (0.6)
Hypothyroidism	27 (8.6)	0	47 (15.0)	1 (0.3)	13 (4.2)	0
Colitis	4 (1.3)	2 (0.6)	37 (11.8)	24 (7.7)	36 (11.6)	27 (8.7)
Arthralgia	24 (7.7)	0	33 (10.5)	1 (0.3)	19 (6.1)	0
Headache	23 (7.3)	0	32 (10.2)	1 (0.3)	24 (7.7)	1 (0.3)
Dyspnea	14 (4.5)	1 (0.3)	32 (10.2)	2 (0.6)	13 (4.2)	0
Treatment-related adverse event leading to discontinuation	24 (7.7)	16 (5.1)	114 (36.4)	92 (29.4)	46 (14.8)	41 (13.2)

* The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

† The treatment-related adverse events listed here were reported in at least 10% of the patients in any of the three study groups.

Table 6. Treatment-related Select Adverse Events (Safety Population).*

Event	Nivolumab alone (N=313)		Nivolumab plus Ipilimumab (N=313)		Ipilimumab alone (N=311)	
	Total	Grade 3 or 4	Total	Grade 3 or 4	Total	Grade 3 or 4
	no. of patients with event (%)					
Any select adverse event	194 (62.0)	24 (7.7)	275 (87.9)	124 (39.6)	229 (73.6)	58 (18.6)
Treatment-related select adverse event†						
Skin	131 (41.9)	5 (1.6)	185 (59.1)	18 (5.8)	168 (54.0)	9 (2.9)
Pruritus	59 (18.8)	0	104 (33.2)	6 (1.9)	110 (35.4)	1 (0.3)
Rash	68 (21.7)	1 (0.3)	89 (28.4)	9 (2.9)	65 (20.9)	5 (1.6)
Rash maculo-papular	13 (4.2)	1 (0.3)	37 (11.8)	6 (1.9)	37 (11.9)	1 (0.3)
Vitiligo	23 (7.3)	1 (0.3)	21 (6.7)	0	12 (3.9)	0
Gastrointestinal	61 (19.5)	7 (2.2)	145 (46.3)	46 (14.7)	114 (36.7)	36 (11.6)
Diarrhea	60 (19.2)	7 (2.2)	138 (44.1)	29 (9.3)	103 (33.1)	19 (6.1)
Colitis	4 (1.3)	2 (0.6)	37 (11.8)	24 (7.7)	36 (11.6)	27 (8.7)
Hepatic	20 (6.4)	8 (2.6)	94 (30.0)	59 (18.8)	22 (7.1)	5 (1.6)
Increase in alanine aminotransferase	12 (3.8)	4 (1.3)	55 (17.6)	26 (8.3)	12 (3.9)	5 (1.6)
Increase in aspartate aminotransferase	12 (3.8)	3 (1.0)	48 (15.3)	19 (6.1)	11 (3.5)	2 (0.6)
Endocrine	45 (14.4)	2 (0.6)	94 (30.0)	15 (4.8)	34 (10.9)	7 (2.3)
Hypothyroidism	27 (8.6)	0	47 (15.0)	1 (0.3)	13 (4.2)	0
Hyperthyroidism	13 (4.2)	0	31 (9.9)	3 (1.0)	3 (1.0)	0
Hypophysitis	2 (0.6)	1 (0.3)	24 (7.7)	5 (1.6)	12 (3.9)	6 (1.9)
Pulmonary	5 (1.6)	1 (0.3)	22 (7.0)	3 (1.0)	6 (1.9)	1 (0.3)
Pneumonitis	4 (1.3)	1 (0.3)	20 (6.4)	3 (1.0)	5 (1.6)	1 (0.3)

* The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

† The treatment-related select adverse events listed here were reported in at least 5% of the patients in any of the three study groups.

Table 7. Management of Treatment-related Select Adverse Events With Immune Modulatory Medication (IMM).

	Nivolumab			Nivolumab plus Ipilimumab			Ipilimumab		
Select Adverse Event Organ Category	**Patients managed with IMM, n (%)**	**Patients with resolution of AE after treatment with IMM, n (%)**	**Median time to resolution, weeks (95% CI)**	**Patients managed with IMM, n (%)**	**Patients with resolution of AE after treatment with IMM, n (%)**	**Median time to resolution, weeks (95% CI)**	**Patients managed with IMM, n (%)**	**Patients with resolution of AE after treatment with IMM, n (%)**	**Median time to resolution, weeks (95% CI)**
Skin Grade 3-4	33/131 (25.2) 3/5 (60.0)	17 (54.8) 3 (75.0)	34.9 (18.0, NE) 2.1 (0.9, NE)	80/185 (43.2) 12/18 (66.7)	55 (75.3) 12 (85.7)	8.6 (7.0, 12.6) 3.4 (1.1, 12.4)	58/168 (34.5) 5/9 (55.6)	41 (74.5) 5 (83.3)	12.4 (8.9, 19.1) 6.1 (4.4, NE)
Gastrointestinal Grade 3-4	9/61 (14.8) 5/7 (71.4)	5 (71.4) 3 (50.0)	4.0 (0.8, NE) NE (0.8, NE)	71/145 (49.0) 41/46 (89.1)	62 (93.9) 41 (97.6)	4.5 (3.5, 5.7) 3.0 (1.9, 4.3)	54/114 (47.4) 33/36 (91.7)	44 (88.0) 31 (88.6)	4.9 (2.9, 7.6) 4.7 (1.9, 6.1)
Endocrine Grade 3-4	5/45 (11.1) 2/2 (100)	2 (40.0) 0	24.3 (7.1, NE) NE (NE, NE)	36/94 (38.3) 10/15 (66.7)	14 (41.2) 5 (45.5)	NE (10.4, NE) NE (4.5, NE)	15/34 (44.1) 7/7 (100.0)	4 (28.6) 3 (42.9)	NE (0.71, NE) NE (0.43, NE)
Hepatic Grade 3-4	8/22 (36.4) 7/8 (87.5)	6 (100.0) 6 (100.0)	7.0 (2.0, 27.1) 7.0 (2.0, 27.1)	46/95 (48.4) 37/60 (61.7)	43 (95.6) 38 (100.0)	5.9 (3.3, 6.6) 4.1 (3.0, 6.1)	3/22 (13.6) 2/5 (40.0)	3 (100.0) 2 (100.0)	4.1 (4.0, 7.7) 5.9 (4.0, 7.7)
Pulmonary Grade 3-4	4/5 (80.0) 1/1 (100)	4 (100.0) 1 (100.0)	3.3 (2.3, 9.1) 2.3 (NE, NE)	17/22 (77.3) 2/3 (66.7)	16 (94.1) 2 (100.0)	6.1 (1.1, 8.3) 4.2 (1.1, 7.3)	3/6 (50.0) 1/1 (100)	2 (66.7) 1 (100.0)	6.1 (6.0, 6.3) 4.7 (NE, NE)
Renal Grade 3-4	1/3 (33.3) 0/1	1 (100.0) 0	0.3 (NE, NE) -	4/17 (23.5) 3/6 (50.0)	3 (100.0) 3 (100.0)	1.7 (0.4, 3.7) 1.7 (0.4, 3.6)	3/8 (37.5) 1/1 (100)	3 (100.0) 1 (100.0)	4.6 (0.6, 16.1) 4.6 (NE, NE)

Discussion

[0143] In this randomized, double-blind, phase 3 study, both nivolumab alone and the combination of nivolumab and ipilimumab significantly increased progression-free survival and objective response rates, as compared with ipilimumab alone, in previously untreated advanced melanoma. These results were observed independently of PD-L1 tumor status, BRAF mutation status, or metastasis stage. Baseline characteristics of study participants were typical of patients with advanced melanoma, although the BRAF mutation rate (31.5%) was lower than the 40-50% generally reported for advanced disease. While not a primary end point of the study, the combination of nivolumab and ipilimumab resulted in numerically longer progression-free survival and a higher response rate as compared with nivolumab alone in the overall study population. While time to response was similar between groups, the first tumor assessment was done at week 12 and thus the possibility that responses may have occurred earlier with the combination remains unknown.

[0144] The median progression-free survival reported for the combination of nivolumab and ipilimumab in this study (11.7 months in BRAF-mutant patients) is similar to that recently reported for the combination of BRAF and MEK inhibition in BRAF-mutant metastatic melanoma (9.9 months for vemurafenib and cobimetinib; 9.3 to 11.4 months for dabrafenib and trametinib). Resistance to such targeted therapies is almost inevitable when used as monotherapy and in many cases is very rapid. The confirmed rate of objective response for combined nivolumab and ipilimumab (57.6%) is numerically higher than observed with PD-1 blockade alone in advanced melanoma (nivolumab [40%] in treatment-naïve patients with wild-type BRAF or pembrolizumab [37%] in ipilimumab-naïve patients).

[0145] The results of subgroup analyses suggest that the greatest benefit for the combination of nivolumab and ipilimumab versus nivolumab alone may occur in the setting of negative PD-L1 tumor expression. In the PD-L1-positive group, both nivolumab alone and nivolumab plus ipilimumab resulted in a similar prolongation of progression-free survival as compared with ipilimumab alone, although objective response rates were numerically higher in the combination group versus either nivolumab or ipilimumab alone. Thus, the use of PD-L1 as a biomarker may allow clinicians to make more informed decisions about the risk-benefit of combination therapy versus

monotherapy. Nonetheless, the observation of at least additive activity of the combination of ipilimumab and nivolumab in the setting of negative PD-L1 expression is of interest in melanoma as well as in other tumor types in which PD-1 checkpoint inhibitors are under evaluation.

[0146] The incidence of adverse events in this study was, in general, lowest in the nivolumab group and highest in the combination group. The overall incidence of grade 3 or 4 drug-related adverse events was higher in the combination group as compared with ipilimumab alone (39.6% versus 18.6%), as a result of a slightly higher incidence in most adverse events, particularly hepatic toxicity, where the rates of grade 3 or 4 ALT/AST elevations were 6-8% for the combination and approximately 1% for ipilimumab alone. One drug-related death was reported in each of the nivolumab and ipilimumab groups but none in the combination group. Overall, the safety profile of the combination of nivolumab and ipilimumab was consistent with previous experience with nivolumab or ipilimumab alone. No new safety signals were identified, and adverse events were manageable with established treatment guidelines as most select adverse events resolved with immune modulatory agents. These data suggest that the combination of nivolumab and ipilimumab can be used safely in a broad range of clinical settings.

[0147] In summary, the report shows increased progression-free survival and objective response rates for nivolumab alone and the combination of nivolumab and ipilimumab, as compared with ipilimumab alone, in previously untreated advanced melanoma. Adverse events with the combination were managed with established algorithms, with no study drug-related deaths. The combination of nivolumab and ipilimumab may represent a means to improve outcomes with either agent as monotherapy, particularly for patients having PD-L1-negative tumors. Overall, nivolumab alone and the combination of nivolumab and ipilimumab are promising treatment options for previously untreated advanced melanoma.

[0148] The present application claims benefit to U.S. Provisional Application No. 61/153,973 filed April 28, 2015, which is incorporated herein by reference in its entirety.

SEQUENCES

V_H amino acid sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:1)

Met Glu Thr Gly Leu Arg Trp Leu Leu Leu Val Ala Val Leu Lys Gly Val Gln Cys Leu Ser Val Glu Glu Ser Gly Gly Arg Leu Val Thr Pro Gly Thr Pro Leu Thr Leu Thr Cys Thr Ala Ser Gly Phe Thr Ile Thr Asn Tyr His Met Phe Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile Gly Val Ile Thr Ser Ser Gly Ile Gly Ser Ser Ser Thr Thr Tyr Tyr Ala Thr Trp Ala Lys Gly Arg Phe Thr Ile Ser Lys Thr Ser Thr Thr Val Asn Leu Arg Ile Thr Ser Pro Thr Thr Glu Asp Thr Ala Thr Tyr Phe Cys Ala Arg Asp Tyr Phe Thr Asn Thr Tyr Tyr Ala Leu Asp Ile Trp Gly Pro Gly Thr Leu Val Thr Val Ser Ser

V_L amino acid sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:2)

Met Asp Thr Arg Ala Pro Thr Gln Leu Leu Gly Leu Leu Leu Leu Trp Leu Pro Gly Ala Arg Cys Ala Leu Val Met Thr Gln Thr Pro Ser Ser Thr Ser Thr Ala Val Gly Gly Thr Val Thr Ile Lys Cys Gln Ala Ser Gln Ser Ile Ser Val Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ser Ala Ser Thr Leu Ala Ser Gly Val Pro Ser Arg Phe Lys Gly Ser Arg Ser Gly Thr Glu Tyr Thr Leu Thr Ile Ser Gly Val Gln Arg Glu Asp Ala Ala Thr Tyr Tyr Cys Leu Gly Ser Ala Gly Ser

Heavy Chain CDR1 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:3)

Asn Tyr His Met Phe

Heavy Chain CDR2 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:4)

Val Ile Thr Ser Ser Gly Ile Gly Ser Ser Ser Thr Thr Tyr Tyr Ala Thr Trp Ala Lys Gly

Heavy Chain CDR3 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:5)

Asp Tyr Phe Thr Asn Thr Tyr Tyr Ala Leu Asp Ile

Light Chain CDR1 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:6)

Gln Ala Ser Gln Ser Ile Ser Val Tyr Leu Ala

Light Chain CDR2 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:7)

Ser Ala Ser Thr Leu Ala Ser

Light Chain CDR3 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:8)

Leu Gly Ser Ala Gly Ser Asp Asp Ala Ala

What is claimed is:

1. A method for treating a melanoma comprising:
   (i) identifying a patient having a PD-L1-negative melanoma tumor; and
   (ii) administering to the patient:
      (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and
      (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

2. A method for treating a melanoma comprising administering to a patient afflicted with a melanoma tumor:
      (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and
      (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4,

   wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration.

3. A method for extending a progression-free survival period for over 8 months in a patient afflicted with a melanoma tumor comprising administering to the patient:
      (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and
      (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4,

   wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the patient demonstrates progression-free survival for over 8 months.

4. The method of claim 3, wherein the progression-free survival of the patient is extended after the administration for over about 11 months, about 12 months, about 13 months, about 14 months, about 15 months, about 16 months, about 17 months, about 18 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, or about 10 years.

5. The method of claim 4, wherein the progression-free survival of the patient is extended for over 11 months.

6. A method for reducing a tumor size at least by 10% in a patient afflicted with a melanoma tumor comprising administering to the patient:

(a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and

(b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4,

wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration.

7. The method of any one of claims 2 to 6, further comprising identifying the patient as having a melanoma tumor that does not express PD-L1 prior to the administration.

8. The method of any one of claims 1 to 7, wherein the patient is characterized by (i) extended progression-free survival for over 8 months, (ii) tumor size reduction at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration, or (iii) both.

9. A method for increasing an objective response rate to be higher than 40% in a patient population, each of whom is afflicted with a melanoma tumor, in a cancer treatment comprising administering to the patient:

(a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and

(b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4,

wherein each patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the objective response rate is higher than 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%.

10. The method of claim 9, further comprising identifying each patient of the patient population as having a PD-L1-negative melanoma tumor prior to the administration.

11. The method of claim 9 or 10, wherein each patient of the patient population is further characterized by (i) extended progression-free survival for over 8 months, (ii) tumor size reduction at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration, or (iii) both.

12. The method of any one of claims 9 to 11, wherein the patient population comprises at least about 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 patients having a PD-L1-negative melanoma tumor.

13. A method for selecting a patient suitable for an anti-PD-1 antibody and anti-CTLA-4 antibody combination therapy comprising:
(i) identifying a patient having a PD-L1-negative melanoma tumor; and
(ii) instructing a healthcare provider to administer to the patient:
(a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and
(b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

14. The method of any one of claims 1, 7, 10, and 13, wherein the identifying comprises measuring a PD-L1 expression on the melanoma tumor.

15. The method of any one of claims 1 to 14, wherein the administration treats the melanoma tumor.

16. The method of claim 14 or 15, wherein the measuring comprises providing a test tissue sample obtained from the patient, the test tissue sample comprising tumor cells and/or tumor-infiltrating inflammatory cells.

17. The method of claim 16, wherein the measuring further comprises assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface.

18. The method of claim 17, wherein the test tissue sample is a formalin-fixed paraffin-embedded (FFPE) tissue sample.

19. The method of claim 18, wherein the presence of PD-L1 is determined using an automated IHC assay.

20. The method of claim 19, wherein the IHC assay is performed using an anti-PD-L1 monoclonal antibody that specifically binds to the PD-L1 and wherein the anti-PD-L1 monoclonal antibody is selected from the group consisting of 28-8, 28-1, 28-12, 29-8, 5H1, and any combination thereof.

21. The method of any one of claims 1 to 20, wherein the PD-L1-negative melanoma tumor is characterized by having less than about 5%, about 4%, about 3%, about 2%, about 1%, or about 0% of tumor cells showing binding to the anti-PD-L1 antibody or an antigen-binding portion thereof.

22. The method of any one of claims 1 to 21, wherein the anti-PD-1 antibody or antigen-binding portion thereof cross-competes with nivolumab for binding to human PD-1.

23. The method of any one of claims 1 to 22, wherein the anti-PD-1 antibody or antigen-binding portion thereof is a chimeric, humanized or human monoclonal antibody or a portion thereof.

24. The method of any one of claims 1 to 23, wherein the anti-PD-1 antibody or antigen-binding portion thereof comprises a heavy chain constant region which is of a human IgG1 or IgG4 isotype.

25. The method of any one of claims 1 to 24, wherein the anti-PD-1 antibody is nivolumab.

26. The method of any one of claims 1 to 24, wherein the anti-PD-1 antibody is pembrolizumab.

27. The method of any one of claims 1 to 26, wherein the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose ranging from 0.1 to 10.0 mg/kg body weight once every 2, 3 or 4 weeks.

28. The method of claim 27, wherein the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of 1 or 3 mg/kg body weight once every 3 weeks.

29. The method of any one of claims 1 to 28, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof cross-competes with ipilimumab for binding to human CTLA-4.

30. The method of any one of claims 1 to 29, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof is a chimeric, humanized or human monoclonal antibody or a portion thereof.

31. The method of any one of claims 1 to 30, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof comprises a heavy chain constant region which is of a human IgG1 isotype.

32. The method of any one of claims 1 to 31, wherein the anti-CTLA-4 antibody is ipilimumab.

33. The method of any one of claims 1 to 31, wherein the anti-CTLA-4 antibody is tremelimumab.

34. The method of any one of claims 1 to 33, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof is administered at a dose ranging from 0.1 to 10.0 mg/kg body weight once every 2, 3 or 4 weeks.

35. The method of claim 34, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof is administered at a dose of 1 or 3 mg/kg body weight once every 3 weeks.

36. The method of claim 35, wherein the anti-PD-1 antibody or an antigen-binding portion thereof is administered at a dose of 1 mg/kg body weight once every 3 weeks and the anti-CTLA-4 antibody or an antigen-binding portion thereof is administered at a dose of 3 mg/kg body weight once every 3 weeks.

37. The method of any of claims 1 to 36, wherein the anti-PD-1 antibody or an antigen-binding portion thereof is administered concurrently with the anti-CTLA-4 antibody or an antigen-binding portion thereof.

38. The method of any of claims 1 to 36, wherein the anti-PD-1 antibody or an antigen-binding portion thereof is administered prior to or after the anti-CTLA-4 antibody or an antigen-binding portion thereof.

39. The method of any of claims 1 to 36, wherein the anti-PD-1 antibody or an antigen-binding portion thereof and the anti-CTLA-4 antibody or an antigen-binding portion thereof are in a single pharmaceutical composition.

40. The method of claim 39, wherein the pharmaceutical composition comprises the anti-PD-1 antibody and the anti-CTLA-4 antibody at a ratio of 50:1 to 1:50.

41. The method of claim 39, wherein the pharmaceutical composition comprises the anti-PD-1 antibody and the anti-CTLA-4 antibody at a ratio of 10:1 to 1:10.

42. The method of claim 39, wherein the pharmaceutical composition comprises the anti-PD-1 antibody and the anti-CTLA-4 antibody at a ratio of 3:1 to 1:3.

43. The method of claim 39, wherein the pharmaceutical composition comprises the anti-PD-1 antibody and the anti-CTLA-4 antibody at a ratio of 1:3.

44. The method of claim 39, wherein the pharmaceutical composition comprises 80 mg of the anti-PD-1 antibody and 240 mg of the anti-CTLA-4 antibody.

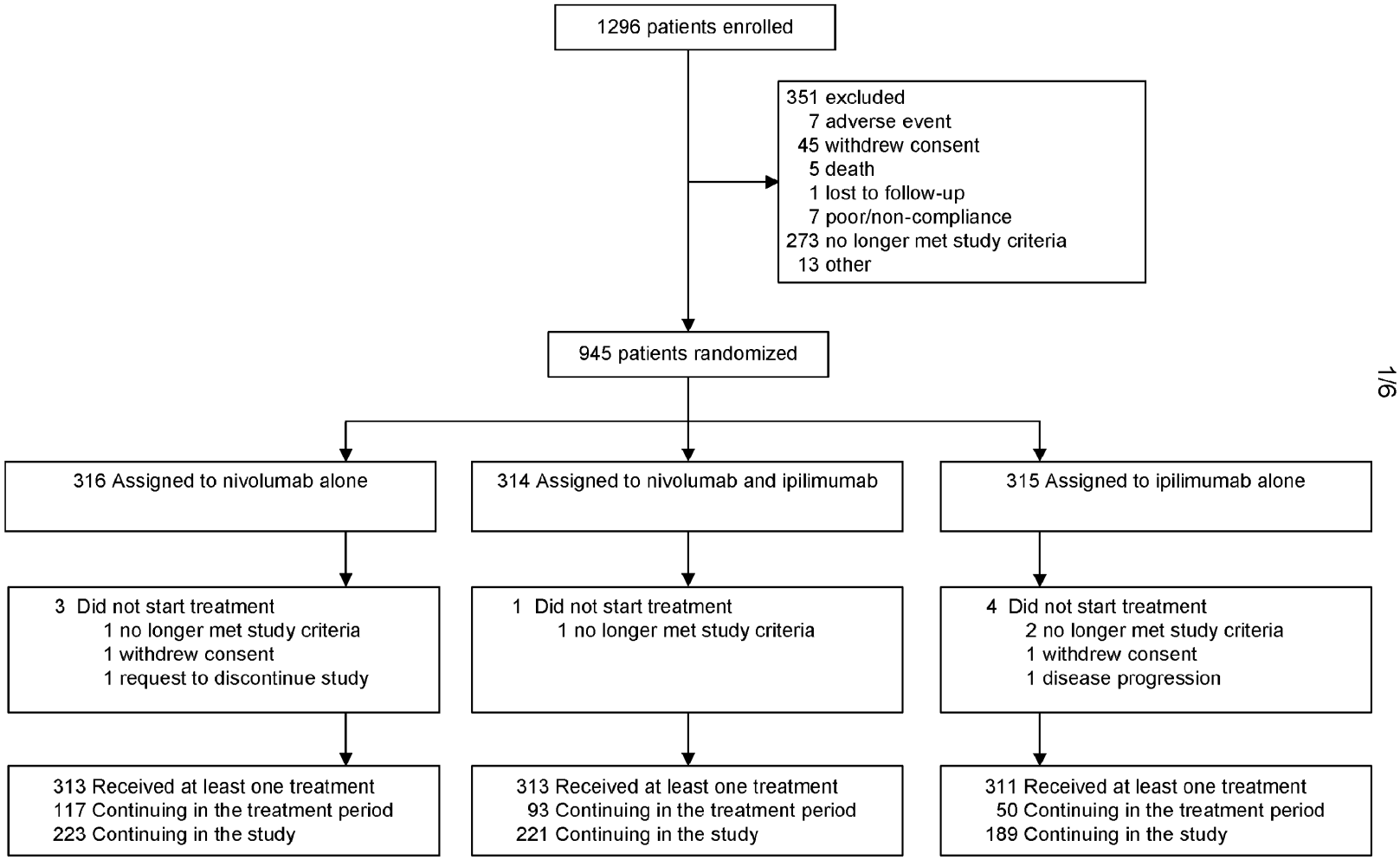
45. The method of claim any of claims 1 to 38, wherein the anti-PD-1 and anti-CTLA-4 antibodies are administered within 30 minutes of each other.

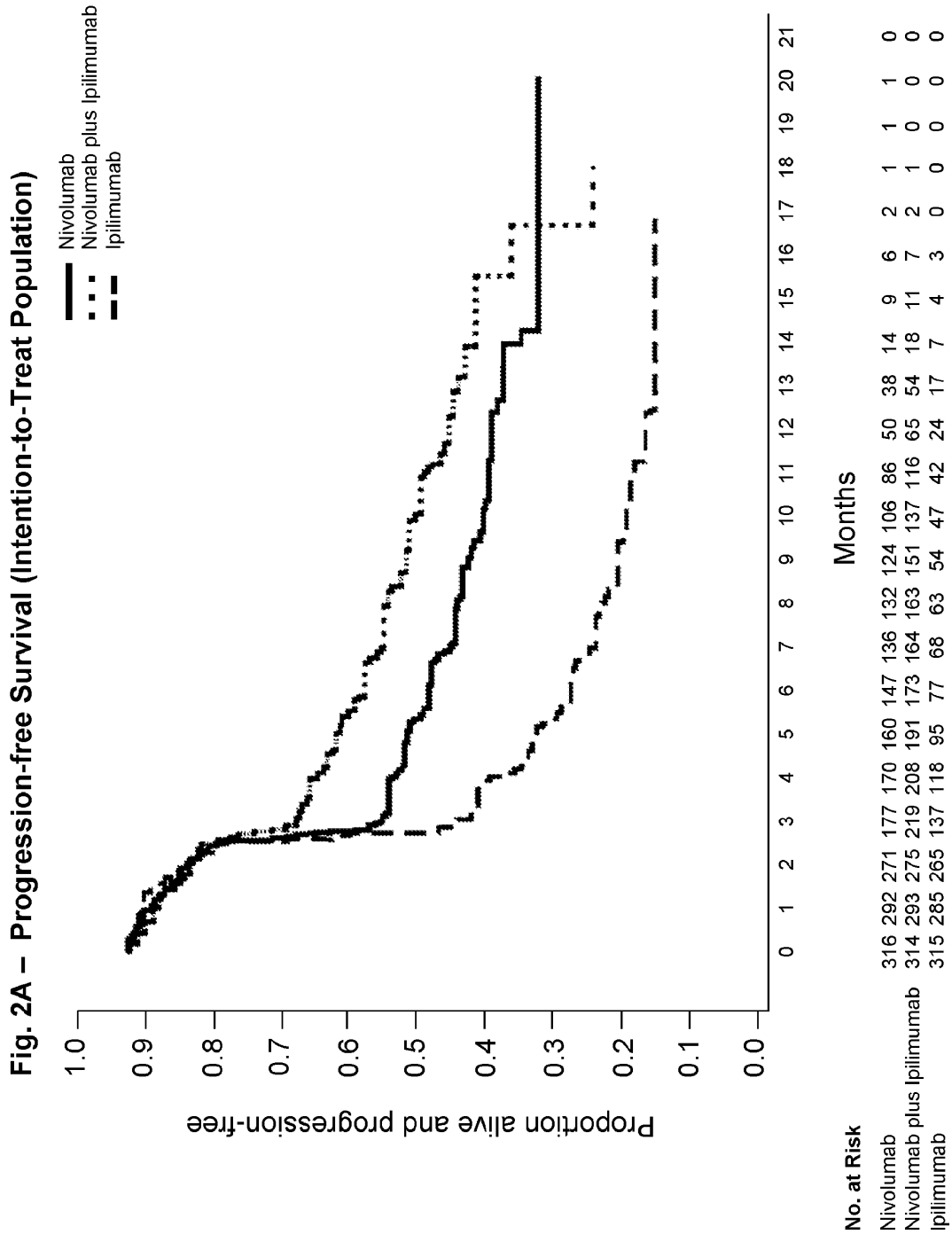
46. The method of any one of claims 1 to 45, further comprising administering an anti-cancer agent.

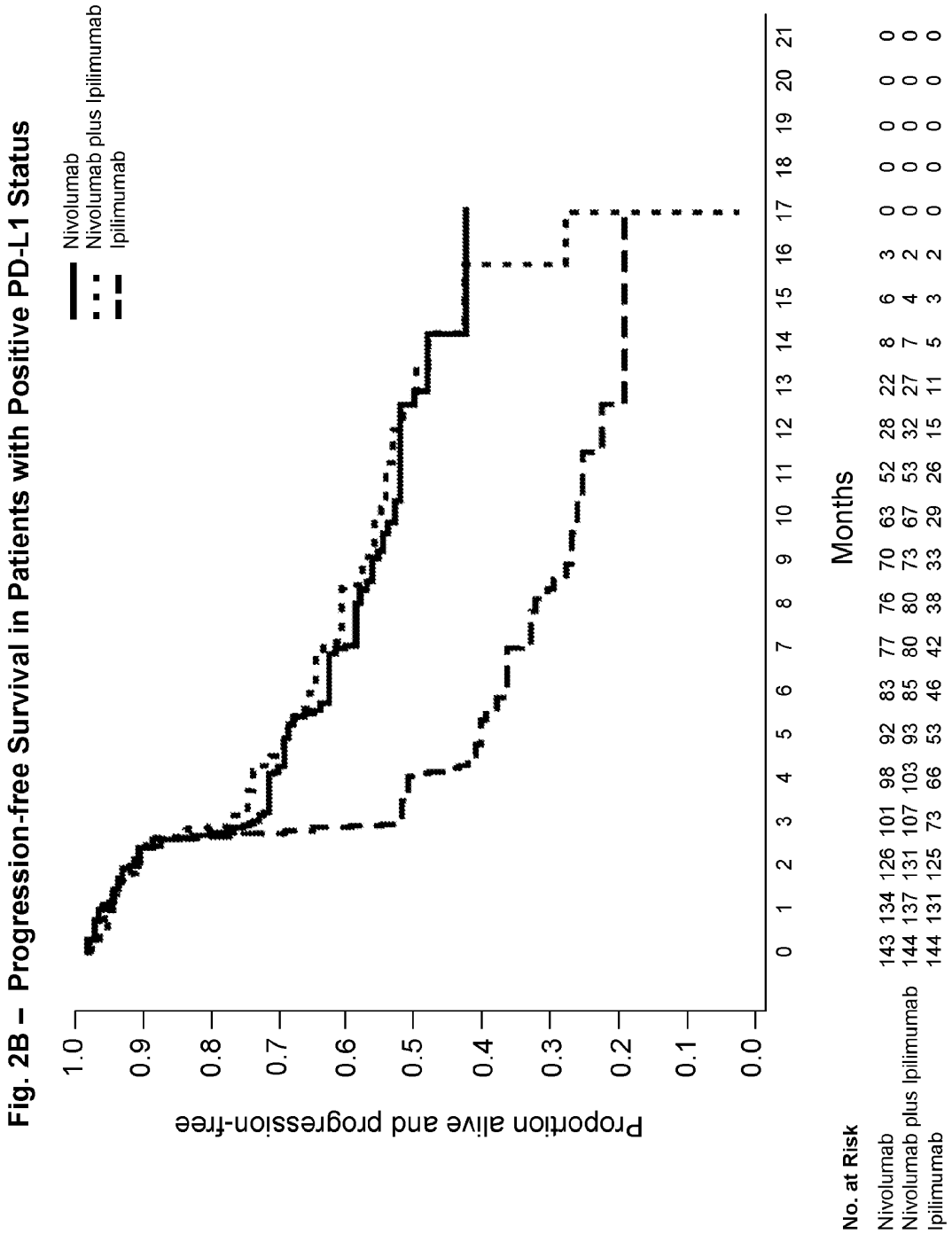
47. A kit for treating a patient afflicted with a melanoma tumor, the kit comprising:
    (a) a dosage ranging from 0.1 to 10 mg/kg body weight of an anti-PD-1 antibody or an antigen-binding portion thereof;
    (b) a dosage ranging from 0.1 to 10 mg/kg body weight of an anti-CTLA-4 antibody or an antigen-binding portion thereof; and
    (c) instructions for using the anti-PD-1 antibody or the antigen-binding portion thereof and the anti-CTLA-4 antibody or the antigen-binding portion thereof in the method of any one of claims 1 to 46.

SUBSTITUTE SHEET (RULE 26)

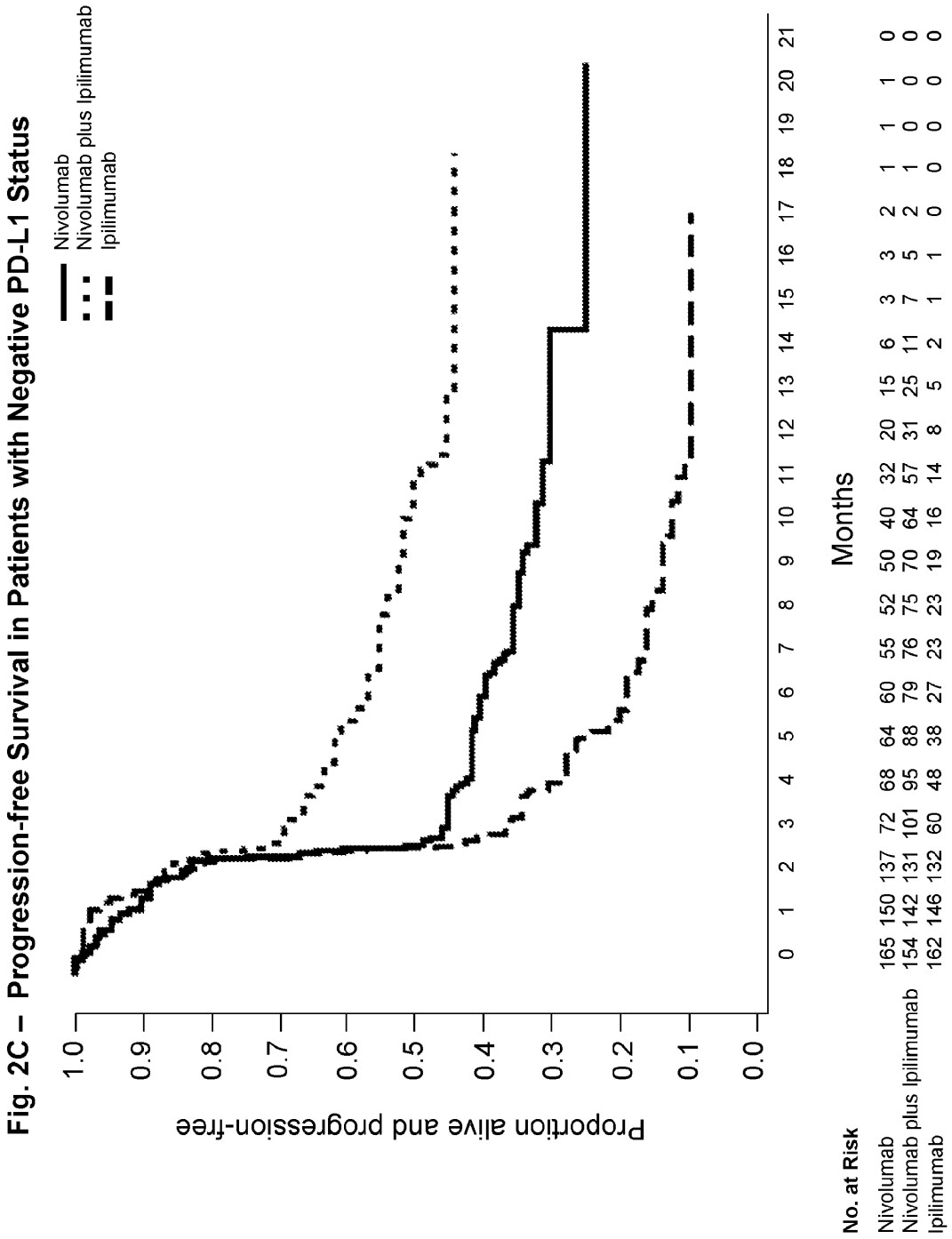
Fig. 1 – Patient Flow Diagram: Enrollment, Randomization, and Follow-up of Patients in the Study




Fig. 2A – Progression-free Survival (Intention-to-Treat Population)
Nivolumab
Nivolumab plus Ipilimumab
Ipilimumab
Proportion alive and progression-free
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Months
No. at Risk
Nivolumab 316 292 271 177 170 160 147 136 132 124 106 86 50 38 14 9 6 2 1 1 1 0
Nivolumab plus Ipilimumab 314 293 275 219 208 191 173 164 163 151 137 116 65 54 18 11 7 2 1 0 0 0
Ipilimumab 315 285 265 137 118 95 77 68 63 54 47 42 24 17 7 4 3 0 0 0 0 0



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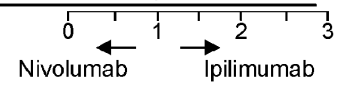
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Fig. 3 – Subgroup Analyses of Progression-free Survival

(A) Nivolumab versus ipilimumab

	N	Nivolumab + Ipilimumab		Ipilimumab		Unstratified Hazard Ratio (95% CI)
		N of events (N of patients)	Median PFS (95% CI)	N of events (N of patients)	Median PFS (95% CI)	
Overall	945	174 (316)	6.87 (4.34, 9.46)	234 (315)	2.89 (2.79, 3.42)	0.57 (0.47, 0.69)
BRAF Mutation Status						
Mutant	300	57 (98)	5.62 (2.79, 9.46)	66 (100)	4.04 (2.79, 5.52)	0.77 (0.54,1.09)
Wild-type	645	117 (218)	7.89 (4.86, 12.68)	168 (215)	2.83 (2.76, 3.09)	0.50 (0.39, 0.63)
M Stage at Study Entry						
M0/M1a/M1b	386	63 (131)	9.30 (5.62, N.A.)	92 (126)	4.17 (2.96, 5.52)	0.54 (0.39, 0.74)
M1c	559	111 (185)	5.39 (2.83, 8.87)	142 (189)	2.79 (2.73, 2.83)	0.59 (0.46, 0.76)



(B) Nivolumab plus ipilimumab versus ipilimumab

	N	Nivolumab + Ipilimumab		Ipilimumab		Unstratified Hazard Ratio (95% CI)
		N of events (N of patients)	Median PFS (95% CI)	N of events (N of patients)	Median PFS (95% CI)	
Overall	945	151 (314)	11.50 (8.90, 16.72)	234 (315)	2.89 (2.79, 3.42)	0.43 (0.35, 0.53)
BRAF Mutation Status						
Mutant	300	48 (102)	11.73 (8.02, N.A.)	66 (100)	4.04 (2.79, 5.52)	0.47 (0.32, 0.68)
Wild-type	645	103 (212)	11.24 (8.34, N.A.)	168 (215)	2.83 (2.76, 3.09)	0.41 (0.32, 0.53)
M Stage at Study Entry						
M0/M1a/M1b	386	51 (129)	15.54 (11.17, N.A.)	92 (126)	4.17 (2.96, 5.52)	0.35 (0.25, 0.50)
M1c	559	100 (185)	8.51 (5.52, 12.35)	142 (189)	2.79 (2.73, 2.83)	0.48 (0.37, 0.62)

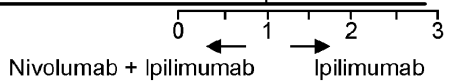
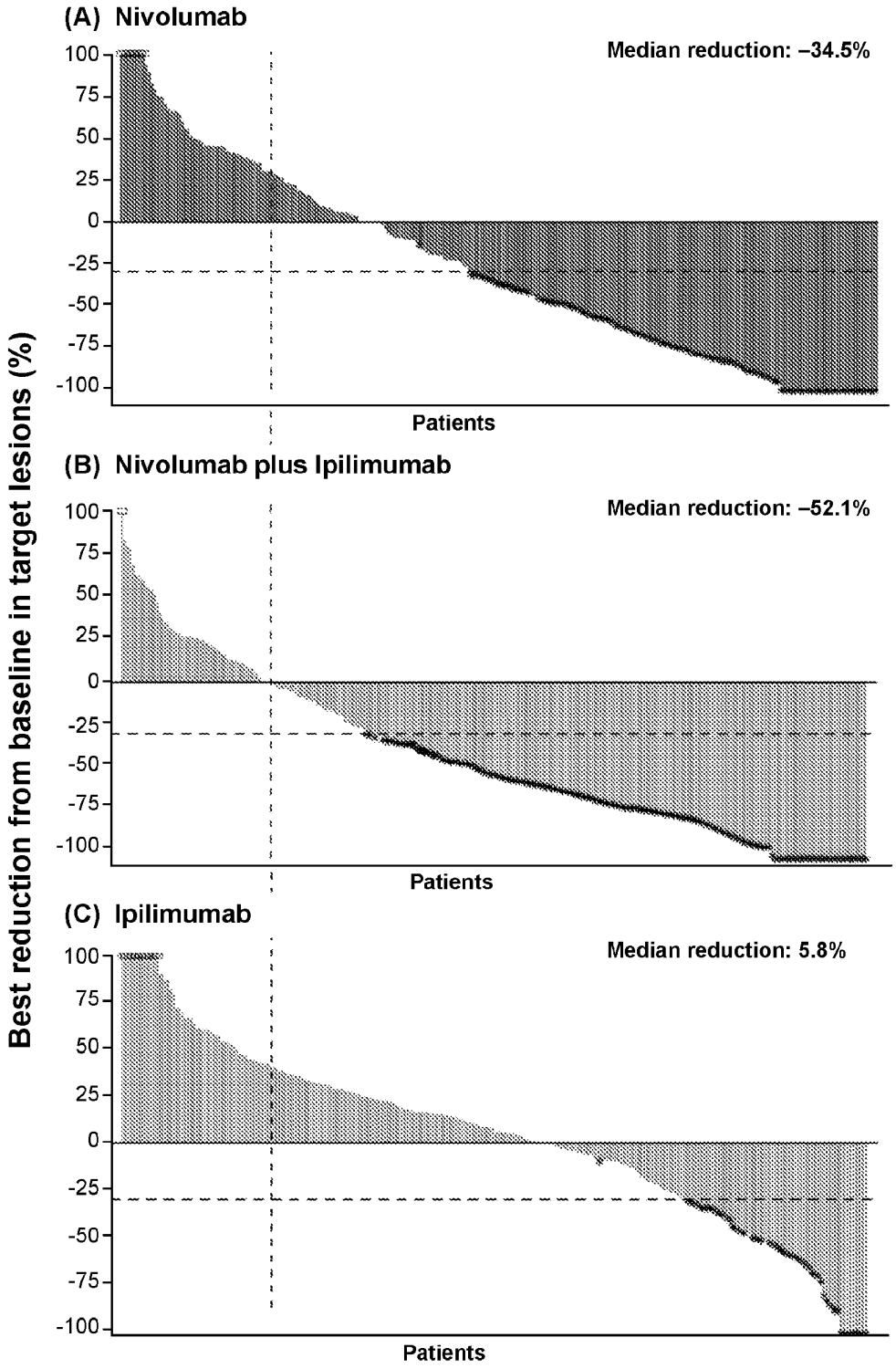


Fig. 4 – Tumor Burden Change in Target Lesions



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/029877

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	P. A. OTT ET AL: "CTLA-4 and PD-1/PD-L1 Blockade: New Immunotherapeutic Modalities with Durable Clinical Benefit in Melanoma Patients", CLINICAL CANCER RESEARCH, vol. 19, no. 19, 1 October 2013 (2013-10-01), pages 5300-5309, XP055200528, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-13-0143 page 5304, left-hand column, paragraph 2 - page 5306, right-hand column, paragraph 1 page 5307, left-hand column, paragraph 2 - right-hand column ----- -/--	1-12, 21-25, 27-32, 34-47

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 July 2016	13/07/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Le Flao, Katell

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G. K. PHILIPS ET AL: "Therapeutic uses of anti-PD-1 and anti-PD-L1 antibodies", INTERNATIONAL IMMUNOLOGY., vol. 27, no. 1, 16 October 2014 (2014-10-16), pages 39-46, XP055217958, GB ISSN: 0953-8178, DOI: 10.1093/intimm/dxu095 page 44, right-hand column, paragraph 2 - page 45, left-hand column, paragraph 2; table 1 page 43, right-hand column, paragraph 2 - page 44, left-hand column, paragraph 2 page 41, right-hand column, paragraph 2 - page 42, left-hand column, paragraph 2; table 1	1-12, 21-32, 34-47
X	MAHONEY KATHLEEN M ET AL: "The Next Immune-Checkpoint Inhibitors: PD-1/PD-L1 Blockade in Melanoma.", CLINICAL THERAPEUTICS 1 APR 2015, vol. 37, no. 4, 1 April 2015 (2015-04-01), pages 764-782, XP055285031, ISSN: 1879-114X page 768, right-hand column, paragraph 3 - page 769, left-hand column, paragraph 2; tables 2-4 page 771, left-hand column, paragraph 2 - page 772, right-hand column, line 13 page 774, right-hand column, paragraph 2 - page 775, left-hand column, paragraph 1 page 778, left-hand column, line 6 - line 15	1-12, 21-47
X	JEDD D. WOLCHOK ET AL: "Nivolumab plus Ipilimumab in Advanced Melanoma", NEW ENGLAND JOURNAL OF MEDICINE, vol. 369, no. 2, 11 July 2013 (2013-07-11) , pages 122-133, XP055182024, ISSN: 0028-4793, DOI: 10.1056/NEJMoa1302369 page 124, right-hand column, paragraph 2 page 128, right-hand column, paragraph 4 - page 130, right-hand column, paragraph 3	1-12, 21-47
X	MARZUKA ALEXANDER ET AL: "Melanoma Treatments: Advances and Mechanisms.", JOURNAL OF CELLULAR PHYSIOLOGY NOV 2015, vol. 230, no. 11, 22 April 2015 (2015-04-22), pages 2626-2633, XP002759396, ISSN: 1097-4652, DOI: 10.1002/jcp.25019 page 2631, left-hand column, paragraph 2 - right-hand column, paragraph 3	13-20

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PAOLO A ASCIERTO ET AL: "The additional facet of immunoscore: immunoprofiling as a possible predictive tool for cancer treatment", JOURNAL OF TRANSLATIONAL MEDICINE, BIOMED CENTRAL, LONDON, GB, vol. 11, no. 1, 3 March 2013 (2013-03-03), page 54, XP021142097, ISSN: 1479-5876, DOI: 10.1186/1479-5876-11-54 page 3/4, line 10 - line 17	13-20
X	US 2013/309250 A1 (COGSWELL JOHN [US] ET AL) 21 November 2013 (2013-11-21) claims 1-17; sequences 35,36	13-20
A	P. A. ASCIERTO ET AL: "Biomarkers for Immunostimulatory Monoclonal Antibodies in Combination Strategies for Melanoma and Other Tumor Types", CLINICAL CANCER RESEARCH, vol. 19, no. 5, 1 March 2013 (2013-03-01), pages 1009-1020, XP055124339, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-12-2982 the whole document	1-47

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/029877

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2013309250 A1	21-11-2013	AU 2013263076 A1	22-01-2015
		CA 2873402 A1	21-11-2013
		CN 104470949 A	25-03-2015
		EA 201492105 A1	30-06-2015
		EP 2850102 A1	25-03-2015
		HK 1203971 A1	06-11-2015
		JP 2015518826 A	06-07-2015
		KR 20150020189 A	25-02-2015
		SG 11201407190T A	30-12-2014
		US 2013309250 A1	21-11-2013
		US 2015125463 A1	07-05-2015
		US 2016090417 A1	31-03-2016
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(71) Applicant: **BRISTOL-MYERS SQUIBB COMPANY** [US/US]; Route 206 and Province Line Road, Princeton, New Jersey 08543 (US).

(72) Inventor: **YANG, Arvin**; Route 206 and Province Line Road, Princeton, New Jersey 08543 (US).

(74) Agents: **KIM, Ji-Eun** et al.; Sterne, Kessler, Goldstein & Fox P.L.L.C., 1100 New York Avenue, NW, Washington, District of Columbia 20005 (US).

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(54) Title: TREATMENT OF PD-L1-POSITIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY

(57) Abstract: The invention provides a method of treating a melanoma comprising (i) identifying a patient having a PD-L1 positive melanoma and (ii) administering to the patient an anti-PD-1 antibody or an antigen-binding portion thereof ("an anti-PD-1 antibody monotherapy"). The methods of the invention can extend progression-free survival for over 12 months and/or reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration.

TREATMENT OF PD-L1-POSITIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY

[0001] Throughout this application, various publications are referenced in parentheses by author name and date, or by Patent No. or Patent Publication No. The disclosures of these publications are hereby incorporated in their entireties by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein. However, the citation of a reference herein should not be construed as an acknowledgement that such reference is prior art to the present invention.

FIELD OF THE INVENTION

[0002] This invention relates to a method of treating PD-L1-positive melanoma comprising administering an anti-PD-1 antibody.

BACKGROUND OF THE INVENTION

[0003] Human cancers harbor numerous genetic and epigenetic alterations, generating neoantigens potentially recognizable by the immune system (Sjoblom *et al.* (2006) *Science* 314:268-74). The adaptive immune system, comprised of T and B lymphocytes, has powerful anti-cancer potential, with a broad capacity and exquisite specificity to respond to diverse tumor antigens. Further, the immune system demonstrates considerable plasticity and a memory component. The successful harnessing of all these attributes of the adaptive immune system would make immunotherapy unique among all cancer treatment modalities.

[0004] Recently, several immune checkpoint pathway inhibitors have begun to provide new immunotherapeutic approaches for treating cancer, including the development of an antibody (Ab), ipilimumab (YERVOY®), that binds to and inhibits Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) for the treatment of patients with advanced melanoma and the development of antibodies such as nivolumab and pembrolizumab (formerly lambrolizumab; USAN Council Statement, (2013) Pembrolizumab: Statement on a nonproprietary name adopted by the USAN Council (ZZ-165), November 27, 2013) that

bind specifically to the Programmed Death-1 (PD-1) receptor and block the inhibitory PD-1/PD-1 ligand pathway.

[0005] The promise of the emerging field of personalized medicine is that advances in pharmacogenomics will increasingly be used to tailor therapeutics to defined sub-populations, and ultimately, individual patients in order to enhance efficacy and minimize adverse effects. Recent successes include, for example, the development of imatinib mesylate (GLEEVEC®), a protein tyrosine kinase inhibitor that inhibits the bcr-abl tyrosine kinase, to treat Philadelphia chromosome-positive chronic myelogenous leukemia (CML); crizotinib (XALKORI®) to treat the 5% of patients with late-stage non-small cell lung cancers who express a mutant anaplastic lymphoma kinase (ALK) gene; and vemurafenib (ZELBORAF®), an inhibitor of mutated B-RAF protein (V600E-BRAF) which is expressed in around half of melanoma tumors. However, unlike the clinical development of small molecule agents that target discrete activating mutations found in select cancer populations, a particular challenge in cancer immunotherapy has been the identification of mechanism-based predictive biomarkers to enable patient selection and guide on-treatment management.

SUMMARY OF THE INVENTION

[0006] The present disclosure provides a method for treating a melanoma comprising (i) identifying a patient having a PD-L1 positive melanoma tumor; and (ii) administering to the patient an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1 ("anti-PD1 antibody monotherapy"), wherein the patient is not administered a combination of an anti-PD-1 antibody or an antigen-binding portion thereof and an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4 ("combination therapy").

[0007] The present disclosure also provides a method for treating a melanoma comprising (i) identifying a patient having a PD-L1 positive melanoma tumor; (ii) administering to the patient an anti-PD-1 antibody monotherapy; and (iii) administering a combination therapy to the patient, wherein the patient does not show efficacy with the anti-PD-1 monotherapy.

[0008] The present disclosure further provides a method for treating a melanoma comprising administering to a patient afflicted with a melanoma tumor an anti-PD-1

antibody monotherapy, not a combination therapy, wherein the patient is identified as having a PD-L1 positive melanoma tumor prior to the administration.

[0009] The present disclosure also provides a method for extending a progression-free survival period for over 12 months in a patient afflicted with a melanoma tumor comprising administering to the patient an anti-PD-1 antibody monotherapy, wherein the patient is identified as having a melanoma tumor expressing PD-L1 prior to the administration and wherein the patient demonstrates progression-free survival for over 12 months. In some embodiments, the progression-free survival of the patient is extended after the administration for over about 13 months, about 14 months, about 15 months, about 16 months, about 17 months, about 18 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, or about 10 years. In one particular embodiment, the progression-free survival of the patient is extended for over 14 months.

[0010] The present disclosure also provides a method for reducing a tumor size at least by 10% in a patient afflicted with a melanoma tumor comprising administering to the patient an anti-PD-1 antibody monotherapy, wherein the patient is identified as having a melanoma tumor expressing PD-L1 prior to the administration and wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration.

[0011] In certain embodiments, the present disclosure provides a method for selecting a patient suitable for an anti-PD-1 antibody monotherapy comprising (i) identifying a patient having a PD-L1 positive melanoma tumor; and (ii) instructing a healthcare provider to administer to the patient an anti-PD-1 antibody monotherapy.

[0012] In some embodiments, the methods disclosed herein further comprise measuring a PD-L1 expression on a melanoma tumor. In certain embodiments, the measuring comprises assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface. In one particular embodiment, the presence of PD-L1 is determined using an automated IHC assay.

[0013] The present disclosure also provides a kit for treating a patient afflicted with a melanoma tumor, the kit comprising (a) a dosage ranging from 0.1 to 10 mg/kg body weight of an anti-PD-1 antibody or an antigen-binding portion thereof; and (b) instructions for using the anti-PD-1 antibody or the antigen-binding portion thereof in a

method disclosed herein. In certain embodiments, the kit further comprises a dosage ranging from 0.1 to 10 mg/kg body weight of an anti-CTLA-4 antibody or an antigen-binding portion thereof.

BRIEF DESCRIPTION OF THE FIGURES

[0014] Figure 1 shows a patient flow diagram of the randomized, double-blind, multicenter, phase 3 trial.

[0015] Figures 2A-C show progression-free survival data in the intention-to-treat population (Figure 2A), in patients with positive PD-L1 status (Figure 2B), and Negative PD-L1 Status (Figure 2C). Each graph shows the progression free survival for patients treated with nivolumab alone (solid line), ipilimumab alone (dashed line), or the combination of nivolumab and ipilimumab (dotted line) in months (Figures 2A-C). The number at risk in months for each of nivolumab, nivolumab plus ipilimumab, and ipilimumab is shown below each x-axis (Figures 2A-C). PD-L1 expression status is based on verified PD-L1 assay data (Figures 2B-C).

[0016] Figures 3A-B show subgroup analyses of progression-free survival among patients treated with nivolumab alone compared to ipilimumab alone (Figure 3A) and patients treated with nivolumab plus ipilimumab compared to ipilimumab alone (Figure 3B).

[0017] Figures 4A-C show the tumor burden change in target lesions in patients treated with nivolumab alone (Figure 4A), nivolumab plus ipilimumab (Figure 4B), and ipilimumab alone (Figure 4C). In each graph, the y-axis shows the best reduction from baseline in target lesions (%) and the x-axis represents each patient (Figures 4A-C).

DETAILED DESCRIPTION OF THE INVENTION

[0018] The present invention relates to identifying an optimal strategy for treating a PD-L1-positive melanoma. In particular, the present invention shows that for a PD-L1-positive melanoma, a monotherapy of an anti-PD-1 antibody or an antigen-binding portion thereof is a better option than a combination therapy of an anti-PD-1 antibody or an antigen-binding portion thereof and an anti-CTLA-4 antibody or an antigen-binding portion thereof. If the monotherapy of an anti-PD-L1 antibody does not work in the

patient, however, a combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody can be used to treat the patient.

Definitions

[0019] In order that the present disclosure may be more readily understood, certain terms are first defined. As used in this application, except as otherwise expressly provided herein, each of the following terms shall have the meaning set forth below. Additional definitions are set forth throughout the application.

[0020] The term "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0021] It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0022] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0023] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. The headings provided herein are not limitations of the various aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0024] "Administering" refers to the physical introduction of a composition comprising a therapeutic agent to a subject, using any of the various methods and delivery systems

known to those skilled in the art. Routes of administration for the formulations disclosed herein include intravenous, intramuscular, subcutaneous, intraperitoneal, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as *in vivo* electroporation. In some embodiments, the formulation is administered via a non-parenteral route, in some embodiments, orally. Other non-parenteral routes include a topical, epidermal or mucosal route of administration, for example, intranasally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

[0025] An "adverse event" (AE) as used herein is any unfavorable and generally unintended or undesirable sign (including an abnormal laboratory finding), symptom, or disease associated with the use of a medical treatment. For example, an adverse event may be associated with activation of the immune system or expansion of immune system cells (*e.g.*, T cells) in response to a treatment. A medical treatment may have one or more associated AEs and each AE may have the same or different level of severity. Reference to methods capable of "altering adverse events" means a treatment regime that decreases the incidence and/or severity of one or more AEs associated with the use of a different treatment regime.

[0026] An "antibody" (Ab) shall include, without limitation, a glycoprotein immunoglobulin which binds specifically to an antigen and comprises at least two heavy (H) chains and two light (L) chains interconnected by disulfide bonds, or an antigen-binding portion thereof. Each H chain comprises a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region comprises three constant domains, C_{H1}, C_{H2} and C_{H3}. Each light chain comprises a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprises one constant domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed

complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L comprises three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system.

[0027] An immunoglobulin may derive from any of the commonly known isotypes, including but not limited to IgA, secretory IgA, IgG and IgM. IgG subclasses are also well known to those in the art and include but are not limited to human IgG1, IgG2, IgG3 and IgG4. "Isotype" refers to the antibody class or subclass (*e.g.*, IgM or IgG1) that is encoded by the heavy chain constant region genes. The term "antibody" includes, by way of example, both naturally occurring and non-naturally occurring antibodies; monoclonal and polyclonal antibodies; chimeric and humanized antibodies; human or nonhuman antibodies; wholly synthetic antibodies; and single chain antibodies. A nonhuman antibody may be humanized by recombinant methods to reduce its immunogenicity in man. Where not expressly stated, and unless the context indicates otherwise, the term "antibody" also includes an antigen-binding fragment or an antigen-binding portion of any of the aforementioned immunoglobulins, and includes a monovalent and a divalent fragment or portion, and a single chain antibody.

[0028] The term "monoclonal antibody" ("mAb") refers to a non-naturally occurring preparation of antibody molecules of single molecular composition, *i.e.*, antibody molecules whose primary sequences are essentially identical, and which exhibits a single binding specificity and affinity for a particular epitope. A mAb is an example of an isolated antibody. MAbs may be produced by hybridoma, recombinant, transgenic or other techniques known to those skilled in the art.

[0029] A "human" antibody (HuMAb) refers to an antibody having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region is also derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by

human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody," as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. The terms "human" antibodies and "fully human" antibodies and are used synonymously.

[0030] A "humanized antibody" refers to an antibody in which some, most or all of the amino acids outside the CDR domains of a non-human antibody are replaced with corresponding amino acids derived from human immunoglobulins. In one embodiment of a humanized form of an antibody, some, most or all of the amino acids outside the CDR domains have been replaced with amino acids from human immunoglobulins, whereas some, most or all amino acids within one or more CDR regions are unchanged. Small additions, deletions, insertions, substitutions or modifications of amino acids are permissible as long as they do not abrogate the ability of the antibody to bind to a particular antigen. A "humanized" antibody retains an antigenic specificity similar to that of the original antibody.

[0031] A "chimeric antibody" refers to an antibody in which the variable regions are derived from one species and the constant regions are derived from another species, such as an antibody in which the variable regions are derived from a mouse antibody and the constant regions are derived from a human antibody.

[0032] An "anti-antigen" antibody refers to an antibody that binds specifically to the antigen. For example, an anti-PD-1 antibody binds specifically to PD-1 and an anti-CTLA-4 antibody binds specifically to CTLA-4.

[0033] An "antigen-binding portion" of an antibody (also called an "antigen-binding fragment") refers to one or more fragments of an antibody that retain the ability to bind specifically to the antigen bound by the whole antibody.

[0034] A "cancer" refers a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth results in the formation of malignant tumors that invade neighboring tissues and may also metastasize to distant parts of the body through the lymphatic system or bloodstream. A "cancer" or "cancer tissue" can include a tumor.

[0035] "Cytotoxic T-Lymphocyte Antigen-4" (CTLA-4) refers to an immunoinhibitory receptor belonging to the CD28 family. CTLA-4 is expressed exclusively on T cells *in vivo*, and binds to two ligands, CD80 and CD86 (also called B7-1 and B7-2, respectively). The term "CTLA-4" as used herein includes human CTLA-4 (hCTLA-4), variants, isoforms, and species homologs of hCTLA-4, and analogs having at least one common epitope with hCTLA-4. The complete hCTLA-4 sequence can be found under GenBank Accession No. AAB59385.

[0036] The term "progression-free survival," which can be abbreviated as PFS, as used herein refers to the length of time during and after the treatment of a solid tumor (*i.e.*, melanoma) that a patient lives with the disease but it does not get worse.

[0037] "Dosing interval," as used herein, means the amount of time that elapses between multiple doses of a formulation disclosed herein being administered to a subject. Dosing interval can thus be indicated as ranges.

[0038] The term "dosing frequency" as used herein refers to the frequency of administering doses of a formulation disclosed herein in a given time. Dosing frequency can be indicated as the number of doses per a given time, *e.g.*, once a week or once in two weeks.

[0039] The use of the term "fixed dose" with regard to a composition of the invention means that two or more different antibodies in a single composition are present in the composition in particular (fixed) ratios with each other. In some embodiments, the fixed dose is based on the weight (*e.g.*, mg) of the antibodies. In certain embodiments, the fixed dose is based on the concentration (*e.g.*, mg/ml) of the antibodies. In some embodiments, the ratio is at least about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9, about 1:10, about 1:15, about 1:20, about 1:30, about 1:40, about 1:50, about 1:60, about 1:70, about 1:80, about 1:90, about 1:100, about 1:120, about 1:140, about 1:160, about 1:180, about 1:200, about 200:1, about 180:1, about 160:1, about 140:1, about 120:1, about 100:1, about 90:1, about 80:1, about 70:1, about 60:1, about 50:1, about 40:1, about 30:1, about 20:1, about 15:1, about 10:1, about 9:1, about 8:1, about 7:1, about 6:1, about 5:1, about 4:1, about 3:1, or about 2:1 mg first antibody to mg second antibody. For example, the 3:1 ratio of a first antibody and a second antibody can mean that a vial can contain about 240 mg of the first antibody and

80 mg of the second antibody or about 3 mg/ml of the first antibody and 1 mg/ml of the second antibody.

[0040] The use of the term "flat dose" with regard to the composition of the invention means a dose that is administered to a patient without regard for the weight or body surface area (BSA) of the patient. The flat dose is therefore not provided as a mg/kg dose, but rather as an absolute amount of the agent (*e.g.,* the anti-CTLA-4 antibody and/or anti-PD-1 antibody). For example, a 60 kg person and a 100 kg person would receive the same dose of the composition (*e.g.*, 240 mg of an anti-PD-1 antibody and 80 mg of an anti-CTLA-4 antibody in a single fixed dosing formulation vial containing both 240 mg of an anti-PD-1 antibody and 80 mg of an anti-CTLA-4 antibody (or two fixed dosing formulation vials containing 120 mg of an anti-PD-1 antibody and 40 mg of an anti-CTLA-4 antibody, etc.)).

[0041] The term "weight based dose" as referred to herein means that a dose that is administered to a patient is calculated based on the weight of the patient. For example, when a patient with 60 kg body weight requires 3 mg/kg of an anti-PD-1 antibody in combination with 1 mg/kg of an anti-CTLA-4 antibody, one can draw the appropriate amounts of the anti-PD-1 antibody (*i.e.*, 180 mg) and the anti-CTLA-4 antibody (*i.e.*, 60 mg) at once from a 3:1 ratio fixed dosing formulation of an anti-PD1 antibody and an anti-CTLA-4 antibody.

[0042] The term "single antibody composition" as used herein refers to a composition comprising antibodies that specifically bind to a single target, e.g., PD-1. As used herein, the single antibody composition can include a mixture of antibodies if the antibodies specifically bind to the same target, e.g., nivolumab and pembrolizumab. In other embodiments, a single antibody composition can include only one specific antibodies, e.g., nivolumab alone or pembrolizumab alone, but not a mixture thereof.

[0043] The term "anti-PD-1 antibody monotherapy" as used herein includes a therapy of an anti-PD-1 antibody without an anti-CTLA-4 antibody therapy. The anti-PD-1 antibody monotherapy comprises, consists essentially of, or consists of administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include administering an anti-CTLA-4 antibody. In one embodiment, the anti-PD-1 antibody monotherapy comprises administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include administering an anti-CTLA-4 antibody. In

another embodiment, the anti-PD-1 antibody monotherapy comprises administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include administering an antibody specifically targeting a protein other than PD-1. In other embodiments, the anti-PD-1 antibody monotherapy comprises administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include administering another anti-cancer agent.

[0044] An "immune response" refers to the action of a cell of the immune system (for example, T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells and neutrophils) and soluble macromolecules produced by any of these cells or the liver (including antibodies, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from a vertebrate's body of invading pathogens, cells or tissues infected with pathogens, cancerous or other abnormal cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

[0045] The term "PD-L1 positive" or "PD-L1 expression positive," relating to cell surface PD-L1 expression, refers to the proportion of cells in a test tissue sample comprising tumor cells and tumor- infiltrating inflammatory cells above which the sample is scored as expressing cell surface PD-L1. For cell surface expression assayed by immunohistochemistry (IHC), e.g., with the mAb 28- 8, the PD-L1 positive tumor or PD-L1 expression positive tumor means that at least about 0.01%, at least about 0.5%, at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 30% of the total number of cells express PD-L1. PD-L1 positive tumor or PD-L1 expression positive tumor can also be expressed herein as tumor expressing PD-L1. In other embodiments, the PD-L1 positive tumor or PD-L1 expression positive tumor means that at least about 0.1% to at least about 20% of the total number of cells express PD-L1. In certain embodiments, the PD-L1 positive tumor or PD-L1 expression positive tumor means that at least about 0.1% to at least about 10% of the total number of cells express PD-L1. In some embodiments, the PD-L1 positive or PD-L1 expression positive tumor means that at least about 1% of the total number of cells express PD-L1 on the cell surface. In other embodiments, the PD-L1 positive or PD-L1 expression positive tumor means that at least

about 5% of the total number of cells express PD-L1 on the cell surface. In one particular embodiment, PD-L1 positive or PD-L1 expression positive tumor means that at least about 1%, or in the range of 1- 5% of the total number of cells express PD-L1 on the cell surface.

[0046] "PD-L1 negative" or "PD-L1 expression negative," relating to cell surface PD-L1 expression, refers to the lack of a detectable amount of cell surface PD-L1. For cell surface expression assayed by IHC, e.g., with the mAb 28-8, a PD-L1 negative tumor or PD-L1 expression negative tumor means that less than 0.01% of cells express a detectable level of PD-L1. In some embodiments, a PD-L1 negative tumor or PD-L1 expression negative tumor means that zero (0) cells express a detectable level of PD-L1. In some embodiments, a PD-L1 negative or a PD-L1 expression negative tumor is any tumor other than a PD-L1 positive or a PD-L1 expression positive tumor.

[0047] "Programmed Death-1 (PD-1)" refers to an immunoinhibitory receptor belonging to the CD28 family. PD-1 is expressed predominantly on previously activated T cells *in vivo*, and binds to two ligands, PD-L1 and PD-L2. The term "PD-1" as used herein includes human PD-1 (hPD-1), variants, isoforms, and species homologs of hPD-1, and analogs having at least one common epitope with hPD-1. The complete hPD-1 sequence can be found under GenBank Accession No. U64863.

[0048] "Programmed Death Ligand-1 (PD-L1)" is one of two cell surface glycoprotein ligands for PD-1 (the other being PD-L2) that down-regulate T cell activation and cytokine secretion upon binding to PD-1. The term "PD-L1" as used herein includes human PD-L1 (hPD-L1), variants, isoforms, and species homologs of hPD-L1, and analogs having at least one common epitope with hPD-L1. The complete hPD-L1 sequence can be found under GenBank Accession No. Q9NZQ7.

[0049] A "patient" as used herein includes any patient who is afflicted with a cancer (*e.g.*, melanoma). The terms "subject" and "patient" are used interchangeably herein.

[0050] A "therapeutically effective amount" or "therapeutically effective dosage" of a drug or therapeutic agent is any amount of the drug that, when used alone or in combination with another therapeutic agent, protects a subject against the onset of a disease or promotes disease regression evidenced by a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. The ability of a

therapeutic agent to promote disease regression can be evaluated using a variety of methods known to the skilled practitioner, such as in human subjects during clinical trials, in animal model systems predictive of efficacy in humans, or by assaying the activity of the agent in *in vitro* assays.

[0051] "Treatment" or "therapy" of a subject refers to any type of intervention or process performed on, or the administration of an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, slowing down or preventing the onset, progression, development, severity or recurrence of a symptom, complication or condition, or biochemical indicia associated with a disease.

[0052] A "tumor-infiltrating inflammatory cell" is any type of cell that typically participates in an inflammatory response in a subject and which infiltrates tumor tissue. Such cells include tumor-infiltrating lymphocytes (TILs), macrophages, monocytes, eosinophils, histiocytes and dendritic cells.

[0053] The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the indefinite articles "a" or "an" should be understood to refer to "one or more" of any recited or enumerated component.

[0054] The terms "about" or "comprising essentially of" refer to a value or composition that is within an acceptable error range for the particular value or composition as determined by one of ordinary skill in the art, which will depend in part on how the value or composition is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" or "comprising essentially of" can mean within 1 or more than 1 standard deviation per the practice in the art. Alternatively, "about" or "comprising essentially of" can mean a range of up to 10% or 20% (*i.e.*, ±10% or ±20%). For example, about 3mg can include any number between 2.7 mg and 3.3 mg (for 10%) or between 2.4 mg and 3.6 mg (for 20%). Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values or compositions are provided in the application and claims, unless otherwise stated, the meaning of "about" or "comprising essentially of" should be assumed to be within an acceptable error range for that particular value or composition.

[0055] The terms "about once a week," "once about every week," "once about every two weeks," or any other similar dosing interval terms as used herein means approximate

number, and "about once a week" or "once about every week" can include every seven days ± two days, *i.e.*, every five days to every nine days. The dosing frequency of "once a week" thus can be every five days, every six days, every seven days, every eight days, or every nine days. "Once about every two weeks" can include every fourteen days ± three days, *i.e.*, every eleven days to every seventeen days. Similar approximations apply, for example, to once about every three weeks, once about every four weeks, once about every five weeks, once about every six weeks and once about every twelve weeks. In some embodiments, a dosing interval of once about every six weeks or once about every twelve weeks means that the first dose can be administered any day in the first week, and then the next dose can be administered any day in the sixth or twelfth week, respectively. In other embodiments, a dosing interval of once about every six weeks or once about every twelve weeks means that the first dose is administered on a particular day of the first week (e.g., Monday) and then the next dose is administered on the same day of the sixth or twelfth weeks (i.e., Monday), respectively.

[0056] As described herein, any concentration range, percentage range, ratio range or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one-tenth and one-hundredth of an integer), unless otherwise indicated.

[0057] Various aspects of the invention are described in further detail in the following subsections.

Methods of the Invention

[0058] The present invention is directed to a method for treating a PD-L1-positive melanoma in a subject in need thereof. In the general melanoma population (mix of PD-L1-negative tumors and PD-L1-positive tumors), a combination therapy of an anti-PD-L1 antibody and an anti-CTLA-4 antibody appears to provide better progression-free survival than a monotherapy of either an anti-PD-1 antibody or an anti-CTLA-4 antibody. See Figure 2A. However, the present invention identifies that in a PD-L1-positive melanoma population, the progression-free survival of an anti-PD-1 antibody monotherapy is substantially the same as a combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody. See Figure 2B. Therefore, in patients having a PD-L1-positive tumor, a monotherapy of an anti-PD-1 antibody can be as effective as a combination therapy. In order to reduce the toxicity of the combination therapy in the patient, the present

invention provides identifying a patient having a PD-L1-positive tumor and providing a monotherapy of an anti-PD-1 antibody for the initial treatment option. The patient can be administered with a combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody if the initial option fails for any reasons.

[0059] In one embodiment, the invention includes a method of treating a melanoma comprising: (i) identifying a patient having a PD-L1-positive melanoma tumor; and (ii) administering to the patient a single antibody composition comprising an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1, wherein the patient is not administered with a combination of an anti-PD-1 antibody or an antigen-binding portion thereof and an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4 ("combination therapy"). The invention can also include a method for treating a melanoma comprising: (i) identifying a patient having a PD-L1-positive melanoma tumor; (ii) administering to the patient an anti-PD-1 antibody monotherapy; and (iii) administering a combination therapy, wherein the patient does not show efficacy with the anti-PD-1 antibody monotherapy.

[0060] In another embodiment, the invention includes a method for treating a melanoma comprising: (i) identifying a patient having a PD-L1 positive melanoma tumor; and (ii) administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1 ("anti-PD1 antibody monotherapy"); or (b) a combination of an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1 and an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4 ("combination therapy"). Selection of either an anti-PD-1 antibody monotherapy or a combination therapy can be based on the patient's prior toxicity profile or prior history with either the monotherapy or the combination therapy.

[0061] In another embodiment, the invention includes a method for treating a melanoma comprising administering to a patient afflicted with a melanoma tumor an anti-PD1 antibody monotherapy, not a combination therapy, wherein the patient is identified as having a PD-L1-positive melanoma tumor prior to the administration. The methods of the present invention can further include (i) identifying a patient who does not show efficacy with the anti-PD-1 monotherapy; and (ii) administering a combination therapy to the patient who does not show efficacy with the anti-PD-1 monotherapy. In one particular

embodiment, the invention includes a method for treating a melanoma comprising: (i) identifying a patient having a PD-L1 positive melanoma tumor; (ii) administering to the patient an anti-PD-1 antibody monotherapy; (iii) identifying a patient that does not show efficacy with the anti-PD-1 monotherapy; and (iv) administering a combination therapy to the patient who does not show efficacy with the anti-PD-1 monotherapy.

[0062] In certain embodiments, the invention includes a method for extending a progression-free survival period for over 12 months in a patient afflicted with a melanoma tumor comprising administering to the patient a monotherapy of an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1, wherein the patient is identified as having a PD-L1-positive melanoma tumor prior to the administration and wherein the patient demonstrates progression-free survival for over 12 months. In other embodiments, the invention provides a method for extending a progression-free survival period for over 12 months in a patient afflicted with a melanoma tumor comprising: (i) identifying a patient having a PD-L1-positive melanoma tumor; (ii) administering to the patient an anti-PD-1 monotherapy ; and (iii) administering a combination therapy to the patient who does not show efficacy with the anti-PD-1 monotherapy, wherein the patient demonstrates progression-free survival for over 12 months. According to the invention, the progression-free survival of the patient can be extended, after the administration, for over about 13 months, about 14 months, about 15 months, about 16 months, about 17 months, about 18 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, or about 10 years. In a particular embodiment, the progression-free survival of the patient is extended for over 14 months.

[0063] In still other embodiments, the invention is directed to a method for reducing a tumor size at least by 10% in a patient afflicted with a melanoma tumor comprising administering to the patient a monotherapy of an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1, wherein the patient is identified as having a PD-L1-positive melanoma tumor prior to the administration and wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration. In yet other embodiments, the method comprises (i) identifying a patient having a PD-L1-positive melanoma tumor; and (ii) administering to the patient an anti-PD-1 antibody

monotherapy; and (iii) administering a combination therapy to the patient who does not show efficacy with the anti-PD-1 monotherapy, wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration. The tumor size, after the administration, can be reduced at least about 60%, 70%, 80%, 90% or 100%. The tumor can be completely eliminated from the patient's body after the administration.

[0064] The invention can also include a method of preventing a relapse and/or induce a remission to a patient comprising administering to the patient a monotherapy of an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1, wherein the patient is identified as having a PD-L1-positive melanoma tumor prior to the administration. In some embodiments, the method of the invention comprises (i) identifying a patient having a PD-L1-positive melanoma tumor; (ii) administering to the patient an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1 as a monotherapy, and not a combination of an anti-PD-1 antibody or an antigen-binding portion thereof and an anti-CTLA-4 antibody or an antigen-binding portion thereof.

[0065] In certain embodiments, the invention includes a method for increasing an objective response rate to be higher than 55% in a patient population, wherein each patient of the patient population is afflicted with a melanoma tumor, in a cancer treatment comprising administering to the patient a monotherapy of an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1, wherein each patient is identified as having a PD-L1 positive melanoma tumor prior to the administration and wherein the objective response rate is higher than 55%, 60%, 65%, 70%, or 75%. The method can further comprise administering a combination therapy of an anti-PD-1 antibody or an antigen-binding portion thereof and an anti-CTLA-4 antibody or an antigen-binding portion thereof to those patients who do not show efficacy with the anti-PD-1 monotherapy. In a particular embodiment, the method further comprises identifying each patient as having a PD-L1-positive melanoma tumor prior to the administration. In other embodiments, each patient in the methods can further be characterized by (i) extended progression-free survival for over 12 months, (ii) tumor size reduction at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration, or (iii) both. In some embodiments, the

patient population can be at least 100 patients having a PD-L1 positive melanoma tumor. In some embodiments, the patient population can be at least 200, 300, 400, 500, 600, 700, 800, 900, or 1000 patients having a PD-L1 positive melanoma tumor.

[0066] In further embodiments, the invention provides a method for selecting a patient suitable for an anti-PD-1 antibody monotherapy comprising: (i) identifying a patient having a PD-L1-positive melanoma tumor; and (ii) instructing a healthcare provider to administer to the patient a monotherapy of an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1. The method can further comprise administering the anti-PD-1 antibody or an antigen-binding portion thereof to the patient.

[0067] The disclosure also provides a method for treatment of a subject afflicted with a melanoma, which method comprises: (a) selecting a patient who has a PD-L1-positive tumor, (b) further selecting a patient who is not suitable for the anti-PD-1 antibody monotherapy, and (c) administering a combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody or a standard-of-care therapeutic, *e.g.*, an anti-CTLA-4 antibody monotherapy, to the selected patient. The further selection of a patient who is not suitable for the monotherapy can be patients who do not respond to the monotherapy or who have too much toxicity to the monotherapy. Those patients may show efficacy to the combination therapy because, not bound by any theory, the combination therapy may utilize a different mechanism of action. In addition, the patients may be able to tolerate the combination therapy, which may have a lower dose of one or both antibodies.

[0068] The methods of the invention as a result of the anti-PD-1 monotherapy and/or the combination therapy can treat the melanoma tumor, reduce the tumor size, prevent growth of the tumor, eliminate the tumor from the patient, prevent a relapse of a tumor, induce a remission in a patient, or any combination thereof. In certain embodiments, the anti-PD-1 monotherapy and/or the combination therapy induces a complete response. In other embodiments, the anti-PD-1 monotherapy and/or the combination therapy induces a partial response.

Melanoma

[0069] Melanoma (MEL) is a malignant tumor of melanocytes, the melanin-producing cells found predominantly in skin. Though less common than other skin cancers, it is the most dangerous of skin cancers if not diagnosed early and causes the majority (75%) of skin cancer deaths. The incidence of MEL is increasing worldwide in Caucasian

populations, especially where peoples with low amounts of skin pigmentation receive excessive ultraviolet light exposure from the sun. In Europe, the incidence rate is < 10-20 per 100,000 population; in the USA 20-30 per 100,000; and in Australia, where the highest incidence is observed, 50-60 per 100,000 (Garbe *et al.*, *Eur. J. Cancer. 48(15)*:2375-90 (2012)). MEL accounts for about 5% of all new cases of cancer in the United States (U.S.), and the incidence continues to rise by almost 3% per year. This translates to an estimated 76,690 new cases in the U.S. in 2013 with 9,480 associated deaths (Siegel *et al.*, *CA Cancer J. Clin. 63(1)*:11-30 (2013)).

[0070] For in situ (stage 0) or early-stage MEL (Stages I-II), surgical excision is the primary treatment. In general, the prognosis is excellent for patients with localized disease and tumors 1.0 mm or less in thickness, with 5-year survival rates of more than 90% (NCCN GUIDELINES®, 2013- Melanoma). Where surgical excision is not feasible for in situ melanoma due to comorbidity or cosmetically sensitive tumor location, topical imiquimod (INN) and radiotherapy are emerging as treatments, especially for *lentigo maligna*. Chemotherapeutic agents for treating MEL include dacarbazine, temozolomide and imatinib for melanoma with a c-KIT mutation, high-dose interleukin-2, and paclitaxel with or without carboplatin. However, these treatments have modest success, with response rates below 20% in first-line (1L) and second-line (2L) settings.

[0071] For patients with localized melanomas more than 1.0 mm in thickness, survival rates range from 50-90%. The likelihood of regional nodal involvement increases with increasing tumor thickness. With Stage III MEL (clinically positive nodes and/or in-transit disease), 5-year survival rates range from 20-70%. By far the most lethal is Stage IV MEL where long-term survival in patients with distant metastatic melanoma is less than 10% (NCCN GUIDELINES®, 2013 - melanoma).

[0072] The types of melanoma that can be treated with the present methods include, but are not limited to, lentigo maligna, lentigo maligna melanoma, superficial spreading melanoma, acral lentiginous melanoma, nucosal melanoma, nodular melanoma, polypoid melanoma, desmoplastic melanoma, amelanotic melanoma, soft-tissue melanoma, melanoma with small nevus-like cells, melanoma with features of a Spitz nevus, or uveal melanoma. The stages of melanoma that can be treated with the present methods include, but are not limited to, (i) Stage I / II (invasive melanoma): T1a characterized by less than 1.0 mm primary tumor thickness, without ulceration, and mitosis < $1/mm^2$; T1b

characterized by less than 1.0 mm primary tumor thickness, with ulceration or mitoses ≥ 1/mm^2; T2a characterized by 1.01–2.0 mm primary tumor thickness, without ulceration; (ii) Stage II (high risk melanoma): T2b characterized by 1.01–2.0 mm primary tumor thickness, with ulceration; T3a characterized by 2.01–4.0 mm primary tumor thickness, without ulceration; T3b characterized by 2.01–4.0 mm primary tumor thickness, with ulceration; T4a characterized by greater than 4.0 mm primary tumor thickness, without ulceration; or T4b characterized by greater than 4.0 mm primary tumor thickness, with ulceration; (iii) Stage III (regional metastasis): N1 characterized by single positive lymph node; N2 characterized by two to three positive lymph nodes or regional skin/in-transit metastasis; or N3 characterized by four positive lymph nodes or one lymph node and regional skin/in-transit metastases; and (iv) Stage IV (distant metastasis): M1a characterized by distant skin metastasis, normal LDH; M1b characterized by Lung metastasis, normal LDH; or M1c characterized by other distant metastasis or any distant metastasis with elevated LDH. PD-L1-positive tumors that are treatable by the present methods can have PD-L1 expression on the surface of tumor cells or tumor infiltrating inflammatory cells.

Measurement of PD-L1 expression

[0073] In certain embodiments, identifying a patient suitable for a PD-1 monotherapy for the present methods includes measuring or assessing a PD-L1 expression on the surface of the melanoma tumor cells or tumor infiltrating inflammatory cells. The phrases "tumors expressing PD-L1," "PD-L1 expressing tumor," "PD-L1 positive tumor," and "PD-L1 expression positive tumor" are used interchangeably herein. The meaning of the phrases is provided elsewhere herein. The methods of measuring or assessing the PD-L1 expression can be achieved by any methods applicable.

[0074] In order to assess the PD-L1 expression, in one embodiment, a test tissue sample is obtained from the patient who is in need of the therapy. In another embodiment, the assessment of PD-L1 expression can be achieved without obtaining a test tissue sample. In some embodiments, selecting a suitable patient includes (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and/or tumor-infiltrating inflammatory cells; and (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the surface of the cells

based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is higher than a predetermined threshold level.

[0075] In any of the methods comprising the measurement of PD-L1 expression in a test tissue sample, however, it should be understood that the step comprising the provision of a test tissue sample obtained from a patient is an optional step. That is, in certain embodiments the method includes this step, and in other embodiments, this step is not included in the method. It should also be understood that in certain embodiments the "measuring" or "assessing" step to identify, or determine the number or proportion of, cells in the test tissue sample that express PD-L1 on the cell surface is performed by a transformative method of assaying for PD-L1 expression, for example by performing a reverse transcriptase-polymerase chain reaction (RT-PCR) assay or an IHC assay. In certain other embodiments, no transformative step is involved and PD-L1 expression is assessed by, for example, reviewing a report of test results from a laboratory. In certain embodiments, the steps of the methods up to, and including, assessing PD-L1 expression provides an intermediate result that may be provided to a physician or other healthcare provider for use in selecting a suitable candidate for the combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody. In certain embodiments, the steps that provide the intermediate result is performed by a medical practitioner or someone acting under the direction of a medical practitioner. In other embodiments, these steps are performed by an independent laboratory or by an independent person such as a laboratory technician.

[0076] In certain embodiments of any of the present methods, the proportion of cells that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 RNA. In further embodiments, the presence of PD-L1 RNA is determined by RT-PCR, *in situ* hybridization or RNase protection. In other embodiments, the proportion of cells that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 polypeptide. In further embodiments, the presence of PD-L1 polypeptide is determined by immunohistochemistry (IHC), enzyme-linked immunosorbent assay (ELISA), *in vivo* imaging, or flow cytometry. In some embodiments, PD-L1 expression is assayed by IHC. In other embodiments of all of these methods, cell surface expression of PD-L1 is assayed using, *e.g.*, IHC or *in vivo* imaging.

[0077] Imaging techniques have provided important tools in cancer research and treatment. Recent developments in molecular imaging systems, including positron emission tomography (PET), single-photon emission computed tomography (SPECT), fluorescence reflectance imaging (FRI), fluorescence-mediated tomography (FMT), bioluminescence imaging (BLI), laser-scanning confocal microscopy (LSCM) and multiphoton microscopy (MPM), will likely herald even greater use of these techniques in cancer research. Some of these molecular imaging systems allow clinicians to not only see where a tumor is located in the body, but also to visualize the expression and activity of specific molecules, cells, and biological processes that influence tumor behavior and/or responsiveness to therapeutic drugs (Condeelis and Weissleder, *Cold Spring Harb. Perspect. Biol. 2(12)*:a003848 (2010)). Antibody specificity, coupled with the sensitivity and resolution of PET, makes immunoPET imaging particularly attractive for monitoring and assaying expression of antigens in tissue samples (McCabe and Wu, *Cancer Biother. Radiopharm. 25(3)*:253-61 (2010); Olafsen *et al.*, *Protein Eng. Des. Sel. 23(4)*:243-9 (2010)). In certain embodiments of any of the present methods, PD-L1 expression is assayed by immunoPET imaging. In certain embodiments of any of the present methods, the proportion of cells in a test tissue sample that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 polypeptide on the surface of cells in the test tissue sample. In certain embodiments, the test tissue sample is a FFPE tissue sample. In other embodiments, the presence of PD-L1 polypeptide is determined by IHC assay. In further embodiments, the IHC assay is performed using an automated process. In some embodiments, the IHC assay is performed using an anti-PD-L1 mAb to bind to the PD-L1 polypeptide.

Assaying Cell-Surface PD-L1 Expression by Automated IHC

[0078] In one embodiment of the present methods, an automated IHC method is used to assay the expression of PD-L1 on the surface of cells in FFPE tissue specimens. This disclosure provides methods for detecting the presence of human PD-L1 antigen in a test tissue sample, or quantifying the level of human PD-L1 antigen or the proportion of cells in the sample that express the antigen, which methods comprise contacting the test sample, and a negative control sample, with a mAb that specifically binds to human PD-L1, under conditions that allow for formation of a complex between the antibody or portion thereof and human PD-L1. In certain embodiments, the test and control tissue

samples are FFPE samples. The formation of a complex is then detected, wherein a difference in complex formation between the test sample and the negative control sample is indicative of the presence of human PD-L1 antigen in the sample. Various methods are used to quantify PD-L1 expression.

[0079] In a particular embodiment, the automated IHC method comprises: (a) deparaffinizing and rehydrating mounted tissue sections in an autostainer; (b) retrieving antigen using a decloaking chamber and pH 6 buffer, heated to 110°C for 10 min; (c) setting up reagents on an autostainer; and (d) running the autostainer to include steps of neutralizing endogenous peroxidase in the tissue specimen; blocking non-specific protein-binding sites on the slides; incubating the slides with primary Ab; incubating with a postprimary blocking agent; incubating with NovoLink Polymer; adding a chromogen substrate and developing; and counterstaining with hematoxylin.

[0080] For assessing PD-L1 expression in tumor tissue samples, a pathologist examines the number of membrane PD-L1$^+$ tumor cells in each field under a microscope and mentally estimates the percentage of cells that are positive, then averages them to come to the final percentage. The different staining intensities are defined as 0/negative, 1+/weak, 2+/moderate, and 3+/strong. Typically, percentage values are first assigned to the 0 and 3+ buckets, and then the intermediate 1+ and 2+ intensities are considered. For highly heterogeneous tissues, the specimen is divided into zones, and each zone is scored separately and then combined into a single set of percentage values. The percentages of negative and positive cells for the different staining intensities are determined from each area and a median value is given to each zone. A final percentage value is given to the tissue for each staining intensity category: negative, 1+, 2+, and 3+. The sum of all staining intensities needs to be 100%.

[0081] Staining is also assessed in tumor-infiltrating inflammatory cells such as macrophages and lymphocytes. In most cases macrophages serve as an internal positive control since staining is observed in a large proportion of macrophages. While not required to stain with 3+ intensity, an absence of staining of macrophages should be taken into account to rule out any technical failure. Macrophages and lymphocytes are assessed for plasma membrane staining and only recorded for all samples as being positive or negative for each cell category. Staining is also characterized according to an outside/inside tumor immune cell designation. "Inside" means the immune cell is within

the tumor tissue and/or on the boundaries of the tumor region without being physically intercalated among the tumor cells. "Outside" means that there is no physical association with the tumor, the immune cells being found in the periphery associated with connective or any associated adjacent tissue.

[0082] In certain embodiments of these scoring methods, the samples are scored by two pathologists operating independently, and the scores are subsequently consolidated. In certain other embodiments, the identification of positive and negative cells is scored using appropriate software.

[0083] A histoscore is used as a more quantitative measure of the IHC data. The histoscore is calculated as follows:

Histoscore = [(% tumor x 1 (low intensity)) + (% tumor x 2 (medium intensity)) + (% tumor x 3 (high intensity)]

[0084] To determine the histoscore, the pathologist estimates the percentage of stained cells in each intensity category within a specimen. Because expression of most biomarkers is heterogeneous the histoscore is a truer representation of the overall expression. The final histoscore range is 0 (no expression) to 300 (maximum expression).

[0085] An alternative means of quantifying PD-L1 expression in a test tissue sample IHC is to determine the adjusted inflammation score (AIS) score defined as the density of inflammation multiplied by the percent PD-L1 expression by tumor-infiltrating inflammatory cells (Taube *et al.*, *Sci. Transl. Med. 4(127)*:127ra37 (2012)).

Anti-PD-1 antibodies

[0086] PD-1 is a key immune checkpoint receptor expressed by activated T and B cells and mediates immunosuppression. PD-1 is a member of the CD28 family of receptors, which includes CD28, CTLA-4, ICOS, PD-1, and BTLA. Two cell surface glycoprotein ligands for PD-1 have been identified, Programmed Death Ligand-1 (PD-L1) and Programmed Death Ligand-2 (PD-L2), that are expressed on antigen-presenting cells as well as many human cancers and have been shown to down regulate T cell activation and cytokine secretion upon binding to PD-1. Inhibition of the PD-1/PD-L1 interaction mediates potent antitumor activity in preclinical models.

[0087] HuMAbs that bind specifically to PD-1 with high affinity have been disclosed in U.S. Patent Nos. 8,008,449 and 8,779,105. Other anti-PD-1 mAbs have been described in, for example, U.S. Patent Nos. 6,808,710, 7,488,802, 8,168,757 and 8,354,509, and PCT

Publication No. WO 2012/145493. Each of the anti-PD-1 HuMAbs disclosed in U.S. Patent No. 8,008,449 has been demonstrated to exhibit one or more of the following characteristics: (a) binds to human PD-1 with a K_D of 1 x 10^{-7} M or less, as determined by surface plasmon resonance using a Biacore biosensor system; (b) does not substantially bind to human CD28, CTLA-4 or ICOS; (c) increases T-cell proliferation in a Mixed Lymphocyte Reaction (MLR) assay; (d) increases interferon-γ production in an MLR assay; (e) increases IL-2 secretion in an MLR assay; (f) binds to human PD-1 and cynomolgus monkey PD-1; (g) inhibits the binding of PD-L1 and/or PD-L2 to PD-1; (h) stimulates antigen-specific memory responses; (i) stimulates antibody responses; and (j) inhibits tumor cell growth *in vivo*. Anti-PD-1 antibodies useful for the present invention include mAbs that bind specifically to human PD-1 and exhibit at least one, at least two, at least three, at least four, or at least five of the preceding characteristics.

[0088] In one embodiment, the anti-PD-1 antibody is nivolumab. Nivolumab (also known as "OPDIVO®"; formerly designated 5C4, BMS-936558, MDX-1106, or ONO-4538) is a fully human IgG4 (S228P) PD-1 immune checkpoint inhibitor antibody that selectively prevents interaction with PD-1 ligands (PD-L1 and PD-L2), thereby blocking the down-regulation of antitumor T-cell functions (U.S. Patent No. 8,008,449; Wang *et al.*, *Cancer Immunol Res.* 2(9):846-56 (2014)). In another embodiment, the anti-PD-1 antibody or fragment thereof cross-competes with nivolumab. In other embodiments, the anti-PD-1 antibody or fragment thereof binds to the same epitope as nivolumab. In certain embodiments, the anti-PD-1 antibody has the same CDRs as nivolumab.

[0089] In another embodiment, the anti-PD-1 antibody or fragment thereof cross-competes with pembrolizumab. In some embodiments, the anti-PD-1 antibody or fragment thereof binds to the same epitope as pembrolizumab. In certain embodiments, the anti-PD-1 antibody has the same CDRs as pembrolizumab. In another embodiment, the anti-PD-1 antibody is pembrolizumab. Pembrolizumab (also known as "KEYTRUDA®", lambrolizumab, and MK-3475) is a humanized monoclonal IgG4 antibody directed against human cell surface receptor PD-1 (programmed death-1 or programmed cell death-1). Pembrolizumab is described, for example, in U.S. Patent Nos. 8,354,509 and 8,900,587; *see also* http://www.cancer.gov/drugdictionary?cdrid=695789 (last accessed: December 14, 2014). Pembrolizumab has been approved by the FDA for the treatment of relapsed or refractory melanoma.

[0090] In other embodiments, the anti-PD-1 antibody or fragment thereof cross-competes with MEDI0608. In still other embodiments, the anti-PD-1 antibody or fragment thereof binds to the same epitope as MEDI0608. In certain embodiments, the anti-PD-1 antibody has the same CDRs as MEDI0608. In other embodiments, the anti-PD-1 antibody is MEDI0608 (formerly AMP-514), which is a monoclonal antibody. MEDI0608 is described, for example, in US Pat. No. 8,609,089B2 or in http://www.cancer.gov/drugdictionary?cdrid=756047 (last accessed December 14, 2014).

[0091] In certain embodiments, the first antibody is an anti-PD-1 antagonist. One example of the anti-PD-1 antagonist is AMP-224, which is a B7-DC Fc fusion protein. AMP-224 is discussed in U.S. Publ. No. 2013/0017199 or in http://www.cancer.gov/publications/dictionaries/cancer-drug?cdrid=700595 (last accessed July 8, 2015).

[0092] In other embodiments, the anti-PD-1 antibody or fragment thereof cross-competes with BGB-A317. In some embodiments, the anti-PD-1 antibody or fragment thereof binds the same epitope as BGB-A317. In certain embodiments, the anti-PD-1 antibody has the same CDRs as BGB-A317. In certain embodiments, the anti-PD-1 antibody is BGB-A317, which is a humanized monoclonal antibody. BGB-A317 is described in U.S. Publ. No. 2015/0079109.

[0093] In some embodiments, the antibody is Pidilizumab (CT-011), which is an antibody previously reported to bind to PD-1 but which is believed to bind to a different target. Pidilizumab is described in US Pat. No. 8,686,119 B2 or WO 2013/014668 A1.

[0094] Anti-PD-1 antibodies useful for the disclosed compositions also include isolated antibodies that bind specifically to human PD-1 and cross-compete for binding to human PD-1 with nivolumab (*see, e.g.*, U.S. Patent Nos. 8,008,449 and 8,779,105; WO 2013/173223). The ability of antibodies to cross-compete for binding to an antigen indicates that these antibodies bind to the same epitope region of the antigen and sterically hinder the binding of other cross-competing antibodies to that particular epitope region. These cross-competing antibodies are expected to have functional properties very similar to those of nivolumab by virtue of their binding to the same epitope region of PD-1. Cross-competing antibodies can be readily identified based on their ability to cross-compete with nivolumab in standard PD-1 binding assays such as Biacore analysis, ELISA assays or flow cytometry (*see, e.g.*, WO 2013/173223).

[0095] In certain embodiments, the antibodies that cross-compete for binding to human PD-1 with, or bind to the same epitope region of human PD-1 as, nivolumab are mAbs. For administration to human subjects, these cross-competing antibodies can be chimeric antibodies, or humanized or human antibodies. Such chimeric, humanized or human mAbs can be prepared and isolated by methods well known in the art.

[0096] Anti-PD-1 antibodies useful for the compositions of the disclosed invention also include antigen-binding portions of the above antibodies. It has been amply demonstrated that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_{H1} domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_{H1} domains; and (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody.

[0097] Anti-PD-1 antibodies suitable for use in the disclosed compositions are antibodies that bind to PD-1 with high specificity and affinity, block the binding of PD-L1 and or PD-L2, and inhibit the immunosuppressive effect of the PD-1 signaling pathway. In any of the compositions or methods disclosed herein, an anti-PD-1 "antibody" includes an antigen-binding portion or fragment that binds to the PD-1 receptor and exhibits the functional properties similar to those of whole antibodies in inhibiting ligand binding and upregulating the immune system. In certain embodiments, the anti-PD-1 antibody or antigen-binding portion thereof cross-competes with nivolumab for binding to human PD-1. In other embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is a chimeric, humanized or human monoclonal antibody or a portion thereof. In certain embodiments, the antibody is a humanized antibody. In other embodiments, the antibody is a human antibody. Antibodies of an IgG1, IgG2, IgG3 or IgG4 isotype can be used.

[0098] In certain embodiments, the anti-PD-1 antibody or antigen-binding portion thereof comprises a heavy chain constant region which is of a human IgG1 or IgG4 isotype. In certain other embodiments, the sequence of the IgG4 heavy chain constant region of the anti-PD-1 antibody or antigen-binding portion thereof contains an S228P mutation which replaces a serine residue in the hinge region with the proline residue normally found at the corresponding position in IgG1 isotype antibodies. This mutation, which is present in

nivolumab, prevents Fab arm exchange with endogenous IgG4 antibodies, while retaining the low affinity for activating Fc receptors associated with wild-type IgG4 antibodies (Wang *et al.*, 2014 *Cancer Immunol Res.* 2(9):846-56). In yet other embodiments, the antibody comprises a light chain constant region which is a human kappa or lambda constant region. In other embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is a mAb or an antigen-binding portion thereof. In certain embodiments of any of the therapeutic methods described herein comprising administration of an anti-PD-1 antibody, the anti-PD-1 antibody is nivolumab. In other embodiments, the anti-PD-1 antibody is pembrolizumab. In other embodiments, the anti-PD-1 antibody is chosen from the human antibodies 17D8, 2D3, 4H1, 4A11, 7D3 and 5F4 described in U.S. Patent No. 8,008,449. In still other embodiments, the anti-PD-1 antibody is MEDI0608 (formerly AMP-514), AMP-224, or BGB-A317.

[0099] Because anti-PD-1 and anti-PD-L1 target the same signaling pathway and have been shown in clinical trials to exhibit similar levels of efficacy in a variety of cancers, including RCC (*see* Brahmer *et al.* (2012) *N Engl J Med* 366:2455-65; Topalian *et al.* (2012a) *N Engl J Med* 366:2443-54; WO 2013/173223), an anti-PD-L1 antibody may be substituted for the anti-PD-1 Ab in any of the therapeutic methods disclosed herein. In certain embodiments, the anti-PD-L1 antibody is BMS-936559 (formerly 12A4 or MDX-1105) (*see, e.g.*, U.S. Patent No. 7,943,743; WO 2013/173223). In other embodiments, the anti-PD-L1 antibody is MPDL3280A (also known as RG7446) (*see, e.g.*, Herbst et al. (2013) *J Clin Oncol* 31(suppl):3000. Abstract; U.S. Patent No. 8,217,149) or MEDI4736 (Khleif (2013) In: Proceedings from the European Cancer Congress 2013; September 27-October 1, 2013; Amsterdam, The Netherlands. Abstract 802). In certain embodiments, the antibodies that cross-compete for binding to human PD-L1 with, or bind to the same epitope region of human PD-L1 as the above-references PD-L1 antibodies are mAbs. For administration to human subjects, these cross-competing antibodies can be chimeric antibodies, or can be humanized or human antibodies. Such chimeric, humanized or human mAbs can be prepared and isolated by methods well known in the art.

Anti-PD-L1 Antibodies

[0100] In certain embodiments, the present application encompasses use of an anti-PD-L1 antibody as a monotherapy in lieu of anti-PD-1 antibody. In one embodiment, the anti-PD-L1 antibody inhibits the binding of PD-L1 receptor, i.e., PD-1 to its ligand PD-L1.

[0101] Anti-PD-L1 antibodies useful for the invention include antibodies engineered starting from antibodies having one or more of the V_H and/or V_L sequences disclosed herein, which engineered antibodies can have altered properties from the starting antibodies. An anti-PD-L1 antibody can be engineered by a variety of modifications as described above for the engineering of modified anti-PD-1 antibodies of the invention.

[0102] Anti-PD-L1 antibodies of the invention also include isolated antibodies selected for their ability to bind to PD-L1 in formalin-fixed, paraffin-embedded (FFPE) tissue specimens. The use of FFPE samples is essential for the long-term follow-up analysis of the correlation between PD-L1 expression in tumors and disease prognosis or progression. The use of different antibodies to stain PD-L1 in frozen versus FFPE tissues, and the ability of certain antibodies to distinguish membranous and/or cytoplasmic forms of PD-L1, may account for some of the disparate data reported in the literature correlating PD-L1 expression with disease prognosis (Hamanishi *et al.*, *Proc. Natl. Acad. Sci. USA 104(9)*:3360-3365 (2007); Gadiot *et al.*, *Cancer 117(10)*:2192-2201 (2011)). This disclosure provides several rabbit mAbs that bind with high affinity specifically to membranous human PD-L1 in FFPE tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells.

[0103] In some embodiments, an anti-PD-L1 antibody useful for the present methods includes mAb 28-8 set forth in SEQ ID NOs. 1 and 2, respectively. The sequences of the heavy and light chain CDR domains of mAb 28-8, as delineated using the Kabat system, are set forth in SEQ ID NOs. 3-8. In other embodiments, an anti-PD-L1 antibody useful for the invention comprises mAbs 28-1, 28-12, 29-8 and 20-12 or an antigen-binding portion thereof, for example, including Fab, $F(ab')_2$ Fd, Fv, and scFv, di-scFv or bi-scFv, and scFv-Fc fragments, diabodies, triabodies, tetrabodies, and isolated CDRs.

Anti-CTLA-4 antibodies

[0104] Anti-CTLA-4 antibodies of the instant invention bind to human CTLA-4 so as to disrupt the interaction of CTLA-4 with a human B7 receptor. Because the interaction of CTLA-4 with B7 transduces a signal leading to inactivation of T-cells bearing the CTLA-4 receptor, disruption of the interaction effectively induces, enhances or prolongs the activation of such T cells, thereby inducing, enhancing or prolonging an immune response.

[0105] HuMAbs that bind specifically to CTLA-4 with high affinity have been disclosed in U.S. Patent Nos. 6,984,720 and 7,605,238. Other anti-PD-1 mAbs have been described in, for example, U.S. Patent Nos. 5,977,318, 6,051,227, 6,682,736, and 7,034,121. The anti-PD-1 HuMAbs disclosed in U.S. Patent No. Nos. 6,984,720 and 7,605,238 have been demonstrated to exhibit one or more of the following characteristics: (a) binds specifically to human CTLA-4 with a binding affinity reflected by an equilibrium association constant (K_a) of at least about 10^7 M^{-1}, or about 10^9 M^{-1}, or about 10^{10} M^{-1} to 10^{11} M^{-1} or higher, as determined by Biacore analysis; (b) a kinetic association constant (k_a) of at least about 10^3, about 10^4, or about 10^5 m^{-1} s^{-1}; (c) a kinetic disassociation constant (k_d) of at least about 10^3, about 10^4, or about 10^5 m^{-1} s^{-1}; and (d) inhibits the binding of CTLA-4 to B7-1 (CD80) and B7-2 (CD86). Anti-CTLA-4 antibodies useful for the present invention include mAbs that bind specifically to human CTLA-4 and exhibit at least one, at least two, or at least three of the preceding characteristics.

[0106] An exemplary clinical anti-CTLA-4 antibody is the human mAb 10D1 (now known as ipilimumab and marketed as YERVOY®) as disclosed in U.S. Patent No. 6,984,720. Ipilimumab is an anti-CTLA-4 antibody for use in the methods disclosed herein. Ipilimumab is a fully human, IgG1 monoclonal antibody that blocks the binding of CTLA-4 to its B7 ligands, thereby stimulating T cell activation and improving overall survival (OS) in patients with advanced melanoma.

[0107] Another anti-CTLA-4 antibody useful for the present methods is tremelimumab (also known as CP-675,206). Tremelimumab is human IgG2 monoclonal anti-CTLA-4 antibody. Tremelimumab is described in WO/2012/122444, U.S. Publ. No. 2012/263677, or WO Publ. No. 2007/113648 A2.

[0108] Anti-CTLA-4 antibodies useful for the disclosed composition also include isolated antibodies that bind specifically to human CTLA-4 and cross-compete for binding to human CTLA-4 with ipilimumab or tremelimumab or bind to the same epitope region of human CTLA-4 as ipilimumab or tremelimumab. In certain embodiments, the antibodies that cross-compete for binding to human CTLA-4 with, or bind to the same epitope region of human CTLA-4 as does ipilimumab or tremelimumab, are antibodies comprising a heavy chain of the human IgG1 isotype. For administration to human subjects, these cross-competing antibodies are chimeric antibodies, or humanized or

human antibodies. Useful anti-CTLA-4 antibodies also include antigen-binding portions of the above antibodies such as Fab, $F(ab')_2$, Fd or Fv fragments.

Dosages

[0109] The anti-PD-1 antibody can be administered to a suitable patient in therapeutically effective amounts. For example, the anti-PD-1 antibody can be administered at a dosage ranging from at least about 0.1 to at least about 20.0 mg/kg body weight. In certain embodiments, the anti-PD-1 antibody is individually administered at a dosage of at least about 0.1, at least about 0.3, at least about 0.5, at least about 1, at least about 3, at least about 5, at least about 10 or at least about 20 mg/kg, *e.g.*, at least about 1 to at least about 10 mg/kg, *e.g.*, at least about 1 to at least about 3 mg/kg, *e.g.*, at least about 3 mg/kg, *e.g.*, at least about 1 mg/kg. The anti-PD-1 antibody can be administered at a dosing frequency of at least once about every week, at least once about every 2 weeks, at least once about every 3 weeks, or at least once about every 4 weeks, or at least once a month, for up to 6 to up to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In some embodiments, the anti-PD-1 antibody is administered at a dosage of about 1 or about 3 mg/kg. In certain embodiments, an anti-PD-1 antibody regimen comprises administering the anti-PD-1 antibody to the subject at a dosing frequency of once about every week, once about every 2 weeks, once about every 3 weeks, or once about every 4 weeks, or once a month for 6 to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In other embodiments, the anti-PD-1 antibody is administered at a dosage of about 1 mg/kg at a dosing frequency of once about every 3 weeks for up to 48 doses.

[0110] In other embodiments, the anti-PD-1 antibody is administered at the following dosages: (a) 0.1 mg/kg anti-PD-1 antibody; (b) 0.3 mg/kg anti-PD-1 antibody; (c) 1 mg/kg anti-PD-1 antibody; (d) 3 mg/kg anti-PD-1 antibody; (e) 5 mg/kg anti-PD-1 antibody; (f) 10 mg/kg anti-PD-1 antibody; (g) 0.1 mg/kg anti-PD-1 antibody; (h) 0.3 mg/kg anti-PD-1 antibody; (i) 1 mg/kg anti-PD-1 antibody; (j) 3 mg/kg anti-PD-1 antibody; (k) 5 mg/kg anti-PD-1 antibody; or (1) 10 mg/kg anti-PD-1 antibody. In a particular embodiment, the method includes administration of 1 mg/kg of an anti-PD-1 antibody.

[0111] In some embodiments, the dosage of the anti-PD-1 antibody is kept constant during the induction dosing schedule and the maintenance dosing schedule. In certain

embodiments, a regimen comprises: (i) an induction dosing schedule comprising administration of the anti-PD-1 antibody at a dosing frequency of at least once about every 2 weeks, once about every 3 weeks, or once about every 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 10 doses, followed by administration of the anti-PD-1 antibody at a dosing frequency of at least once about every 2 weeks, once about every 3 weeks, or once about every 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 12 doses; followed by (ii) a maintenance dosing schedule comprising administration of the anti-PD-1 antibody at a dosing frequency of at least once about every 8 weeks, once about every 12 weeks, or once about every 16 weeks, or at least about once a quarter, for at least 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In one particular embodiment, the dosing schedule comprises administering about 3 mg/kg of an anti-PD-1 antibody every 2 weeks. In another embodiment, the dosing schedule comprises administering about 1 mg/kg of an anti-PD-1 antibody every 3 weeks for 4 doses followed by about 3 mg/kg of an anti-PD-1 antibody every 2 weeks.

[0112] In certain embodiments of this method, the maintenance dosing schedule comprises combined administration of up to 4, 6, 8, 10, 12 or 16 doses of the anti-PD-1 antibody. In other embodiments, the concurrent regimen comprises: (i) an induction dosing schedule comprising administration of the anti-PD-1 antibody at a dosing frequency of once about every 2 weeks, once about every 3 weeks, or once about every 4 weeks, or once a month, for 2, 4, 6 or 8 doses, followed by administration of the anti-PD-1 antibody at a dosing frequency of once about every 2 weeks, once about every 3 weeks, or once about every 4 weeks, or once a month, for 2, 4, 6, 8 or 12 doses; followed by (ii) a maintenance dosing schedule comprising administration of the anti-PD-1 antibody at a dosing frequency of once about every 8 weeks, once about every 12 weeks, or once about every 16 weeks, or about once a quarter, for 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs.

[0113] In a particular embodiment, the anti-PD-1 antibody is administered at dosages of about 1 mg/kg anti-PD-1 antibody every three weeks.

[0114] In certain embodiments of the present methods, the anti-PD-1 antibody is formulated for parenteral administration, *e.g.*, intravenous administration.

[0115] In some embodiments, the therapeutically effective dosage of the anti-PD-1 antibody or antigen-binding portion thereof is a flat dosing (not a bodyweight based dosing) and comprises about 60 mg, about 80 mg, about 100 mg, about 120 mg, about 140 mg, about 160 mg, about 180 mg, about 200 mg, about 220 mg, about 240 mg, about 260 mg, about 280 mg, or about 300 mg. In other embodiments, the therapeutically effective dosage of the anti-PD-1 antibody or antigen-binding portion thereof comprises about about 320 mg, 360mg, 400 mg, 420mg, 480 mg, 500mg, 540 mg, 550mg, 600mg, 620 mg, 650mg, 680mg, 700 mg, 720mg, 780 mg, 800mg, 840mg, or 900mg. In some embodiments, the dose of the anti-PD-1 antibody in the composition is between about 60 mg and about 300 mg, between about 60 mg and about 100 mg, between about 100 mg and about 200 mg, or between about 200 mg and about 300 mg. In some embodiments, the amount of the anti-PD-1 antibody in the composition is at least about 80 mg, about 160 mg, or about 240 mg. In some embodiments, the dose of the anti-PD-1 antibody in the composition is at least about 240 mg or at least about 80 mg.

[0116] In other embodiments, the dosages are given every week, every two weeks, every three weeks, every four weeks, every five weeks, every six weeks, every seven weeks, every eight weeks, every nine weeks, or every 10 weeks.

[0117] In some embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of about 240 mg. In embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of about 360 mg. In embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of about 480 mg. In one embodiment, 360mg of the anti-PD-1 antibody or antigen binding fragment is administered once every 3 weeks. In another embodiment, 480mg of the anti-PD-1 antibody or antigen binding fragment is administered once every 4 weeks.

[0118] The combination of an anti-PD-1 antibody and an anti-CTLA-4 antibody can be administered to a suitable patient in therapeutically effective amounts. For example, each antibody can be administered at a dosage ranging from at least about 0.1 to at least about 20.0 mg/kg body weight. In certain embodiments, each of the anti-PD-1 and anti-CTLA-4 antibodies is individually administered at a dosage of at least about 0.1, at least about 0.3, at least about 0.5, at least about 1, at least about 3, at least about 5, at least about 10 or at least about 20 mg/kg, *e.g.*, at least about 1 to at least about 10 mg/kg, *e.g.*, at least about 1

to at least about 3 mg/kg, *e.g.*, at least about 3 mg/kg, *e.g.*, at least about 1 mg/kg. Each of the anti-PD-1 antibody and anti-CTLA-4 antibody can be administered at a dosing frequency of at least once about every week, at least once about every 2 weeks, at least once about every 3 weeks, or at least once about every 4 weeks, or at least once a month, for up to 6 to up to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In some embodiments, the anti-PD-1 antibody is administered at a dosage of about 1 or about 3 mg/kg. In certain embodiments, the sequenced regimen comprises administering the anti-PD-1 antibody to the subject at a dosing frequency of once about every week, once about every 2 weeks, once about every 3 weeks, or once about every 4 weeks, or once a month for 6 to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In other embodiments, the anti-PD-1 is administered at a dosage of about 1 mg/kg at a dosing frequency of once about every 3 weeks for up to 48 doses. In some embodiments, the anti-CTLA-4 antibody is administered at a dosage of 1 or 3 mg/kg. In certain embodiments, the sequenced regimen comprises administering the anti-CTLA-4 antibody to the subject at a dosing frequency of once about every week, once about every 2 weeks, once about every 3 weeks, or once about every 4 weeks, or once a month for 6 to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In other embodiments, the anti-CTLA-4 antibody is administered at a dosage of about 3 mg/kg at a dosing frequency of once about every 3 weeks for up to 48 doses.

[0119] In other embodiments, the anti-PD-1 and anti-CTLA-4 antibodies are administered at the following dosages: (a) 0.1 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (b) 0.3 mg/kg anti-PD-1 antibody and 3 mg/kg of anti- CTLA-4 antibody; (c) 1 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (d) 3 mg/kg anti-PD-1 A antibody b and 3 mg/kg of anti-CTLA-4 antibody; (e) 5 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (f) 10 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (g) 0.1 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; (h) 0.3 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; (i) 1 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; (j) 3 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; (k) 5 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; or (1) 10 mg/kg anti-PD-1 antibody and

1 mg/kg of anti-CTLA-4 antibody. In a particular embodiment, the methods include administration of 1 mg/kg of an anti-PD-1 antibody and 3 mg/kg of an anti-CTLA-4 antibody.

[0120] In some embodiments, the dosage of each of the anti-PD-1 and anti-CTLA-4 antibodies is kept constant during the induction dosing schedule and the maintenance dosing schedule. In certain embodiments, a regimen comprises: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of at least once about every 2 weeks, once about every 3 weeks, or once about every 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 10 doses, followed by administration of the anti-PD-1 antibody alone at a dosing frequency of at least once about every 2 weeks, once about every 3 weeks, or once about every 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 12 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of at least once about every 8 weeks, once about every 12 weeks, or once about every 16 weeks, or at least about once a quarter, for at least 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs.

[0121] In certain embodiments of this method, the maintenance dosing schedule comprises combined administration of up to 4, 6, 8, 10, 12 or 16 doses of the anti-PD-1 and anti-CTLA-4 antibodies. In other embodiments, the concurrent regimen comprises: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti- CTLA-4 antibodies at a dosing frequency of once about every 2 weeks, once about every 3 weeks, or once about every 4 weeks, or once a month, for 2, 4, 6 or 8 doses, followed by administration of the anti-PD-1 antibody alone at a dosing frequency of once about every 2 weeks, once about every 3 weeks, or once about every 4 weeks, or once a month, for 2, 4, 6, 8 or 12 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of once about every 8 weeks, once about every 12 weeks, or once about every 16 weeks, or about once a quarter, for 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs.

[0122] In a particular embodiment, the anti-PD-1 and anti-CTLA-4 antibodies are administered at dosages of about 1 mg/kg anti-PD-1 antibody every three weeks and about 3 mg/kg of anti-CTLA-4 antibody every three weeks.

[0123] In certain embodiments of the present methods, the anti-PD-1 and anti-CTLA-4 antibodies are formulated for parenteral administration, *e.g.*, intravenous administration. In certain other embodiments, when the anti-PD-1 and anti-CTLA-4 antibodies are administered in combination, they are administered within about 30 minutes of each other. Either antibody may be administered first, that is, in certain embodiments, the anti-PD-1 antibody is administered before the anti-CTLA-4 antibody, whereas in other embodiments, the anti-CTLA-4 antibody is administered before the anti-PD-1 antibody. Typically, each antibody is administered intravenously over a period of about 60 minutes. In further embodiments, the anti-PD-1 and anti-CTLA-4 antibodies are administered concurrently, either admixed as a single composition in a pharmaceutically acceptable formulation for concurrent administration, or concurrently as separate compositions with each antibody in a pharmaceutically acceptable formulation.

[0124] In some embodiments, the anti-PD-1 antibody and the anti-CTLA-4 antibody are formulated in a single composition. The ratio of the amount of the anti-PD-1 antibody and the amount of the anti-CTLA-4 antibody in the single composition can be 10:1 to 1:10. In other embodiments, the ratio of the amount of the anti-PD-1 antibody and the amount of the anti-CTLA-4 antibody in the single composition is 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, or 1:5. In a particular embodiment, the ratio of the amount of the anti-PD-1 antibody and the amount of the anti-CTLA-4 antibody in the single composition is 1:3.

[0125] In some embodiments, the composition is administered at a flat dose regardless of the weight of the patient. For example, each of the anti-PD-1 antibody with the anti-CTLA-4 antibody may be administered at a flat dose of 20, 50, 75, 80, 160, 200, 240, 300, 360, 400, 480, 500, 750 or 1500 mg, without regard to the patient's weight. In some embodiments the composition is administered at a weight-based dose at any dose disclosed herein. In some embodiments, the amount of the anti-PD-1 antibody and the amount of the anti-CTLA-4 antibody administered to the patient at a single dose are identical.

[0126] In other embodiments, the therapeutically effective dosage of the anti-CTLA-4 antibody or antigen-binding portion thereof comprises about 60 mg, about 80 mg, about

100 mg, about 120 mg, about 140 mg, about 160 mg, about 180 mg, about 200 mg, about 220 mg, about 240 mg, about 260 mg, about 280 mg, or about 300 mg. In some embodiments, the dose of the anti-CTLA-4 antibody in the composition is between about 60 mg and about 300 mg, between about 60 mg and about 100 mg, between about 100 mg and about 200 mg, or between about 200 mg and about 300 mg. In some embodiments, the amount of the anti-CTLA-4 antibody in the composition is at least about 80 mg, about 160 mg, or about 240 mg. In some embodiments, the dose of the anti-CTLA-4 antibody in the composition is at least about 240 mg or at least about 80 mg.

[0127] In some embodiments, a flat dose of an anti-PD-1 antibody is 80 mg, and a flat dose of an anti-CTLA-4 antibody is 240 mg.

[0128] In some embodiments, the anti-PD-1 antibody is administered at a subtherapeutic dose, *i.e.*, a dose of the therapeutic agent that is significantly lower than the usual or FDA-approved dose when administered as monotherapy for the treatment of the cancer. The quantity of the second antibody in the composition is calculated based on the desired ratio.

[0129] In some embodiments, the composition is administered by intravenous infusion about once per week, about once every 2 weeks, about once every 3 weeks, or about once a month. In certain embodiments, the composition is administered once every 3 weeks. In some embodiments, the infusion occurs over at least about 10 minutes, about 20 minutes, about 30 minutes, about 45 minutes, about 60 minutes, about 90 minutes, about 2 hours, about 3 hours, about 4 hours or about 5 hours.

[0130] Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention can be flat or varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being unduly toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts. A composition of

the present invention can be administered via one or more routes of administration using one or more of a variety of methods well known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

Kits

[0131] Also within the scope of the present invention are kits, including pharmaceutical kits, comprising an anti-PD-1 antibody, for therapeutic uses, and diagnostic kits comprising an anti-PD-L1 antibody for assaying membranous PD-L1 expression as a biomarker for screening patients for the combination therapy or for predicting the efficacy of the anti-PD-1 monotherapy. In some embodiments, the pharmaceutical kits further comprise a combination of an anti-PD-1 antibody and an anti-CTLA-4 Ab, for therapeutic uses. Kits typically include a label indicating the intended use of the contents of the kit and instructions for use. The term "label" includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit. In certain embodiments of a pharmaceutical kit, the anti-PD-1 antibody is co-packaged with an anti-CTLA-4 antibody in unit dosage form. In certain embodiments of a diagnostic kit, the anti-PD-L1 antibody is co-packaged with an anti-PD-1 antibody for performing an assay to detect and/or quantify PD-L1 expression. In one particular embodiment, the diagnostic kit further comprises an anti-CTLA-4 antibody.

[0132] In certain embodiments, the pharmaceutical kit comprises the anti-human PD-1 HuMAb, nivolumab. In other embodiments, the pharmaceutical kit comprises the anti-human PD-L1 HuMAb, BMS-936559. In yet other embodiments, the pharmaceutical kit comprises the anti-human CTLA-4 HuMAb, ipilimumab. In certain embodiments, the diagnostic kit comprises the rabbit anti-human PD-L1 mAb, 28-8, comprising the V_H and V_L regions whose amino acid sequences are set forth in SEQ ID NOs. 1 and 2, respectively. In other embodiments, the diagnostic kit comprises the murine anti-human PD-L1 mAb, 5H1 (Dong *et al.*, *Nature Med. 8(8)*:793-800 (2002)).

[0133] In some embodiments, the invention provides a kit for treating a patient afflicted with a melanoma tumor, the kit comprising:

(a) a dosage ranging from at least about 0.1 to at least about 10 mg/kg body weight of an anti-PD-1 antibody or an antigen-binding portion thereof; and

(b) instructions for using the anti-PD-1 antibody or the antigen-binding portion thereof in the methods disclosed herein.

[0134] In another embodiment, the invention provides a kit for treating a patient afflicted with a melanoma tumor, the kit comprising:

(a) a dosage ranging from at least about 0.1 to at least about 10 mg/kg body weight of an anti-PD-1 antibody or an antigen-binding portion thereof;

(b) a dosage ranging from at least about 0.1 to at least about 10 mg/kg body weight of an anti-CTLA-4 antibody or an antigen-binding portion thereof; and

(c) instructions for using the anti-PD-1 antibody or the antigen-binding portion thereof and the anti-CTLA-4 antibody or the antigen-binding portion thereof in the methods disclosed herein.

[0135] The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of all references cited throughout this application are expressly incorporated herein by reference.

EXAMPLES

EXAMPLE 1

[0136] A randomized, double-blind, multicenter, phase 3 trial was conducted to evaluate the safety and efficacy of nivolumab alone or nivolumab combined with ipilimumab in comparison with ipilimumab alone in previously untreated metastatic melanoma.

Patients

[0137] Eligible patients had histologically confirmed stage III (unresectable) or stage IV melanoma, and no prior systemic treatment for unresectable or metastatic melanoma. Other eligibility criteria included an age of at least 18 years, an Eastern Cooperative Oncology Group (ECOG) performance status score of 0 (indicating no symptoms) or 1 (indicating mild symptoms), measurable disease by computed tomography or magnetic resonance imaging per RECIST v1.1, availability of tissue collected from metastatic or unresectable tumors for the assessment of PD-L1 status, and known BRAF V600 mutation status (or consent to BRAF V600 mutation testing per local standards). Key exclusion criteria were presence of active brain metastases, ocular melanoma, or autoimmune disease, and any prior treatment with an anti-PD-1, anti-PD-L1, anti-PD-L2,

or anti-CTLA-4 antibody. Patients who required systemic corticosteroid treatment or other immunosuppressive medications within 14 days of study drug administration were excluded.

Study Design and Treatment

[0138] In the double-blind, phase 3 study, enrolled patients were randomly assigned in a 1:1:1 ratio to receive 3 mg of nivolumab per kilogram of body weight every 2 weeks (plus ipilimumab-matched placebo), or 1 mg of nivolumab per kilogram every 3 weeks plus 3 mg of ipilimumab per kilogram every 3 weeks for 4 doses (plus nivolumab-matched placebo), followed by 3 mg of nivolumab per kilogram every 2 weeks for cycle 3 and beyond, or 3 mg of ipilimumab per kilogram every 3 weeks for 4 doses (plus nivolumab-matched placebo).

[0139] Both nivolumab and ipilimumab were administered by intravenous infusion. Randomization was stratified according to tumor PD-L1 status (positive vs. negative or indeterminate), BRAF mutation status (V600 mutation positive vs. wild-type), and American Joint Committee on Cancer metastasis stage (M0, M1a, or M1b vs. M1c). Treatment continued until RECIST v1.1-defined disease progression, unacceptable toxicity, or withdrawal of consent. Patients could be treated beyond progression provided they had a clinical benefit without clinical deterioration, and did not have substantial adverse effects, as assessed by the investigator.

[0140] Progression-free survival and overall survival were co-primary end points. Secondary end points included objective response rate, tumor PD-L1 expression as a predictive biomarker for progression-free and overall survival, and health-related quality of life. Exploratory end points include duration of objective response and safety/tolerability of study drug therapy.

Assessments

[0141] Patients were assessed for tumor response, according to RECIST v1.1 at 12 weeks after randomization and continuing every 6 weeks for 49 weeks, and then every 12 weeks until progression or treatment discontinuation, whichever occurred later. Progression-free survival was defined as the time between the date of randomization and the first date of documented progression or death, whichever occurred first. Patients treated beyond progression were considered to have progressive disease at the time of the initial

progression event, as assessed by the investigator, regardless of subsequent tumor responses. Expression of PD-L1 on the surface of the tumor cells was assessed in a central laboratory by immunohistochemistry in formalin-fixed, paraffin-embedded tumor specimens using a rabbit monoclonal anti-human PD-L1 antibody (clone 28-8) and an analytically validated automated assay developed by Dako (Carpinteria, CA). PD-L1 positivity was defined as at least 5% of tumor cells showing cell surface PD-L1 staining of any intensity in a section containing at least 100 tumor cells that could be evaluated. Indeterminate status was attributed to samples for which tumor cell–surface expression could not be discerned because of melanin content or strong cytoplasmic staining.

[0142] Any patient who received at least one dose of study drug in each of the 3 treatment groups was included in the assessment of safety. The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.18. Safety assessments were made continuously during the treatment phase, and up to 100 days after the last dose of study drug.

Statistical Analysis

[0143] A study sample size of approximately 915 patients, randomized to the 3 treatment arms in a 1:1:1 ratio, was planned. For the comparison of progression-free survival, the number of events projected to be observed at a follow-up of at least 9 months provided approximately 83% power to detect an average hazard ratio of 0.71 with a type I error of 0.005 (two-sided). Progression-free survival was compared between nivolumab plus ipilimumab and ipilimumab alone, and between nivolumab alone and ipilimumab alone with the use of a two-sided log-rank test stratified according to PD-L1 status, BRAF mutation status, and metastasis stage (as described above). The study was not designed for a formal statistical comparison between the nivolumab alone and nivolumab plus ipilimumab groups. Hazard ratios and corresponding two-sided 99.5% confidence intervals (CIs) were estimated using a Cox proportional hazards model, with treatment group as a single covariate, stratified by the above factors. Progression-free survival curves, medians with 95% CIs, and progression-free survival rates at 6, 12, and 18 months with 95% CIs were estimated using Kaplan-Meier methodology. Overall survival will be analyzed when all patients have a minimum follow-up of 22 months.

Results

Patients and Treatment

[0144] From July 2013 through March 2014, a total of 1296 patients were enrolled at 137 centers in Australia, Europe, Israel, New Zealand, and North America. A total of 945 patients underwent randomization: 316 patients were assigned to the nivolumab group, 314 to the nivolumab plus ipilimumab group, and 315 to the ipilimumab group (Figure 1). Baseline characteristics were balanced across the three groups. A total of 58.0% had stage M1c disease, 36.1% had an elevated lactate dehydrogenase level, 31.5% had a BRAF mutation, and 26.5% had positive PD-L1 status (Table 1).

Table 1. Baseline Characteristics of the Patients.

Characteristic	Nivolumab alone (N=316)	Nivolumab plus Ipilimumab (N=314)	Ipilimumab alone (N=315)	Total (N=945)
Age — yr				
Mean	58.7	59.3	60.8	59.6
Range	25–90	18–88	18–89	18–90
Age groups — no. (%)				
<65 yr	198 (62.7)	185 (58.9)	182 (57.8)	565 (59.8)
≥ 65, < 75 yr	79 (25.0)	94 (29.9)	89 (28.3)	262 (27.7)
≥ 75 yr	39 (12.3)	35 (11.1)	44 (14.0)	118 (12.5)
Sex — no. (%)				
Male	202 (63.9)	206 (65.6)	202 (64.1)	610 (64.6)
Female	114 (36.1)	108 (34.4)	113 (35.9)	335 (35.4)
ECOG performance status — no. (%)				
0	238 (75.3)	230 (73.2)	224 (71.1)	692 (73.2)
1	77 (24.4)	83 (26.4)	91 (28.9)	251 (26.6)
2	1 (0.3)	0	0	1 (0.1)
Not reported	0	1 (0.3)	0	1 (0.1)
M stage — no. (%)				
M1c	184 (58.2)	181 (57.6)	183 (58.1)	548 (58.0)
M0, M1a, or M1b	132 (41.8)	133 (42.4)	132 (41.9)	397 (42.0)
Lactate dehydrogenase — no. (%)*				
≤ ULN	196 (62.0)	199 (63.4)	194 (61.6)	589 (62.3)
>ULN	112 (35.4)	114 (36.3)	115 (36.5)	341 (36.1)
≤2x ULN	271 (85.8)	276 (87.9)	279 (88.6)	826 (87.4)

Characteristic	Nivolumab alone (N=316)	Nivolumab plus Ipilimumab (N=314)	Ipilimumab alone (N=315)	Total (N=945)
>2x ULN	37 (11.7)	37 (11.8)	30 (9.5)	104 (11.0)
Unknown	8 (2.5)	1 (0.3)	6 (1.9)	15 (1.6)
Brain metastases at baseline — no. (%)				
Yes	8 (2.5)	11 (3.5)	15 (4.8)	34 (3.6)
No	308 (37.5)	303 (96.5)	300 (95.2)	911 (96.4)
PD-L1 status — no. (%)				
Positive	80 (27.8)	68 (24.5)	75 (27.1)	223(26.4)
Negative	208 (72.2)	210 (75.5)	202 (72.9)	620 (73.5)
BRAF status — no. (%)				
Mutation	100 (31.6)	101 (32.2)	97 (30.8)	298 (31.5)
No mutation	216 (68.4)	213 (67.8)	218 (69.2)	647 (68.5)

* ULN denotes upper limit of normal.

[0145] All randomized patients had been followed for a minimum of 9 months at the time of database lock (February 17, 2015); 117 of 313 patients (37.4%) in the nivolumab group, 93 of 313 patients (29.7%) in the nivolumab plus ipilimumab group, and 50 of 311 patients (16.1%) in the ipilimumab group were continuing study treatment (Table 2). The most frequent reason for discontinuation was disease progression in the nivolumab and ipilimumab monotherapy groups (154 of 313 patients [49.2%] and 202 of 311 patients [65.0%], respectively), versus study drug toxicity in the nivolumab plus ipilimumab group (120 of 313 patients [38.3%]). The number of patients who had died was 85 (27.2%) in the nivolumab group, 86 (27.5%) in the nivolumab plus ipilimumab group, and 114 (36.7%) in the ipilimumab group.

[0146] The median number of doses in patients who received nivolumab alone or ipilimumab alone was 15 (range 1-38) and 4 (1-4), respectively. In the combination group, the median number of doses was 4 (range 1–39) for nivolumab and 4 (range 1–4) for ipilimumab; 147 of 313 patients (47%) received four or more doses of nivolumab monotherapy after combination treatment.

Efficacy

[0147] The median progression-free survival was 6.5 months (95% confidence interval [CI], 4.3 to 9.5) in the nivolumab group, 11.5 months (95% CI, 8.9 to 16.5) in the nivolumab plus ipilimumab group, and 2.9 months (95% CI, 2.8 to 3.4) in the ipilimumab group (Figure 2A). A significant improvement in progression-free survival was observed in the nivolumab plus ipilimumab group as compared with the ipilimumab group (hazard ratio, 0.42; 95% CI, 0.31 to 0.57; $P<0.0001$) (Figure 2A). A significant improvement in progression-free survival was also observed in the nivolumab group as compared with the ipilimumab group (hazard ratio, 0.57; 95% CI, 0.43 to 0.76; $P<0.00001$) (Figure 2A). The hazard ratio for the comparison between nivolumab plus ipilimumab and nivolumab groups was 0.74 (95% CI, 0.60 to 0.92).

[0148] Analyses of progression-free survival among prespecified patient subgroups showed a consistent improvement with nivolumab or nivolumab plus ipilimumab as compared with ipilimumab, including subgroups defined by PD-L1 status, BRAF mutation status, and metastasis stage (Figure 3). In the combination group, median PFS was 11.7 months (95% CI, 8.0 to not reached) among patients with a BRAF mutation and was 11.2 months (95% CI, 8.3 to not reached) in patients with wild-type BRAF (Figure

3B). For patients with a positive PD-L1 tumor status, median progression-free survival in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups was 14.0 months (95% CI, 9.1 to not reached), 14.0 months (95% CI, 9.7 to not reached), and 3.9 months (95% CI, 2.8 to 4.2), respectively (Figure 2B). For patients with a negative PD-L1 tumor status, median progression-free survival in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups was 5.3 months (95% CI, 2.8 to 7.1 months), 11.2 months (95% CI, 8.0 to not reached) and 2.8 months (95% CI, 2.8 to 3.1), respectively (Fig. 2C).

[0149] Investigator-assessed objective response rates were 43.7% (95% CI, 38.1 to 49.3%), 57.6% (95% CI, 52.0 to 63.2), and 19.0% (95% CI, 14.9 to 23.8) in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively (Table 3). The percentage of patients with a complete response was higher in the nivolumab plus ipilimumab group than in either the nivolumab or ipilimumab alone groups (11.5% vs. 8.9% and 2.2%) (Table 3). Time to objective response was similar in each group (Table 3), and the median duration of response was not reached in any of the groups.

[0150] Median reduction in the sum of the longest diameters of tumor target lesions was -34.5% (interquartile range: -75.4 to 15.4), -51.1% (-75.8 to -10.2), and 5.8% (-28.0 to 33.3) in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively (Figure 4). Among patients with PD-L1-positive tumors, the objective response rates were 57.5% (95% CI, 45.9 to 68.5), 72.1% (95% CI, 59.9 to 82.3), and 21.3% (95% CI, 12.7 to 32.3) for the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively; in patients with PD-L1-negative tumors, the objective response rates were 41.3% (95% CI, 34.6 to 48.4), 54.8% (95% CI, 47.8 to 61.6), and 17.8% (95% CI, 12.8 to 23.8) (Table 4).

Table 2. Patient Disposition.*

	Nivolumab alone (N=313)	Nivolumab plus Ipilimumab (N=313)	Ipilimumab alone (N=311)
Patients in the treatment period — no. (%)			
Continuing	117 (37.4)	93 (29.7)	50 (16.1)
Not continuing	196 (62.6)	220 (70.3)	261 (83.9)
Reason for not continuing the treatment — no. (%)			
Disease progression	154 (49.2)	69 (22.0)	202 (65.0)
Study drug toxicity	27 (8.6)	120 (38.3)	47 (15.1)
Adverse event unrelated to study drug	5 (1.6)	12 (3.8)	4 (1.3)
Patient request to discontinue treatment	5 (1.6)	5 (1.6)	4 (1.3)
Death	5 (1.6)	5 (1.6)	4 (1.3)
Maximum clinical benefit	2 (0.6)	2 (0.6)	0
Poor/non-compliance	1 (0.3)	1 (0.3)	1 (0.3)
Patient withdrew consent	0	3 (1.0)	0
Lost to follow-up	1 (0.3)	0	0
Patient no longer meets study criteria	0	1 (0.3)	0
Other	0	3 (1.0)	2 (0.6)
Patients in the study — no. (%)			
Continuing	223 (71.2)	221 (70.6)	189 (60.8)
Died	85 (27.2)	86 (27.5)	114 (36.7)
Not continuing	5 (1.6)	6 (1.9)	8 (2.6)

***At the time of database lock on February 17, 2015**.

Table 3. Response to Treatment.

Response	Nivolumab alone (N=316)	Nivolumab plus Ipilimumab (N=314)	Ipilimumab alone (N=315)
Best overall response — no. (%)[†]			
Complete response	28 (8.9)	36 (11.5)	7 (2.2)
Partial response	110 (34.8)	145 (46.2)	53 (16.8)
Stable disease	34 (10.8)	41 (13.1)	69 (21.9)
Progressive disease	119 (37.7)	71 (22.6)	154 (48.9)
Could not be determined	25 (7.9)	21 (6.7)	32 (10.2)
Objective response[‡]			
No. of patients (% [95% CI])	138 (43.7 [38.1–49.3])	181 (57.6 [52.0–63.2])	60 (19.0 [14.9–23.8])
Estimated odds ratio (95% CI)[§]	3.40 (2.02–5.72)	6.11 (3.59–10.38)	---
Two-sided P value	<0.00001	<0.00001	---
Time to objective response — mo			
No. of responders	138	181	60
Median	2.78	2.76	2.79
Range	2.3–12.5	1.1–11.6	2.5–12.4

[†] ***Best overall response were assessed by the investigators with the use of RECIST v1.1.***

[‡] ***Data include patients with a complete response and those with a partial response.***

[§] ***Relative to ipilimumab alone.***

Table 4. Objective Response Rate by PD-L1 Status.

PD-L1 Positive			
	Nivolumab alone (N=80)	**Nivolumab plus Ipilimumab (N=68)**	**Ipilimumab alone (N=75)**
Objective response[†]			
No. of patients (% [95% CI])	46 (57.5 [45.9–68.5])	49 (72.1 [59.9–82.3])	16 (21.3 [12.7–32.3])
Estimated odds ratio (95% CI)[§]	5.03 (2.44–10.37)	10.41 (4.63–23.40)	---
PD-L1 Negative			
	Nivolumab alone (N=208)	**Nivolumab plus Ipilimumab (N=210)**	**Ipilimumab alone (N=202)**
Objective response[†]			
No. of patients (% [95% CI])	86 (41.3 [34.6–48.4])	115 (54.8 [47.8–61.6])	36 (17.8 [12.8–23.8])
Estimated odds ratio (95% CI)[§]	3.25 (2.05–5.13)	5.90 (3.71–9.38)	---

[†] ***Best overall response was assessed by the investigators with the use of RECIST v1.1.***

[§] Relative to ipilimumab alone.

Adverse Events

[0151] Treatment-related adverse events of any grade occurred in 82.1%, 95.5%, and 86.2% of patients in the nivolumab, nivolumab plus ipilimumab groups, and ipilimumab groups, respectively (Table 5). The most common adverse events in the nivolumab group were fatigue (in 34.2% of patients), rash (in 21.7%), and diarrhea (in 19.2%). In the nivolumab plus ipilimumab and ipilimumab groups, diarrhea (in 44.1% and 33.1% of patients, respectively), fatigue (in 35.1% and 28.0%), and pruritus (in 33.2% and 35.4%) were most common (Table 5). The incidence of treatment-related adverse events of grade 3 or 4 was also higher in the nivolumab plus ipilimumab group than in either the nivolumab or ipilimumab groups (55.0% vs. 16.3% and 27.3%), with diarrhea being the most common (2.2% , 9.3%, and 6.1% in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively) (Table 5). Treatment-related adverse events of any grade leading to discontinuation occurred in 7.7%, 36.4%, and 14.8% of patients in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively, with the most common being diarrhea (in 1.9%, 8.3%, and 4.5%, respectively) and colitis (in 0.6%, 8.3%, and 7.7%, respectively) (Table 5). One death due to study-drug toxicity was reported in the nivolumab group (neutropenia) and one in the ipilimumab group (cardiac arrest), although such adverse events have not been associated with these drugs in prior studies. No treatment-related deaths were reported in the combination group.

[0152] The most frequent grade 3 or 4 treatment-related select adverse events were diarrhea (2.2%, 9.3%, and 6.1% of patients in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively), colitis (in 0.6%, 7.7%, and 8.7%, respectively), increased alanine aminotransferase (in 1.3%, 8.3%, and 1.6%, respectively), and increased aspartate aminotransferase (in 1.0%, 6.1%, and 0.6%, respectively) (Table 6). With the use of immune modulatory agents, resolution rates for grade 3 or 4 select adverse events were generally similar across treatment groups, and were between 85-100% across organ categories in the nivolumab plus ipilimumab group. As observed in prior studies, most endocrine events in all treatment groups did not resolve (Table 7).

Table 5. Adverse Events (Safety Population).*

Event	Nivolumab alone (N=313)		Nivolumab plus Ipilimumab (N=313)		Ipilimumab alone (N=311)	
	Total	Grade 3 or 4	Total	Grade 3 or 4	Total	Grade 3 or 4
	no. of patients with event (%)					
Any adverse event	311 (99.4)	136 (43.5)	312 (99.7)	215 (68.7)	308 (99.0)	173 (55.6)
Treatment-related adverse event†	257 (82.1)	51 (16.3)	299 (95.5)	172 (55.0)	268 (86.2)	85 (27.3)
Diarrhea	60 (19.2)	7 (2.2)	138 (44.1)	29 (9.3)	103 (33.1)	19 (6.1)
Fatigue	107 (34.2)	4 (1.3)	110 (35.1)	13 (4.2)	87 (28.0)	3 (1.0)
Pruritus	59 (18.8)	0	104 (33.2)	6 (1.9)	110 (35.4)	1 (0.3)
Rash	81 (25.9)	2 (0.6)	126 (40.3)	15 (4.8)	102 (32.8)	6 (1.9)
Nausea	41 (13.1)	0	81 (25.9)	7 (2.2)	50 (16.1)	2 (0.6)
Pyrexia	18 (5.8)	0	58 (18.5)	2 (0.6)	21 (6.8)	1 (0.3)
Decreased appetite	34 (10.9)	0	56 (17.9)	4 (1.3)	39 (12.5)	1 (0.3)
Increase in alanine aminotransferase	12 (3.8)	4 (1.3)	55 (17.6)	26 (8.3)	12 (3.9)	5 (1.6)
Vomiting	20 (6.4)	1 (0.3)	48 (15.3)	8 (2.6)	23 (7.4)	1 (0.3)
Increase in aspartate aminotransferase	12 (3.8)	3 (1.0)	48 (15.3)	19 (6.1)	11 (3.5)	2 (0.6)
Hypothyroidism	27 (8.6)	0	47 (15.0)	1 (0.3)	13 (4.2)	0
Colitis	4 (1.3)	2 (0.6)	37 (11.8)	24 (7.7)	36 (11.6)	27 (8.7)
Arthralgia	24 (7.7)	0	33 (10.5)	1 (0.3)	19 (6.1)	0
Headache	23 (7.3)	0	32 (10.2)	1 (0.3)	24 (7.7)	1 (0.3)
Dyspnea	14 (4.5)	1 (0.3)	32 (10.2)	2 (0.6)	13 (4.2)	0
Treatment-related adverse event leading to discontinuation	24 (7.7)	16 (5.1)	114 (36.4)	92 (29.4)	46 (14.8)	41 (13.2)

* The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

† The treatment-related adverse events listed here were reported in at least 10% of the patients in any of the three study groups.

Table 6. Treatment-related Select Adverse Events (Safety Population).*

Event	Nivolumab alone (N=313)		Nivolumab plus Ipilimumab (N=313)		Ipilimumab alone (N=311)	
	Total	Grade 3 or 4	Total	Grade 3 or 4	Total	Grade 3 or 4
	no. of patients with event (%)					
Any select adverse event	194 (62.0)	24 (7.7)	275 (87.9)	124 (39.6)	229 (73.6)	58 (18.6)
Treatment-related select adverse event†						
Skin	131 (41.9)	5 (1.6)	185 (59.1)	18 (5.8)	168 (54.0)	9 (2.9)
Pruritus	59 (18.8)	0	104 (33.2)	6 (1.9)	110 (35.4)	1 (0.3)
Rash	68 (21.7)	1 (0.3)	89 (28.4)	9 (2.9)	65 (20.9)	5 (1.6)
Rash maculo-papular	13 (4.2)	1 (0.3)	37 (11.8)	6 (1.9)	37 (11.9)	1 (0.3)
Vitiligo	23 (7.3)	1 (0.3)	21 (6.7)	0	12 (3.9)	0
Gastrointestinal	61 (19.5)	7 (2.2)	145 (46.3)	46 (14.7)	114 (36.7)	36 (11.6)
Diarrhea	60 (19.2)	7 (2.2)	138 (44.1)	29 (9.3)	103 (33.1)	19 (6.1)
Colitis	4 (1.3)	2 (0.6)	37 (11.8)	24 (7.7)	36 (11.6)	27 (8.7)
Hepatic	20 (6.4)	8 (2.6)	94 (30.0)	59 (18.8)	22 (7.1)	5 (1.6)
Increase in alanine aminotransferase	12 (3.8)	4 (1.3)	55 (17.6)	26 (8.3)	12 (3.9)	5 (1.6)
Increase in aspartate aminotransferase	12 (3.8)	3 (1.0)	48 (15.3)	19 (6.1)	11 (3.5)	2 (0.6)
Endocrine	45 (14.4)	2 (0.6)	94 (30.0)	15 (4.8)	34 (10.9)	7 (2.3)
Hypothyroidism	27 (8.6)	0	47 (15.0)	1 (0.3)	13 (4.2)	0
Hyperthyroidism	13 (4.2)	0	31 (9.9)	3 (1.0)	3 (1.0)	0
Hypophysitis	2 (0.6)	1 (0.3)	24 (7.7)	5 (1.6)	12 (3.9)	6 (1.9)
Pulmonary	5 (1.6)	1 (0.3)	22 (7.0)	3 (1.0)	6 (1.9)	1 (0.3)
Pneumonitis	4 (1.3)	1 (0.3)	20 (6.4)	3 (1.0)	5 (1.6)	1 (0.3)

* The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

† The treatment-related select adverse events listed here were reported in at least 5% of the patients in any of the three study groups.

Table 7. Management of Treatment-related Select Adverse Events With Immune Modulatory Medication (IMM).

	Nivolumab			Nivolumab plus Ipilimumab			Ipilimumab		
Select Adverse Event Organ Category	**Patients managed with IMM, n (%)**	**Patients with resolution of AE after treatment with IMM, n (%)**	**Median time to resolution, weeks (95% CI)**	**Patients managed with IMM, n (%)**	**Patients with resolution of AE after treatment with IMM, n (%)**	**Median time to resolution, weeks (95% CI)**	**Patients managed with IMM, n (%)**	**Patients with resolution of AE after treatment with IMM, n (%)**	**Median time to resolution, weeks (95% CI)**
Skin Grade 3-4	33/131 (25.2) 3/5 (60.0)	17 (54.8) 3 (75.0)	34.9 (18.0, NE) 2.1 (0.9, NE)	80/185 (43.2) 12/18 (66.7)	55 (75.3) 12 (85.7)	8.6 (7.0, 12.6) 3.4 (1.1, 12.4)	58/168 (34.5) 5/9 (55.6)	41 (74.5) 5 (83.3)	12.4 (8.9, 19.1) 6.1 (4.4, NE)
Gastrointestinal Grade 3-4	9/61 (14.8) 5/7 (71.4)	5 (71.4) 3 (50.0)	4.0 (0.8, NE) NE (0.8, NE)	71/145 (49.0) 41/46 (89.1)	62 (93.9) 41 (97.6)	4.5 (3.5, 5.7) 3.0 (1.9, 4.3)	54/114 (47.4) 33/36 (91.7)	44 (88.0) 31 (88.6)	4.9 (2.9, 7.6) 4.7 (1.9, 6.1)
Endocrine Grade 3-4	5/45 (11.1) 2/2 (100)	2 (40.0) 0	24.3 (7.1, NE) NE (NE, NE)	36/94 (38.3) 10/15 (66.7)	14 (41.2) 5 (45.5)	NE (10.4, NE) NE (4.5, NE)	15/34 (44.1) 7/7 (100.0)	4 (28.6) 3 (42.9)	NE (0.71, NE) NE (0.43, NE)
Hepatic Grade 3-4	8/22 (36.4) 7/8 (87.5)	6 (100.0) 6 (100.0)	7.0 (2.0, 27.1) 7.0 (2.0, 27.1)	46/95 (48.4) 37/60 (61.7)	43 (95.6) 38 (100.0)	5.9 (3.3, 6.6) 4.1 (3.0, 6.1)	3/22 (13.6) 2/5 (40.0)	3 (100.0) 2 (100.0)	4.1 (4.0, 7.7) 5.9 (4.0, 7.7)
Pulmonary Grade 3-4	4/5 (80.0) 1/1 (100)	4 (100.0) 1 (100.0)	3.3 (2.3, 9.1) 2.3 (NE, NE)	17/22 (77.3) 2/3 (66.7)	16 (94.1) 2 (100.0)	6.1 (1.1, 8.3) 4.2 (1.1, 7.3)	3/6 (50.0) 1/1 (100)	2 (66.7) 1 (100.0)	6.1 (6.0, 6.3) 4.7 (NE, NE)
Renal Grade 3-4	1/3 (33.3) 0/1	1 (100.0) 0	0.3 (NE, NE) -	4/17 (23.5) 3/6 (50.0)	3 (100.0) 3 (100.0)	1.7 (0.4, 3.7) 1.7 (0.4, 3.6)	3/8 (37.5) 1/1 (100)	3 (100.0) 1 (100.0)	4.6 (0.6, 16.1) 4.6 (NE, NE)

Discussion

[0153] In this randomized, double-blind, phase 3 study, both nivolumab alone and the combination of nivolumab and ipilimumab significantly increased progression-free survival and objective response rates, as compared with ipilimumab alone, in previously untreated advanced melanoma. These results were observed independently of PD-L1 tumor status, BRAF mutation status, or metastasis stage. Baseline characteristics of study participants were typical of patients with advanced melanoma, although the BRAF mutation rate (31.5%) was lower than the 40-50% generally reported for advanced disease. While not a primary end point of the study, the combination of nivolumab and ipilimumab resulted in numerically longer progression-free survival and a higher response rate as compared with nivolumab alone in the overall study population. While time to response was similar between groups, the first tumor assessment was done at week 12 and thus the possibility that responses may have occurred earlier with the combination remains unknown.

[0154] The median progression-free survival reported for the combination of nivolumab and ipilimumab in this study (11.7 months in BRAF-mutant patients) is similar to that recently reported for the combination of BRAF and MEK inhibition in BRAF-mutant metastatic melanoma (9.9 months for vemurafenib and cobimetinib; 9.3 to 11.4 months for dabrafenib and trametinib). Resistance to such targeted therapies is almost inevitable when used as monotherapy and in many cases is very rapid. The confirmed rate of objective response for combined nivolumab and ipilimumab (57.6%) is numerically higher than observed with PD-1 blockade alone in advanced melanoma (nivolumab [40%] in treatment-naïve patients with wild-type BRAF or pembrolizumab [37%] in ipilimumab-naïve patients).

[0155] The results of subgroup analyses suggest that the greatest benefit for the combination of nivolumab and ipilimumab versus nivolumab alone may occur in the setting of negative PD-L1 tumor expression. In the PD-L1-positive group, both nivolumab alone and nivolumab plus ipilimumab resulted in a similar prolongation of progression-free survival as compared with ipilimumab alone, although objective response rates were numerically higher in the combination group versus either nivolumab or ipilimumab alone. Thus, the use of PD-L1 as a biomarker may allow clinicians to make more informed decisions about the risk-benefit of combination therapy versus

monotherapy. Nonetheless, the observation of at least additive activity of the combination of ipilimumab and nivolumab in the setting of negative PD-L1 expression is of interest in melanoma as well as in other tumor types in which PD-1 checkpoint inhibitors are under evaluation.

[0156] The incidence of adverse events in this study was, in general, lowest in the nivolumab group and highest in the combination group. The overall incidence of grade 3 or 4 drug-related adverse events was higher in the combination group as compared with ipilimumab alone (39.6% versus 18.6%), as a result of a slightly higher incidence in most adverse events, particularly hepatic toxicity, where the rates of grade 3 or 4 ALT/AST elevations were 6-8% for the combination and approximately 1% for ipilimumab alone. One drug-related death was reported in each of the nivolumab and ipilimumab groups but none in the combination group. Overall, the safety profile of the combination of nivolumab and ipilimumab was consistent with previous experience with nivolumab or ipilimumab alone. No new safety signals were identified, and adverse events were manageable with established treatment guidelines as most select adverse events resolved with immune modulatory agents. These data suggest that the combination of nivolumab and ipilimumab can be used safely in a broad range of clinical settings.

[0157] In summary, the report shows increased progression-free survival and objective response rates for nivolumab alone and the combination of nivolumab and ipilimumab, as compared with ipilimumab alone, in previously untreated advanced melanoma. Adverse events with the combination were managed with established algorithms, with no study drug-related deaths. Overall, nivolumab alone and the combination of nivolumab and ipilimumab are promising treatment options for previously untreated advanced melanoma.

[0158] The present application claims benefit to U.S. Provisional Application No. 62/153,954 filed April 28, 2015, which is incorporated herein by reference in its entirety.

SEQUENCES

V_H amino acid sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:1)

Met Glu Thr Gly Leu Arg Trp Leu Leu Leu Val Ala Val Leu Lys Gly Val Gln Cys Leu Ser Val Glu Glu Ser Gly Gly Arg Leu Val Thr Pro Gly Thr Pro Leu Thr Leu Thr Cys Thr Ala Ser Gly Phe Thr Ile Thr Asn Tyr His Met Phe Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile Gly Val Ile Thr Ser Ser Gly Ile Gly Ser Ser Ser Thr Thr Tyr Tyr Ala Thr Trp Ala Lys Gly Arg Phe Thr Ile Ser Lys Thr Ser Thr Thr Val Asn Leu Arg Ile Thr Ser Pro Thr Thr Glu Asp Thr Ala Thr Tyr Phe Cys Ala Arg Asp Tyr Phe Thr Asn Thr Tyr Tyr Ala Leu Asp Ile Trp Gly Pro Gly Thr Leu Val Thr Val Ser Ser

V_L amino acid sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:2)

Met Asp Thr Arg Ala Pro Thr Gln Leu Leu Gly Leu Leu Leu Leu Trp Leu Pro Gly Ala Arg Cys Ala Leu Val Met Thr Gln Thr Pro Ser Ser Thr Ser Thr Ala Val Gly Gly Thr Val Thr Ile Lys Cys Gln Ala Ser Gln Ser Ile Ser Val Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ser Ala Ser Thr Leu Ala Ser Gly Val Pro Ser Arg Phe Lys Gly Ser Arg Ser Gly Thr Glu Tyr Thr Leu Thr Ile Ser Gly Val Gln Arg Glu Asp Ala Ala Thr Tyr Tyr Cys Leu Gly Ser Ala Gly Ser

Heavy Chain CDR1 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:3)

Asn Tyr His Met Phe

Heavy Chain CDR2 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:4)

Val Ile Thr Ser Ser Gly Ile Gly Ser Ser Ser Thr Thr Tyr Tyr Ala Thr Trp Ala Lys Gly

Heavy Chain CDR3 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:5)

Asp Tyr Phe Thr Asn Thr Tyr Tyr Ala Leu Asp Ile

Light Chain CDR1 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:6)

Gln Ala Ser Gln Ser Ile Ser Val Tyr Leu Ala

Light Chain CDR2 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:7)

Ser Ala Ser Thr Leu Ala Ser

Light Chain CDR3 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:8)

Leu Gly Ser Ala Gly Ser Asp Asp Ala Ala

What is claimed is:

1. A method for treating a melanoma comprising:

(i) identifying a patient having a PD-L1 positive melanoma tumor; and

(ii) administering to the patient an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1 ("anti-PD1 antibody monotherapy"), wherein the patient is not administered a combination of an anti-PD-1 antibody or an antigen-binding portion thereof and an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4 ("combination therapy").

2. A method for treating a melanoma comprising:

(i) identifying a patient having a PD-L1 positive melanoma tumor;

(ii) administering to the patient an anti-PD-1 antibody monotherapy; and

(iii) administering a combination therapy to the patient,

wherein the patient does not show efficacy with the anti-PD-1 antibody monotherapy after (ii).

3. A method for treating a melanoma comprising administering to a patient afflicted with a melanoma tumor an anti-PD-1 antibody monotherapy, not a combination therapy, wherein the patient is identified as having a PD-L1 positive melanoma tumor prior to the administration.

4. A method for extending a progression-free survival period for over 12 months in a patient afflicted with a melanoma tumor comprising administering to the patient an anti-PD-1 antibody monotherapy, wherein the patient is identified as having a melanoma tumor expressing PD-L1 prior to the administration and wherein the patient demonstrates progression-free survival for over 12 months.

5. The method of claim 4, wherein the progression-free survival of the patient is extended after the administration for over about 13 months, about 14 months, about 15 months, about 16 months, about 17 months, about 18 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, or about 10 years.

6. The method of claim 5, wherein the progression-free survival of the patient is extended for over 14 months.

7. A method for reducing a tumor size at least by 10% in a patient afflicted with a melanoma tumor comprising administering to the patient an anti-PD-1 antibody monotherapy, wherein the patient is identified as having a melanoma tumor expressing PD-L1 prior to the administration and wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration.

8. The method of any one of claims 2 to 7, further comprising identifying the patient as having a melanoma tumor expressing PD-L1 prior to the administration.

9. The method of any one of claims 1 to 8, wherein the patient is characterized by (i) extended progression-free survival for over 12 months, (ii) tumor size reduction at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration, or (iii) both.

10. A method for increasing an objective response rate to be higher than 55% in a patient population, each of whom is afflicted with a melanoma tumor, in a cancer treatment comprising administering to the patient an anti-PD-1 antibody monotherapy, wherein each patient is identified as having a melanoma tumor expressing PD-L1 prior to the administration and wherein the objective response rate is higher than 55%, 60%, 65%, 70%, or 75%.

11. The method of claim 10, further comprising identifying each patient of the patient population as having a melanoma tumor expressing PD-L1 prior to the administration.

12. The method of claim 10 or 11, wherein each patient of the patient population is further characterized by (i) extended progression-free survival for over 12 months, (ii) tumor size reduction at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration, or (iii) both.

13. The method of any one of claims 10 to 12, wherein the patient population comprises at least about 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 patients having a PD-L1 positive melanoma tumor.

14. A method for selecting a patient suitable for an anti-PD-1 antibody monotherapy comprising:

(i) identifying a patient having a PD-L1 positive melanoma tumor; and

(ii) instructing a healthcare provider to administer to the patient an anti-PD-1 antibody monotherapy.

15. The method of any one of claims 1 to 4 7, 8, 11, and 14, wherein the identifying comprises measuring a PD-L1 expression on the melanoma tumor.

16. The method of any one of claims 1 to 15, wherein the administration treats the melanoma tumor.

17. The method of claim 15 or 16, wherein the measuring comprises providing a test tissue sample obtained from the patient, the test tissue sample comprising tumor cells and/or tumor-infiltrating inflammatory cells.

18. The method of claim 17, wherein the measuring further comprises assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface.

19. The method of claim 18, wherein the test tissue sample is a formalin-fixed paraffin-embedded (FFPE) tissue sample.

20. The method of claim 19, wherein the presence of PD-L1 is determined using an automated IHC assay.

21. The method of claim 20, wherein the IHC assay is performed using an anti-PD-L1 monoclonal antibody that specifically binds to the PD-L1 and wherein the anti-PD-L1 monoclonal antibody is selected from the group consisting of 28-8, 28-1, 28-12, 29-8, 5H1, and any combination thereof.

22. The method of any one of claims 1 to 21, wherein the PD-L1 positive melanoma tumor is characterized by having at least about 1%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, or about 90% of tumor cells showing binding to the anti-PD-L1 antibody or an antigen-binding portion thereof.

23. The method of any one of claims 1 to 22, wherein the anti-PD-1 antibody or antigen-binding portion thereof cross-competes with nivolumab for binding to human PD-1.

24. The method of any one of claims 1 to 23, wherein the anti-PD-1 antibody or antigen-binding portion thereof is a chimeric, humanized or human monoclonal antibody or a portion thereof.

25. The method of any one of claims 1 to 24, wherein the anti-PD-1 antibody or antigen-binding portion thereof comprises a heavy chain constant region which is of a human IgG1 or IgG4 isotype.

26. The method of any one of claims 1 to 25, wherein the anti-PD-1 antibody is nivolumab.

27. The method of any one of claims 1 to 25, wherein the anti-PD-1 antibody is pembrolizumab.

28. The method of any one of claims 1 to 27, wherein the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose ranging from 0.1 to 10.0 mg/kg body weight once every 2, 3 or 4 weeks.

29. The method of claim 28, wherein the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of 1 or 3 mg/kg body weight once every 3 weeks.

30. The method of any one of claims 1 and 3 to 29, further comprising (i) identifying a patient that does not show efficacy with the anti-PD-1 monotherapy; and (ii) administering a combination therapy to the patient who does not show efficacy with the anti-PD-1 monotherapy.

31. The method of claim 2 or 30, wherein the combination therapy comprises (i) an anti-PD-1 antibody therapy or antigen-binding portion thereof and (ii) an anti-CTLA-4 antibody or antigen-binding portion thereof.

32. The method of claim 31, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof cross-competes with ipilimumab for binding to human CTLA-4.

33. The method of any one of claims 31, and 32, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof is a chimeric, humanized or human monoclonal antibody or a portion thereof.

34. The method of any one of claims 31 to 33, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof comprises a heavy chain constant region which is of a human IgG1 isotype.

35. The method of any one of claims 31 to 34, wherein the anti-CTLA-4 antibody is ipilimumab.

36. The method of any one of claims 31 to 34, wherein the anti-CTLA-4 antibody is tremelimumab.

37. The method of any one of claims 31 to 36, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof is administered at a dose ranging from 0.1 to 10.0 mg/kg body weight once every 2, 3 or 4 weeks.

38. The method of claim 37, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof is administered at a dose of 1 or 3 mg/kg body weight once every 3 weeks.

39. The method of claim 38, wherein the anti-PD-1 antibody or an antigen-binding portion thereof is administered at a dose of 1 mg/kg body weight once every 3 weeks and the anti-CTLA-4 antibody or an antigen-binding portion thereof is administered at a dose of 3 mg/kg body weight once every 3 weeks.

40. The method of any of claims 31 to 39, wherein the anti-PD-1 antibody or an antigen-binding portion thereof is administered concurrently with the anti-CTLA-4 antibody or an antigen-binding portion thereof.

41. The method of any of claims 31 to 39, wherein the anti-PD-1 antibody or an antigen-binding portion thereof is administered prior to or after the anti-CTLA-4 antibody or an antigen-binding portion thereof.

42. The method of claim 40, wherein the anti-PD-1 antibody or an antigen-binding portion thereof and the anti-CTLA-4 antibody or an antigen-binding portion thereof are in a single pharmaceutical composition.

43. The method of claim 42, wherein the pharmaceutical composition comprises the anti-PD-1 antibody and the anti-CTLA-4 antibody at a ratio of 50:1 to 1:50.

44. The method of claim 42, wherein the pharmaceutical composition comprises the anti-PD-1 antibody and the anti-CTLA-4 antibody at a ratio of 10:1 to 1:10.

45. The method of claim 42, wherein the pharmaceutical composition comprises the anti-PD-1 antibody and the anti-CTLA-4 antibody at a ratio of 3:1 to 1:3.

46. The method of claim 42, wherein the pharmaceutical composition comprises the anti-PD-1 antibody and the anti-CTLA-4 antibody at a ratio of 1:3.

47. The method of claim 42, wherein the pharmaceutical composition comprises 80 mg of the anti-PD-1 antibody and 240 mg of the anti-CTLA-4 antibody.

48. The method of claim any of claims 31 to 47, wherein the anti-PD-1 and anti-CTLA-4 antibodies are administered within 30 minutes of each other.

49. The method of any one of claims 1 to 48, further comprising administering an anti-cancer agent.

50. A kit for treating a patient afflicted with a melanoma tumor, the kit comprising:
    (a) a dosage ranging from 0.1 to 10 mg/kg body weight of an anti-PD-1 antibody or an antigen-binding portion thereof; and
    (b) instructions for using the anti-PD-1 antibody or the antigen-binding portion thereof in the method of any one of claims 1 to 49.

51. A kit for treating a patient afflicted with a melanoma tumor, the kit comprising:
    (a) a dosage ranging from 0.1 to 10 mg/kg body weight of an anti-PD-1 antibody or an antigen-binding portion thereof;
    (b) a dosage ranging from 0.1 to 10 mg/kg body weight of an anti-CTLA-4 antibody or an antigen-binding portion thereof; and

(c) instructions for using the anti-PD-1 antibody or the antigen-binding portion thereof and the anti-CTLA-4 antibody or the antigen-binding portion thereof in the method of any one of claims 2 and 30 to 48.

Fig. 1 – Patient Flow Diagram: Enrollment, Randomization, and Follow-up of Patients in the Study

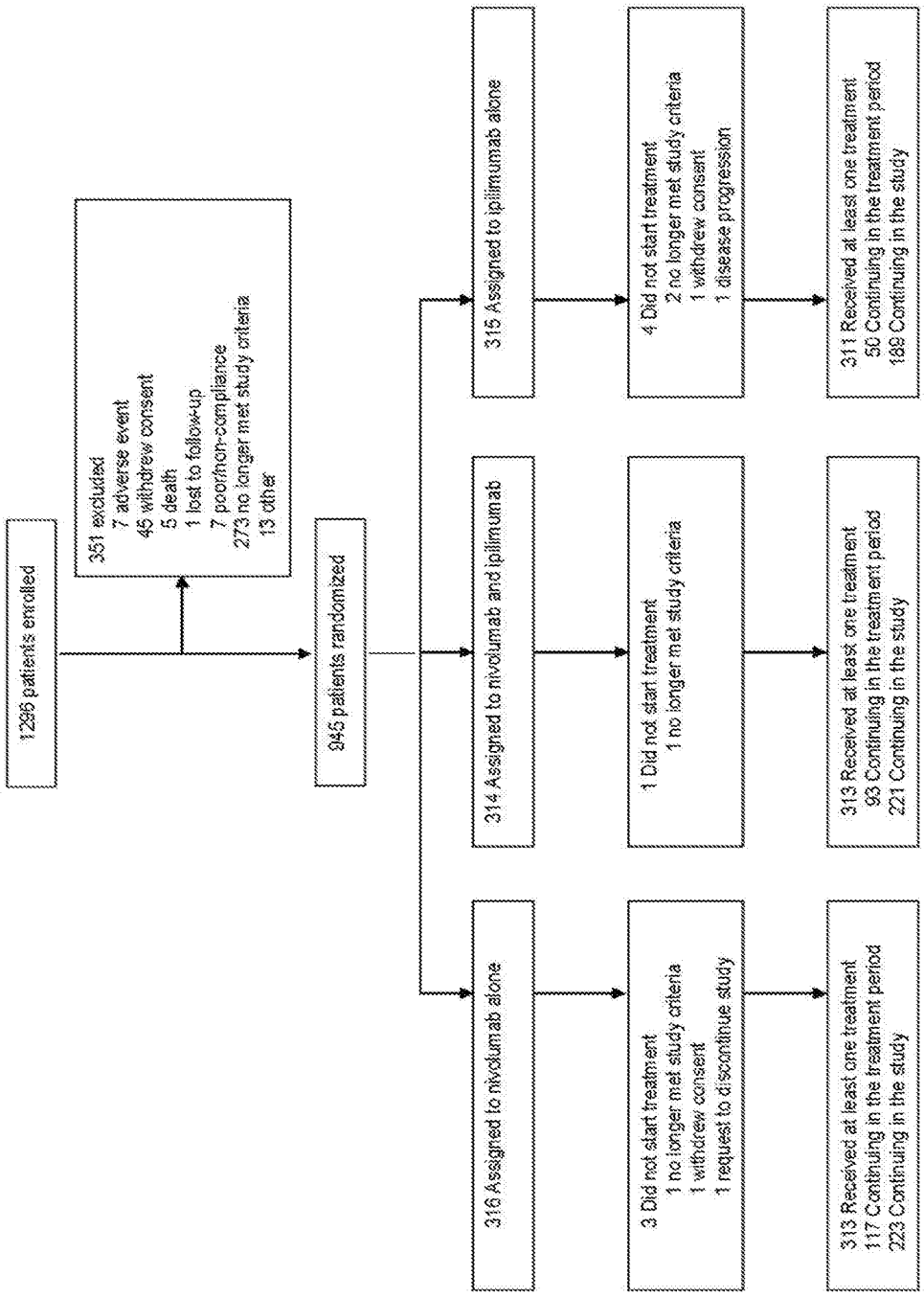
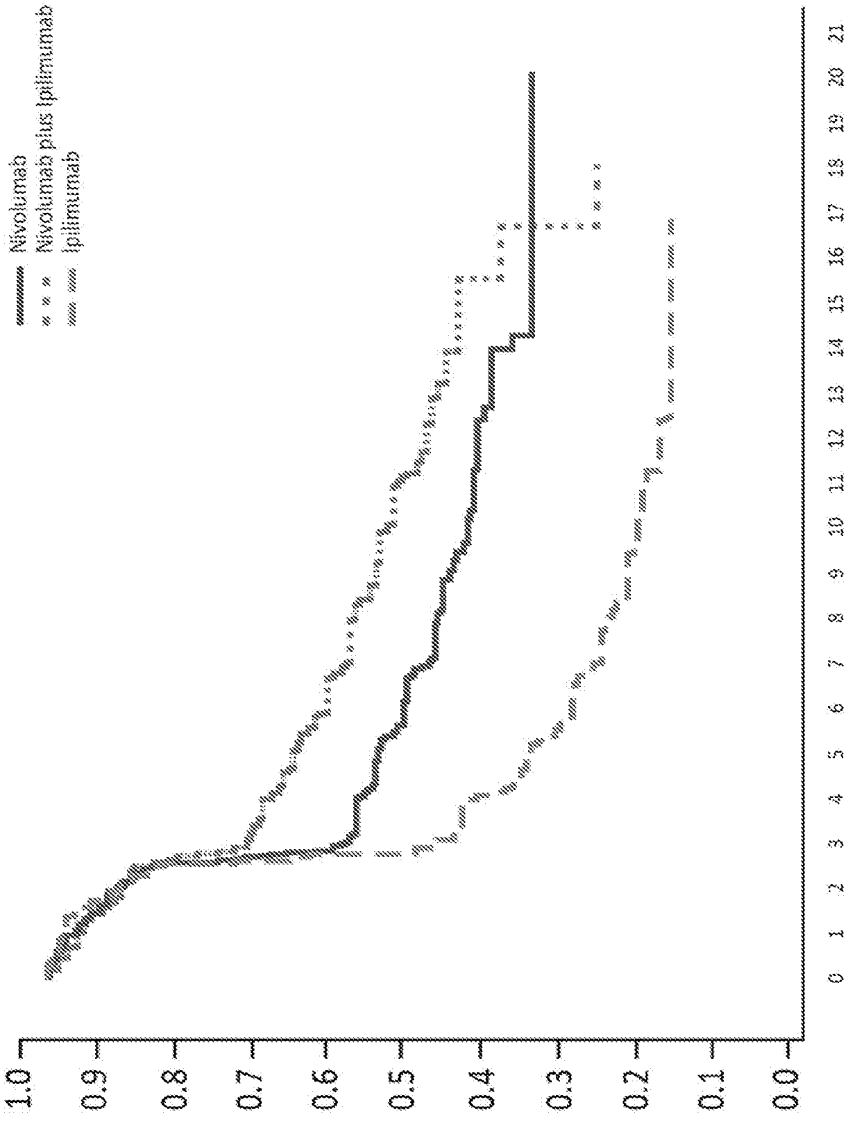


Fig. 2A – Progression-free Survival (Intention-to-Treat Population)



No. at Risk

Nivolumab	316	292	271	177	170	160	147	136	132	124	106	86	50	38	14	9	6	2	1	1	1	0
Nivolumab plus Ipilimumab	314	293	275	219	208	191	173	164	163	151	137	116	65	54	18	11	7	2	1	0	0	0
Ipilimumab	315	285	265	137	118	95	77	68	63	54	47	42	24	17	7	4	3	0	0	0	0	0

Fig. 2B – Progression-free Survival in Patients with Positive PD-L1 Status

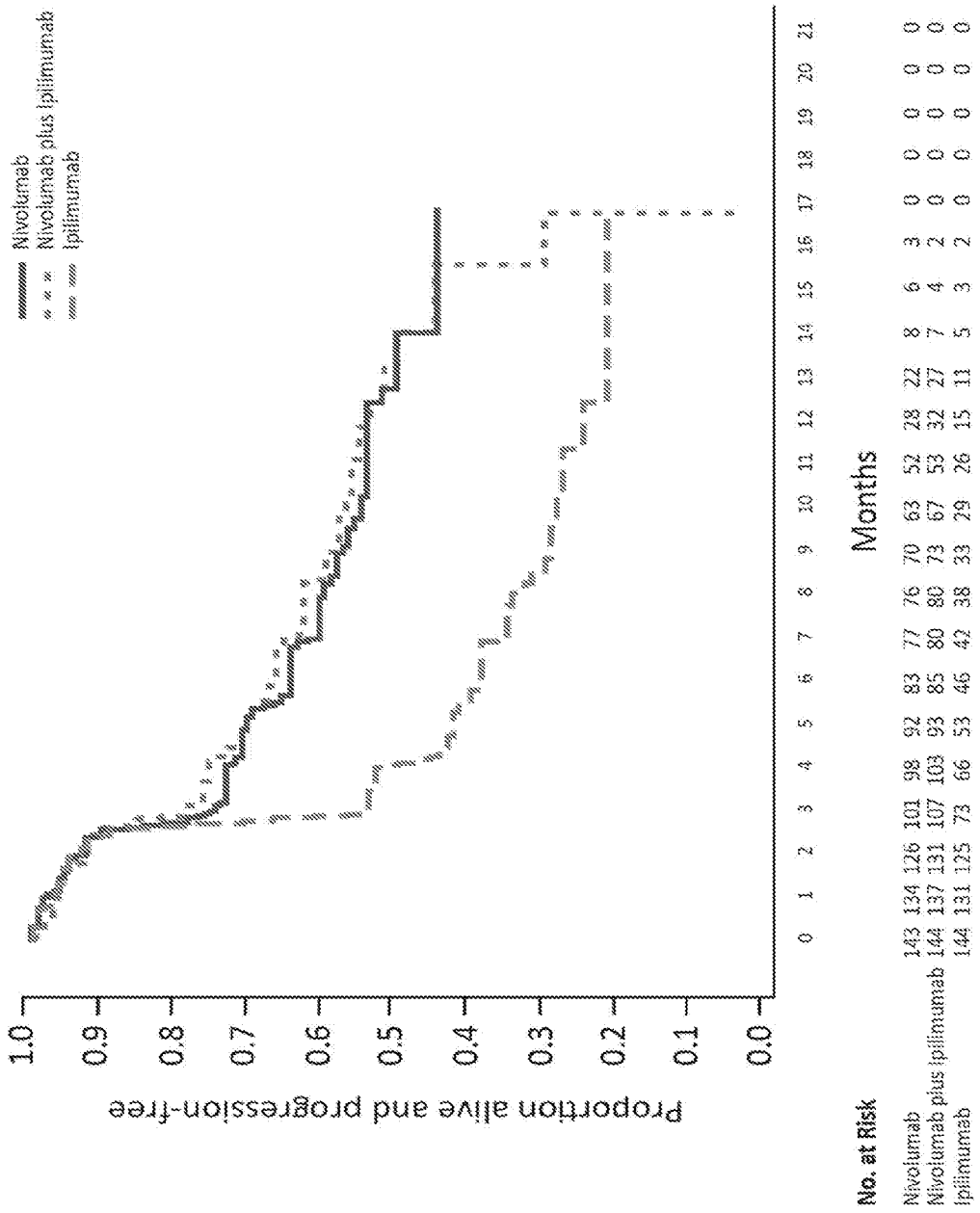


Fig. 2C – Progression-free Survival in Patients with Negative PD-L1 Status

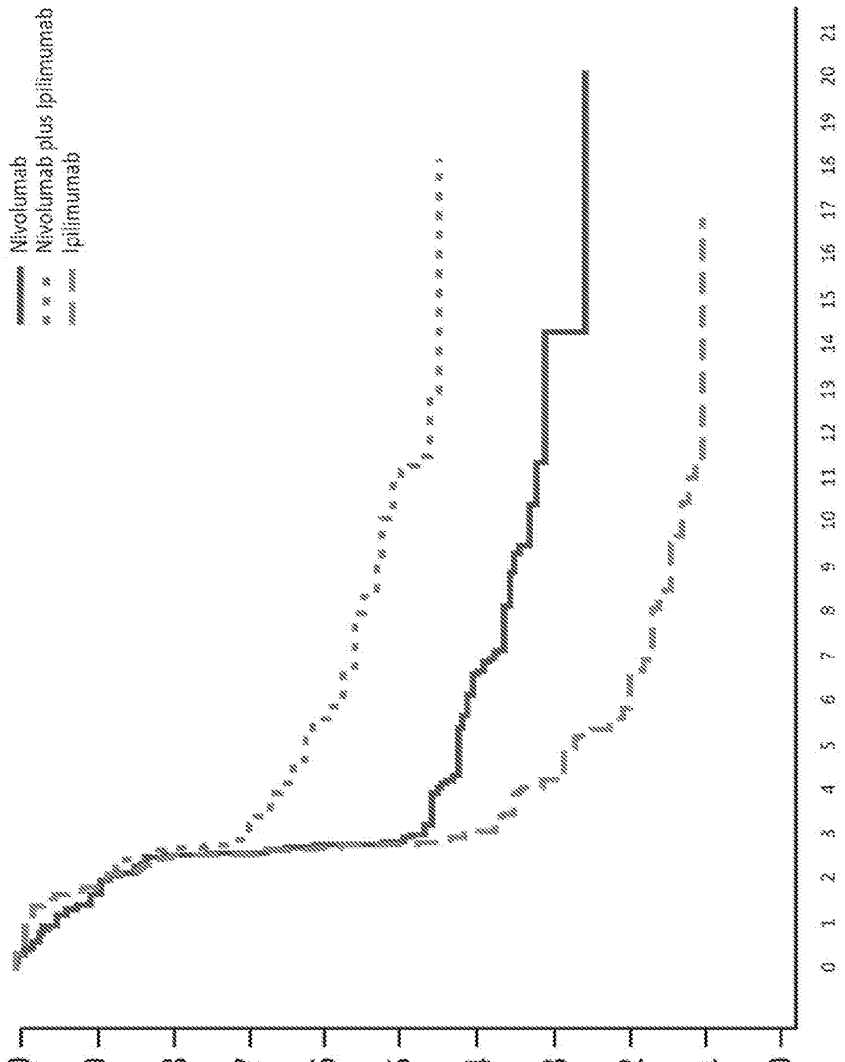
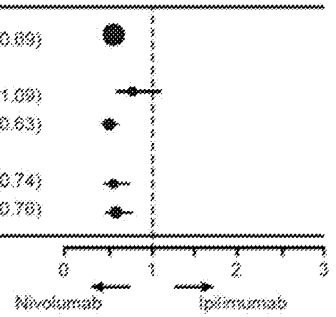


Fig. 3 – Subgroup Analyses of Progression-free Survival

(A) Nivolumab versus ipilimumab

	N	Nivolumab: N of events (N of patients)	Nivolumab: Median PFS (95% CI)	Ipilimumab: N of events (N of patients)	Ipilimumab: Median PFS (95% CI)	Unstratified Hazard Ratio (95% CI)
Overall	945	174 (316)	6.87 (4.34, 9.46)	234 (315)	2.68 (2.79, 3.42)	0.57 (0.47, 0.69)
BRAF Mutation Status						
Mutant	300	57 (98)	5.62 (2.79, 9.46)	68 (100)	4.04 (2.79, 5.52)	0.77 (0.54, 1.09)
Wild-type	645	117 (218)	7.89 (4.86, 12.68)	166 (215)	2.83 (2.76, 3.09)	0.50 (0.39, 0.63)
M Stage at Study Entry						
M0/M1a/M1b	366	63 (131)	9.30 (5.62, N.A.)	82 (126)	4.17 (2.96, 5.52)	0.54 (0.39, 0.74)
M1c	559	111 (185)	5.39 (2.83, 8.87)	142 (189)	2.79 (2.73, 2.83)	0.59 (0.48, 0.76)



(B) Nivolumab plus ipilimumab versus ipilimumab

	N	Nivolumab + Ipilimumab: N of events (N of patients)	Nivolumab + Ipilimumab: Median PFS (95% CI)	Ipilimumab: N of events (N of patients)	Ipilimumab: Median PFS (95% CI)	Unstratified Hazard Ratio (95% CI)
Overall	945	151 (314)	11.50 (8.80, 16.72)	334 (315)	2.68 (2.79, 3.42)	0.43 (0.35, 0.53)
BRAF Mutation Status						
Mutant	300	48 (102)	11.73 (8.02, N.A.)	68 (100)	4.04 (2.79, 5.52)	0.47 (0.32, 0.68)
Wild-type	645	103 (212)	11.24 (8.34, N.A.)	166 (215)	2.83 (2.76, 3.09)	0.41 (0.32, 0.53)
M Stage at Study Entry						
M0/M1a/M1b	366	51 (129)	15.54 (11.17, N.A.)	82 (126)	4.17 (2.96, 5.52)	0.35 (0.25, 0.50)
M1c	559	100 (185)	8.51 (5.52, 12.35)	142 (189)	2.79 (2.73, 2.83)	0.48 (0.37, 0.62)

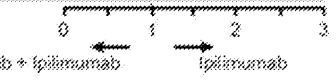
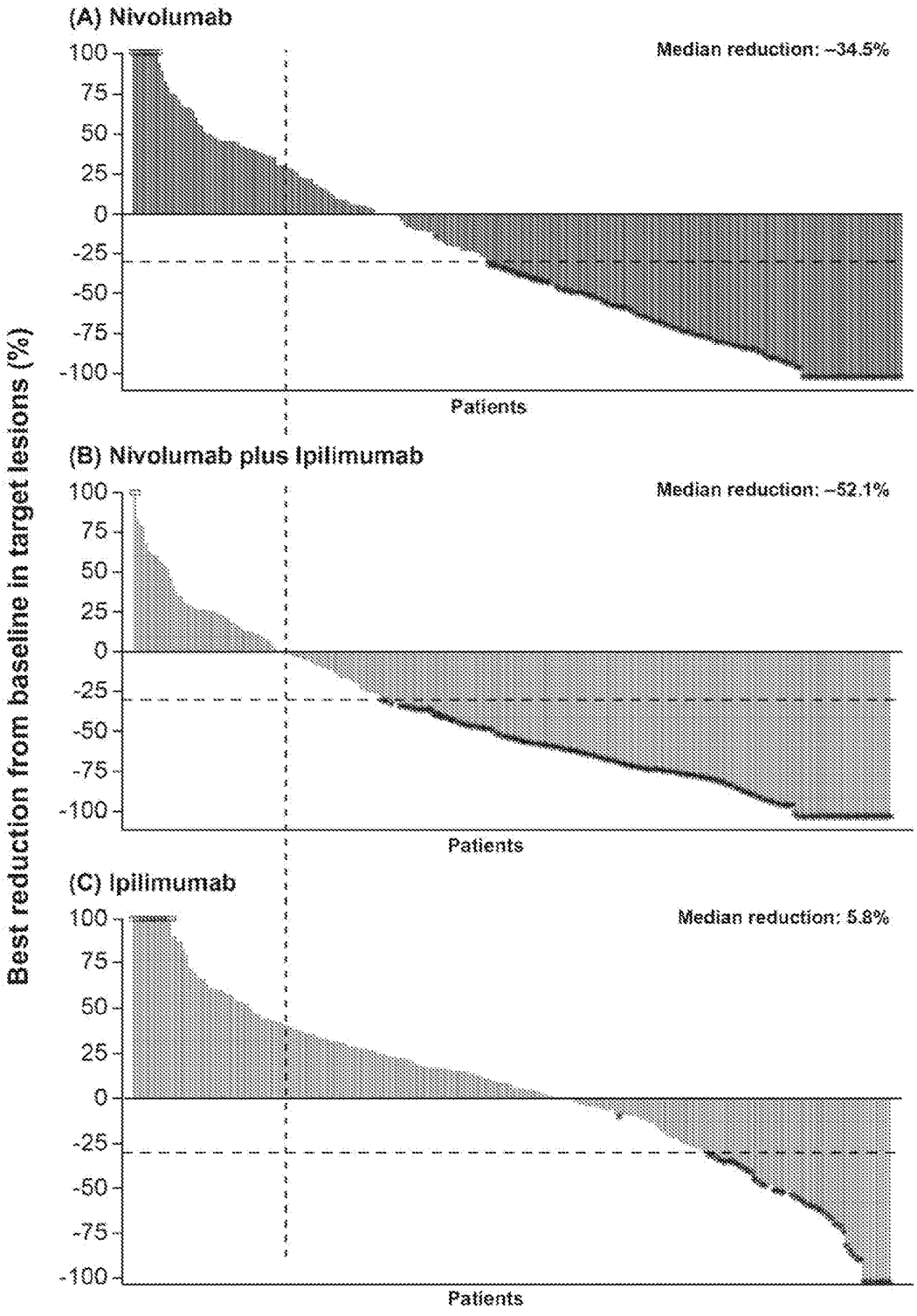


Fig. 4 – Tumor Burden Change in Target Lesions



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/029878

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	P. A. OTT ET AL: "CTLA-4 and PD-1/PD-L1 Blockade: New Immunotherapeutic Modalities with Durable Clinical Benefit in Melanoma Patients", CLINICAL CANCER RESEARCH, vol. 19, no. 19, 1 October 2013 (2013-10-01), pages 5300-5309, XP055200528, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-13-0143 page 5306, left-hand column, paragraph 2 - right-hand column, paragraph 1 page 5307, left-hand column, paragraph 2 - right-hand column ----- -/--	1-51

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 June 2016	13/07/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Le Flao, Katell

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G. K. PHILIPS ET AL: "Therapeutic uses of anti-PD-1 and anti-PD-L1 antibodies", INTERNATIONAL IMMUNOLOGY., vol. 27, no. 1, 16 October 2014 (2014-10-16), pages 39-46, XP55217958, GB ISSN: 0953-8178, DOI: 10.1093/intimm/dxu095 page 44, right-hand column, paragraph 2 - page 45, left-hand column, paragraph 2; table 1 -----	1-51
X	MAHONEY KATHLEEN M ET AL: "The Next Immune-Checkpoint Inhibitors: PD-1/PD-L1 Blockade in Melanoma.", CLINICAL THERAPEUTICS 1 APR 2015, vol. 37, no. 4, 1 April 2015 (2015-04-01), pages 764-782, XP055285031, ISSN: 1879-114X page 771, left-hand column, paragraph 2 - page 774, right-hand column, paragraph 1 -----	1-51
X	JEDD D. WOLCHOK ET AL: "Nivolumab plus Ipilimumab in Advanced Melanoma", NEW ENGLAND JOURNAL OF MEDICINE, vol. 369, no. 2, 11 July 2013 (2013-07-11) , pages 122-133, XP55182024, ISSN: 0028-4793, DOI: 10.1056/NEJMoa1302369 page 124, right-hand column, paragraph 2 page 128, right-hand column, paragraph 4 - page 130, right-hand column, paragraph 3 -----	1-51
X	MARZUKA ALEXANDER ET AL: "Melanoma Treatments: Advances and Mechanisms.", JOURNAL OF CELLULAR PHYSIOLOGY NOV 2015, vol. 230, no. 11, 22 April 2015 (2015-04-22), pages 2626-2633, XP002759396, ISSN: 1097-4652, DOI: 10.1002/jcp.25019 page 2631, left-hand column, paragraph 2 - right-hand column, paragraph 3 -----	1-51
X	PAOLO A ASCIERTO ET AL: "The additional facet of immunoscore: immunoprofiling as a possible predictive tool for cancer treatment", JOURNAL OF TRANSLATIONAL MEDICINE, BIOMED CENTRAL, LONDON, GB, vol. 11, no. 1, 3 March 2013 (2013-03-03), page 54, XP021142097, ISSN: 1479-5876, DOI: 10.1186/1479-5876-11-54 page 3/4, line 10 - line 17 -----	1-51
1	-/--	

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2013/309250 A1 (COGSWELL JOHN [US] ET AL) 21 November 2013 (2013-11-21) claims 1-17; sequences 35,36	1-51
X	P. A. ASCIERTO ET AL: "Biomarkers for Immunostimulatory Monoclonal Antibodies in Combination Strategies for Melanoma and Other Tumor Types", CLINICAL CANCER RESEARCH, vol. 19, no. 5, 1 March 2013 (2013-03-01), pages 1009-1020, XP55124339, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-12-2982 the whole document	1-51
T	BOWYER S ET AL: "Efficacy and toxicity of treatment with the anti-CTLA-4 antibody ipilimumab in patients with metastatic melanoma after prior anti-PD-1 therapy.", BRITISH JOURNAL OF CANCER 10 MAY 2016, vol. 114, no. 10, 10 May 2016 (2016-05-10) , pages 1084-1089, XP002759397, ISSN: 1532-1827 the whole document	

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/029878

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2013309250 A1	21-11-2013	AU 2013263076 A1	22-01-2015
		CA 2873402 A1	21-11-2013
		CN 104470949 A	25-03-2015
		EA 201492105 A1	30-06-2015
		EP 2850102 A1	25-03-2015
		HK 1203971 A1	06-11-2015
		JP 2015518826 A	06-07-2015
		KR 20150020189 A	25-02-2015
		SG 11201407190T A	30-12-2014
		US 2013309250 A1	21-11-2013
		US 2015125463 A1	07-05-2015
		US 2016090417 A1	31-03-2016
		WO 2013173223 A1	21-11-2013

Form PCT/ISA/210 (patent family annex) (April 2005)

PATENT COOPERATION TREATY

RECEIVED
JUL 19 2016
Sterne Kessler Goldstein & Fox PLLC

PCT C. WARD

From the INTERNATIONAL SEARCHING AUTHORITY

To:
Kim, Ji-Eun
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
ETATS-UNIS D'AMERIQUE

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference 3338032PC02	Date of mailing *(day/month/year)* 13 July 2016 (13-07-2016)
International application No. PCT/US2016/029878	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
Applicant BRISTOL-MYERS SQUIBB COMPANY	International filing date *(day/month/year)* 28 April 2016 (28-04-2016)

1. [X] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the International Search Report.

   How? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Fascimile No.: (41-22) 338.82.70

   For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 - 9.011.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. [ ] **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   [ ] the protest together with the decision thereon has been transmitted to the International Bureau together with any applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   [ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   The applicant may **submit comments on an informal basis on the written opinion of the International Searching Authority** to the International Bureau. These comments will be made available to the public after international publication. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established.

   Shortly after the expiration of **18 months from the priority date, the international application will be published** by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for **entry into the national phase** before those designated Offices. In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months. For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

   Within **19 months from the priority date, the applicant may request that a supplementary international search be carried out** by a different International Searching Authority that offers this service (Rule 45*bis*.1). The procedure for requesting supplementary international search is described in the *PCT Applicant's Guide*, International Phase, paragraphs 8.006-8.032.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040 Fax: (+31-70) 340-3016	RODRIGUEZ NóVOA, María Tel: +31 (0)70 340-2591

Form PCT/ISA/220 (July 2014)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US2016/029876	28 April 2016 (28-04-2016)	28 April 2015 (28-04-2015)

Applicant

BRISTOL-MYERS SQUIBB COMPANY

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language,** the international search was carried out on the basis of:
      [X] the international application in the language in which it was filed
      [ ] a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
   b. [ ] This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. [X] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. [ ] **Certain claims were found unsearchable** (See Box No. II)

3. [ ] **Unity of invention is lacking** (see Box No III)

4. With regard to the **title,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the **drawings,**
   a. the figure of the **drawings** to be published with the abstract is Figure No. _____
      [ ] as suggested by the applicant
      [ ] as selected by this Authority, because the applicant failed to suggest a figure
      [ ] as selected by this Authority, because this figure better characterizes the invention
   b. [X] none of the figures is to be published with the abstract

Form PCT/ISA/210 (first sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/029878

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/029878

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	P. A. OTT ET AL: "CTLA-4 and PD-1/PD-L1 Blockade: New Immunotherapeutic Modalities with Durable Clinical Benefit in Melanoma Patients", CLINICAL CANCER RESEARCH, vol. 19, no. 19, 1 October 2013 (2013-10-01), pages 5300-5309, XP055200528, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-13-0143 page 5306, left-hand column, paragraph 2 - right-hand column, paragraph 1 page 5307, left-hand column, paragraph 2 - right-hand column	1-51

-/--

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 June 2016	13/07/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Le Flao, Katell

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G. K. PHILIPS ET AL: "Therapeutic uses of anti-PD-1 and anti-PD-L1 antibodies", INTERNATIONAL IMMUNOLOGY., vol. 27, no. 1, 16 October 2014 (2014-10-16), pages 39-46, XP55217958, GB ISSN: 0953-8178, DOI: 10.1093/intimm/dxu095 page 44, right-hand column, paragraph 2 - page 45, left-hand column, paragraph 2; table 1 -----	1-51
X	MAHONEY KATHLEEN M ET AL: "The Next Immune-Checkpoint Inhibitors: PD-1/PD-L1 Blockade in Melanoma.", CLINICAL THERAPEUTICS 1 APR 2015, vol. 37, no. 4, 1 April 2015 (2015-04-01), pages 764-782, XP055285031, ISSN: 1879-114X page 771, left-hand column, paragraph 2 - page 774, right-hand column, paragraph 1 -----	1-51
X	JEDD D. WOLCHOK ET AL: "Nivolumab plus Ipilimumab in Advanced Melanoma", NEW ENGLAND JOURNAL OF MEDICINE, vol. 369, no. 2, 11 July 2013 (2013-07-11) , pages 122-133, XP55182024, ISSN: 0028-4793, DOI: 10.1056/NEJMoa1302369 page 124, right-hand column, paragraph 2 page 128, right-hand column, paragraph 4 - page 130, right-hand column, paragraph 3 -----	1-51
X	MARZUKA ALEXANDER ET AL: "Melanoma Treatments: Advances and Mechanisms.", JOURNAL OF CELLULAR PHYSIOLOGY NOV 2015, vol. 230, no. 11, 22 April 2015 (2015-04-22), pages 2626-2633, XP002759396, ISSN: 1097-4652, DOI: 10.1002/jcp.25019 page 2631, left-hand column, paragraph 2 - right-hand column, paragraph 3 -----	1-51
X	PAOLO A ASCIERTO ET AL: "The additional facet of immunoscore: immunoprofiling as a possible predictive tool for cancer treatment", JOURNAL OF TRANSLATIONAL MEDICINE, BIOMED CENTRAL, LONDON, GB, vol. 11, no. 1, 3 March 2013 (2013-03-03), page 54, XP021142097, ISSN: 1479-5876, DOI: 10.1186/1479-5876-11-54 page 3/4, line 10 - line 17 -----	1-51
	-/--	

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2013/309250 A1 (COGSWELL JOHN [US] ET AL) 21 November 2013 (2013-11-21) claims 1-17; sequences 35,36	1-51
X	P. A. ASCIERTO ET AL: "Biomarkers for Immunostimulatory Monoclonal Antibodies in Combination Strategies for Melanoma and Other Tumor Types", CLINICAL CANCER RESEARCH, vol. 19, no. 5, 1 March 2013 (2013-03-01), pages 1009-1020, XP55124339, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-12-2982 the whole document	1-51
T	BOWYER S ET AL: "Efficacy and toxicity of treatment with the anti-CTLA-4 antibody ipilimumab in patients with metastatic melanoma after prior anti-PD-1 therapy.", BRITISH JOURNAL OF CANCER 10 MAY 2016, vol. 114, no. 10, 10 May 2016 (2016-05-10) , pages 1084-1089, XP002759397, ISSN: 1532-1827 the whole document	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/029878

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2013309250 A1	21-11-2013	AU 2013263076 A1	22-01-2015
		CA 2873402 A1	21-11-2013
		CN 104470949 A	25-03-2015
		EA 201492105 A1	30-06-2015
		EP 2850102 A1	25-03-2015
		HK 1203971 A1	06-11-2015
		JP 2015518826 A	06-07-2015
		KR 20150020189 A	25-02-2015
		SG 11201407190T A	30-12-2014
		US 2013309250 A1	21-11-2013
		US 2015125463 A1	07-05-2015
		US 2016090417 A1	31-03-2016
		WO 2013173223 A1	21-11-2013

Form PCT/ISA/210 (patent family annex) (April 2005)

Information on Search Strategy - Pilot phase (see OJ 2015, A86)
The type of information contained in this sheet may change during the pilot for improving the usefulness of this new service.

Application Number
PCT/US2016/029878

TITLE: TREATMENT OF PD-L1-POSITIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY

APPLICANT: BRISTOL-MYERS SQUIBB COMPANY

IPC CLASSIFICATION: C07K16/28, A61P35/00

EXAMINER: Le Flao, Katell

CONSULTED DATABASES: DOSYS, EPODOC, MEDLINE, NPL, WPI

CLASSIFICATION SYMBOLS DEFINING EXTENT OF THE SEARCH:

IPC:

CPC: C07K16/2818, A61K2039/505, A61K2039/55, A61K2039/507, A61K2039/545

FI/F-TERMS:

KEYWORDS OR OTHER ELEMENTS FEATURING THE INVENTION:

EPO FORM P04A42

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2016/029878	28.04.2016	28.04.2015

International Patent Classification (IPC) or both national classification and IPC
INV. C07K16/28 A61P35/00

Applicant
BRISTOL-MYERS SQUIBB COMPANY

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Fax: +31 70 340 - 3016	see form PCT/ISA/210	Le Flao, Katell Telephone No. +31 70 340-0

Form PCT/ISA/237 (Cover Sheet) (January 2015)

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-51
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-51
Industrial applicability (IA)	Yes:	Claims	1-51
	No:	Claims	

2. Citations and explanations

see separate sheet

Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Claims 1-49 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 39.1(iv) / 67.1(iv) PCT.

1.1 The patentability can be dependent upon the formulation of the claims. The EPO, for example, does not recognise as patentable claims to the use of a compound in medical treatment, but may allow claims to a product, in particular substances or compositions for use in a first or further medical treatment.

2 Reference is made to the following documents:

D1 P. A. OTT ET AL: "CTLA-4 and PD-1/PD-L1 Blockade: New Immunotherapeutic Modalities with Durable Clinical Benefit in Melanoma Patients", CLINICAL CANCER RESEARCH, vol. 19, no. 19, 1 October 2013, pages 5300-5309, XP055200528, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-13-0143

D2 G. K. PHILIPS ET AL: "Therapeutic uses of anti-PD-1 and anti-PD-L1 antibodies", INTERNATIONAL IMMUNOLOGY., vol. 27, no. 1, 16 October 2014, pages 39-46, XP55217958, GB ISSN: 0953-8178, DOI: 10.1093/intimm/dxu095

D3 MAHONEY KATHLEEN M ET AL: "The Next Immune-Checkpoint Inhibitors: PD-1/PD-L1 Blockade in Melanoma.", CLINICAL THERAPEUTICS, vol. 37, no. 4, 1 April 2015, pages 764-782, XP055285031, ISSN: 1879-114X

D4 JEDD D. WOLCHOK ET AL: "Nivolumab plus Ipilimumab in Advanced Melanoma", NEW ENGLAND JOURNAL OF MEDICINE, vol. 369, no. 2, 11 July 2013, pages 122-133, XP55182024, ISSN: 0028-4793, DOI: 10.1056/NEJMoa1302369

D5 MARZUKA ALEXANDER ET AL: "Melanoma Treatments: Advances and Mechanisms.", JOURNAL OF CELLULAR PHYSIOLOGY, vol. 230, no. 11, 22 April 2015, pages 2626-2633, XP002759396, ISSN: 1097-4652, DOI: 10.1002/jcp.25019

D6 PAOLO A ASCIERTO ET AL: "The additional facet of immunoscore: immunoprofiling as a possible predictive tool for cancer treatment", JOURNAL OF TRANSLATIONAL MEDICINE, vol. 11, no. 1, 3 March 2013, page 54, XP021142097, ISSN: 1479-5876, DOI: 10.1186/1479-5876-11-54

D7 US 2013/309250 A1 (COGSWELL JOHN [US] ET AL) 21 November 2013

D8 P. A. ASCIERTO ET AL: "Biomarkers for Immunostimulatory Monoclonal Antibodies in Combination Strategies for Melanoma and Other Tumor Types", CLINICAL CANCER RESEARCH, vol. 19, no. 5, 1 March 2013, pages 1009-1020, XP55124339, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-12-2982

3 The present application does not meet the criteria of Article 33(2) PCT, because the subject-matter of claims 1-51 is not new.

3.1 Claims 1 relates to a method for treating a melanoma comprising: (i) identifying a patient having a PD-L positive melanoma tumor; and (ii) administering to the patient an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1 ("anti-PD1 antibody monotherapy"), wherein the patient is not administered a combination of an anti-PD-1 antibody or an antigen-binding portion thereof and an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4 ("combination therapy"). This is novel over the following documents D1 to D7.

3.2 Document D1 identifies PD-L1 as a biomarker suitable to discriminate the patients suffering from melanoma that would respond to the therapy with anti PD-1 antibody from the others (page 5306, left-hand column, para. 2 to right-hand column, para. 1). The authors of D1 refers to combined therapy with anti PD-1 and anti CTLA-4 antibodies as a way to overcome the lack of response observed in some patients with either monotherapy (page 5307, left-hand column, para.2 to right-hand column, para. 3).

3.3 Document D2 discloses the use of nivolumab in Phase III clinical development in patients having melanoma, RCC, NSCLC and HNSCC (Table 1) as well as the rational for selecting patients with PD-L1 positive tumor before treating them with anti PD-1 antibody (page 44, right-hand column, para.2) in spite of some limitation and drawback associated with this restriction due to the method and products used to determine the level of PD-L1 (page 44, right-hand column, para. 3 - page 45, left-hand column, para. 2).

3.4 Document D3 reviews the clinical studies of PD-1/PD-L1-targeted therapies in melanoma. The correlation between the expression of PD-L1 and the objective rate response to nivolumab is reported and its relevance is discussed (page 771, left-hand column, para. 2 to page 774, right-hand column, para.2).

3.5 Document D4 discloses the combined therapy of patients with melanoma based upon ipilimumab (anti-CTLA-4 antibody) and nivolimumab (anti PD-1 antibody) as well as the immunohistochemical assessment of PD-L1 (page 124, right-hand column, para. 2 and page 128, right-hand column, last para. to page 130, right-hand column, para. 3).

3.6 Document D5 discusses the advances and mechanisms in melanoma treatments with some focus on the clinical studies of PD-1/PD-L1-targeted therapies in melanoma. The correlation between the expression of PD-L1 and the objective rate response to nivolumab is reported (page 2631, left-hand column, para. 2 to right-hand column, para.3).

3.7 Document D6 discloses the concept of immunoprofiling patients suffering from cancer as "using an individual's immune system signature (or profile) to predict that patient's response to therapy" and refers to PD-L1 as a possible predictive biomarker of therapeutic response to anti PD-1 treatment (abstract and page 3/4, lines 10-17).

3.8 Document D7 discloses the treatment of melanoma with anti-PD-1 antibody following the determination of PD-L1 levels (claims 1-17).

3.9 Independent claims 1, 3, and 4 which relate to the treatment of melanoma in PD-L1 positive patient with anti PD-1 antibody are thus not novel over Documents D1-D7. The same reasoning applies to dependent claims and kit claims. Indepdent claim 2 is also anticipated by the disclosures cited in the search report (see above the relevant passages).

Bitte beachten Sie, dass angeführte Nichtpatentliteratur (wie z. B. wissenschaftliche oder technische Dokumente) je nach geltendem Recht dem Urheberrechtsschutz und/oder anderen Schutzarten für schriftliche Werke unterliegen könnte. Die Vervielfältigung urheberrechtlich geschützter Texte, ihre Verwendung in anderen elektronischen oder gedruckten Publikationen und ihre Weitergabe an Dritte ist ohne ausdrückliche Zustimmung des Rechtsinhabers nicht gestattet.

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XS CPRTENFRDE

Electronic Patent Application Fee Transmittal

Application Number:	15141769
Filing Date:	28-Apr-2016
Title of Invention:	Treatment of PD-L1-Negative Melanoma Using an Anti-PD-1 Antibody and an Anti-CTLA-4 Antibody
First Named Inventor/Applicant Name:	Arvin YANG
Filer:	Ji Eun Kim/Susan Hylton
Attorney Docket Number:	3338.0330001/ELE/C-K

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
SUBMISSION- INFORMATION DISCLOSURE STMT	1806	1	240	240
		Total in USD ($)		**240**

Electronic Acknowledgement Receipt	
EFS ID:	32748447
Application Number:	15141769
International Application Number:	
Confirmation Number:	6926
Title of Invention:	Treatment of PD-L1-Negative Melanoma Using an Anti-PD-1 Antibody and an Anti-CTLA-4 Antibody
First Named Inventor/Applicant Name:	Arvin YANG
Customer Number:	115018
Filer:	Ji Eun Kim/Susan Hylton
Filer Authorized By:	Ji Eun Kim
Attorney Docket Number:	3338.0330001/ELE/C-K
Receipt Date:	29-MAY-2018
Filing Date:	28-APR-2016
Time Stamp:	17:49:21
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$240
RAM confirmation Number	053018INTEFSW17500900
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		33380330001_Amendment.pdf	199251 4b575af60400a44e7ab9e8bdc0d1f920fef42f84	yes	13
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Amendment/Req. Reconsideration-After Non-Final Reject		1	1	
	Claims		2	5	
	Applicant Arguments/Remarks Made in an Amendment		6	13	
Warnings:					
Information:					
2	Transmittal Letter	33380330001_IDS.pdf	151812 4d6a515cfc5019ab135ae7484dce9f6c993017cd	no	3
Warnings:					
Information:					
3	Information Disclosure Statement (IDS) Form (SB08)	33380330001_SB08a_b.pdf	180806 54ba1ee6de0286e8cbe4d2e5b09f4fae25e9ac21	no	3
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
4	Foreign Reference	FP8_WO2004004771A1.PDF	3741041 660eaf08fe06d8a8035a9c284f42a4559c5b6763	no	75
Warnings:					
Information:					

5	Foreign Reference	FP9_WO2015042246_A1.PDF	4325296 c1f45b6a4ed86705a76cac030ca621e685d7867f	no	94
Warnings:					
Information:					
6	Foreign Reference	FP10_WO2016176503A1.PDF	3414630 7a2c49d96b238d7d46355c84e69bd744de71b4f6	no	72
Warnings:					
Information:					
7	Foreign Reference	FP11_WO2016176504A1.PDF	3674626 8ad951282b80eb73c08cceaba3e4279c47b934b6	no	75
Warnings:					
Information:					
8	Non Patent Literature	NPL32_BOWYER_BRIJCANCER_2016_114_1084_EFS.PDF	3661271 82dff7ae96d3610bc8041a7bfd6822902c53b840	no	7
Warnings:					
Information:					
9	Non Patent Literature	NPL33_NPLNCCN_GUIDELINES_2014_4_EFS.PDF	1103885 5d71b82750b47b57bdc1af22515bd0b837636c40	no	4
Warnings:					
Information:					
10	Non Patent Literature	NPL34_NFOA_15141772_6December2017.PDF	447348 88d3725994d078fa318fc10dee49ed12155bda6a	no	17
Warnings:					
Information:					
11	Non Patent Literature	NPL35_OA_15141772_12April2018.PDF	468290 187982ffcf7f2ad6c5c54034edd06be494038107	no	13
Warnings:					
Information:					

12	Non Patent Literature	NPL36_Tzartos.PDF	550057 8aae9b2688fa6e116cc0ba599c9d324f366e84b6	no	12
Warnings:					
Information:					
13	Non Patent Literature	NPL37_ISR.PDF	618240 585870222aca3d530e7410a41e34e718831bf03b	no	15
Warnings:					
Information:					
14	Fee Worksheet (SB06)	fee-info.pdf	30847 48e635eac705ef2e434ae048487c2f0e64db6a0f	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	22567400		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/141,769	YANG, Arvin
	Examiner	Art Unit
	ILIA I OUSPENSKI	1644

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
Priority application	02/27/2018	io
WEST: attached	02/27/2018	io
ProQuest: same as WEST	02/27/2018	io
Inventor search	02/27/2018	io

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

Notice of References Cited	Application/Control No. 15/141,769	Applicant(s)/Patent Under Reexamination YANG, Arvin	
	Examiner ILIA I OUSPENSKI	Art Unit 1644	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-9856320-B2	01-2018	Cogswell; John P.	C07K16/2818	1/1
	B					
	C					
	D					
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/141,769	04/28/2016	Arvin YANG	3338.0330001/ELE/C-K	6926

115018 7590 02/28/2018

Sterne, Kessler, Goldstein & Fox P.L.L.C.
1100 New York Avenue NW
Washington, DISTRICT OF COLUMBIA 20005

EXAMINER	
OUSPENSKI, ILIA I	

ART UNIT	PAPER NUMBER
1644	

MAIL DATE	DELIVERY MODE
02/28/2018	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 15/141,769	Applicant(s) YANG, Arvin	
	Examiner ILIA I OUSPENSKI	Art Unit 1644	AIA Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 08/05/2016 .

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____ .

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 1-2,8,13-14,16-18,20-22,25,27-29,32,34,36 and 46-47 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 1-2,8,13-14,16-18,20-22,25,27-29,32,34,36 and 46-47 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☑ The drawing(s) filed on 05 August 2016 is/are: a)☑ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED ACTION

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

2. Applicant's preliminary amendment filed on 08/05/2016 is acknowledged.

Claims 1, 2, 8, 13, 14, 16-18, 20-22, 25, 27-29, 32, 34, 36, 46 and 47 are pending.

3. Applicant is invited to consider amending claim 8 to recite "(ii) tumor size reduction of at least about 10%" to potentially improve the form and/or style of the claim.

Applicant is further invited to consider amending claim 21 to substitute the term "fewer" for "less" to potentially improve the form and/or style of the claim.

4. **35 U.S.C. § 101** reads as follows:

"Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter or any new and useful improvement thereof, may obtain a patent therefore, subject to the conditions and requirements of this title".

5. Claim 13 is rejected under **35 U.S.C. 101** because the claimed invention is directed to a judicial exception (i.e., a law of nature, a natural phenomenon, or an abstract idea) without significantly more.

Claim 13 is directed to a process, specifically to a method for "selecting" a patient suitable for therapy with anti-PD-1 and anti-CTLA4 antibodies. The method comprises two method steps, i.e. (i) a step of "identifying" a patient having a PD-L1-negative melanoma tumor, and (ii) a step of "instructing" a healthcare provider to administer the therapy to the patient.

The claim is directed to an abstract idea, for the following reasons:

"Selecting" a patient suitable for a specific treatment is a mental process, and as such is an abstract Idea. "Identifying" a patient having a PD-L1-negative melanoma tumor is also a mental step, since it involves only manipulating information. In this step, the percentage of PD-L1-positive cells in the patient's tumor, as determined by any of the numerous methods routine in the art, is compared to a predetermined threshold, and the patient is classified as having a PD-L1 negative tumor if the percentage of PD-L1-positive cells is below the threshold.

The claim is also directed to a natural phenomenon, for the following reasons:

The healthcare provider is instructed to administer to the patient identified as having a PD-L1-negative melanoma tumor a combination of an anti-PD-1 and anti-CTLA4 antibodies, because there is a reasonable likelihood that the patient will benefit from such treatment. The specification discloses in Table 4 the results of a clinical study, demonstrating that approximately 55% of melanoma patients with PD-L1-negative tumors show an objective response to treatment with a combination of nivolumab (an anti-PD-1 antibody) and ipilimumab (an anti-CTLA4 antibody). The fact that PD-L1-negative melanoma tumors respond to the recited treatment is a law of nature because it describes a consequence of natural processes in the human body. The property of PD-L1-negative melanoma tumors is a natural phenomenon, because it occurs in nature and exists apart from any human action, regardless of whether the patient is treated or not.

The claim does not include additional elements that are sufficient to amount to significantly more than the judicial exception because the additional elements, when considered both individually and as an ordered combination, do not amount to significantly more than the abstract idea or a law of nature.

Implicit in step (i) of the claimed method is a process of determining the percentage of PD-L1-positive cells in the patient's tumor. No specific method to

accomplish this is recited in the claim, and there is a multitude of suitable methods which are well-understood and conventional. The instructions given to the healthcare provider in step (ii) of the claimed methods are recited at a high level of generality, since any generic anti-PD-1 and anti-CTLA4 antibody can be used in the claimed method, and no specific dosage or administration regimen are indicated. Both anti-PD-1 and anti-CTLA4 antibodies are well-understood and conventional in the art.

Therefore, neither an unspecified process of determining the percentage of PD-L1-positive cells in the patient's tumor, nor the instructions to the healthcare provider to administer to the patient generic anti-PD-1 and anti-CTLA4 antibodies in an unspecified dosage using an unspecified administration schedule, impose any meaningful limit on the abstract idea or a law of nature. Thus, taken either alone or as an ordered combination, the additional elements do not amount to significantly more than the above-identified judicial exceptions.

Accordingly, claim 13 is not drawn to eligible subject matter as it is directed to an abstract idea and to a law of nature or natural phenomenon without significantly more.

6. The following is a quotation of **35 U.S.C. 112(b)**:

(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

7. Claims 1, 2, 8, 13, 14, 16-18, 20-22, 25, 27-29, 32, 34, 36, 46 and 47 are rejected under **35 U.S.C. 112(b)** or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

(i) Claim 1 is indefinite, because the terminology used in the preamble of the claim is not consistent with that used in the body of the claim. Specifically, the

preamble recites a method for treating a tumor "in a subject," whereas steps (i) and (ii) of the claimed method are performed on a "patient." Therefore, it is not entirely clear whether the "subject" recited in the preamble is the same person as the "patient" recited in the method steps.

(ii) Claim 2 is indefinite, because on view of the definition of "PD-L1-negative melanoma tumor" in dependent claim 21, the meaning of the same expression on claim 2 is unclear. Absent a specific definition, the skilled artisan would reasonably interpret the phrase "a PD-L1-negative melanoma tumor" as a tumor in which expression of PD-L1 is not detectable. Claim 21 depends on claim 2, and stipulates that "the PD-L1-negative melanoma tumor is characterized by having less than about 5% of tumor cells showing binding to the anti-PD-L1 antibody," which creates ambiguity as to the specific meaning of "PD-L1-negative melanoma tumor" in claim 2.

(iii) Claim 13 is indefinite, because the preamble of the claim is incompatible with the recited method steps. The preamble states that the claimed method is for selecting a patient; therefore, the outcome of performing the method is a selected patient. In contrast, performing the method steps of identifying a patient and instructing a healthcare professional produces an instructed healthcare professional. Accordingly, a person of ordinary skill in the art would not be reasonably apprised as to what is being claimed.

(iV) Claim 20 is indefinite because it recites laboratory designations of antibodies "28-8," "28-1," etc. The use of a laboratory designation as the sole means of identifying the claimed/recited antibody renders the claim indefinite because different laboratories may use the same laboratory designation to define completely distinct biological materials. Amending the claims to recite the appropriate Deposit Accession Numbers, SEQ ID Numbers and/or other sufficiently specific identifying information would obviate this rejection.

(v) Claims 8, 13, 14, 16-18, 20-22, 25, 27-29, 32, 34, 36, 46 and 47 are indefinite, because they encompasses the indefinite limitations of the claims on which they depend.

In view of the above, a person of ordinary skill in the art cannot unequivocally interpret the metes and bounds of the claim so as to understand how to avoid infringement. Applicant is reminded that any amendment must point to a basis in the specification so as not to add New Matter. See MPEP 714.02 and 2163.06.

8. The following is a quotation of **35 U.S.C. 112(a)**:

(a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

9. Claim 20 is rejected under **35 U.S.C. 112(a)** or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the enablement requirement. The claim(s) contain(s) subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

It is apparent that the antibodies specifically recited in claim 20 ("28-8," "28-1," etc.) are required to practice the claimed invention. As required elements, they must be known and readily available to the public or obtainable by a repeatable method set forth in the specification. If they are not so obtainable or available, the enablement requirements of 35 USC 112, first paragraph, may be satisfied by a deposit of the pertinent cell lines which produce the antibodies. See 37 CFR 1.801-1.809.

There does not appear to be any evidence on this record to indicate whether any of the cell lines producing antibodies"28-8," "28-1," etc. are known and readily available to the public, obtainable by a repeatable method set forth in the specification, or have been deposited as required by 37 CFR 1.801-1.809.

If the deposits have been made under the terms of the Budapest Treaty, an affidavit or declaration by applicants or someone associated with the patent owner who is in a position to make such assurances, or a statement by an attorney of record over his or her signature, stating that the materials have been deposited under the terms of the Budapest Treaty would satisfy the deposit requirement made herein.

In addition to the conditions under the Budapest Treaty, applicant is required to provide assurance that all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of a patent in U.S. patent applications.

Amendment of the specification to recite the complete name and address of the depository is required. As an additional means for completing the record, applicant is invited to submit a copy of the contract with the depository for deposit and maintenance of each deposit.

10. The following is a quotation of the appropriate paragraphs of **35 U.S.C. 102** that form the basis for the rejections under this section made in this Office action:

> *A person shall be entitled to a patent unless –*
> ***(a)(1)*** *the claimed invention was patented, described in a printed publication, or in public use, on sale or otherwise available to the public before the effective filing date of the claimed invention.*

11. Claims 1, 2, 8, 13, 14, 16-18, 20-22, 25, 27-29, 32, 34, 36, 46 and 47 are rejected under **35 U.S.C. 102(a)(1)** as being anticipated by Wolchok et al. (N Engl J Med 2013; 369:122-133; cited on IDS).

Wolchok teaches treating melanoma patients by administering a combination of an anti-PD-1 antibody nivolumab and an anti-CTLA4 antibody ipilimumab. In particular, Wolchok reports the results of a Phase I clinical study wherein the two antibodies were administered concurrently every 3 weeks at doses 0.3, 1. 3 or 10 mg/kg for nivolumab and 1, 3 or 10 mg/kg for ipilimumab (e.g. p. 123). Expression PD-L1 before treatment was measured by means of immunohistochemical testing in formalin-fixed, paraffin-embedded tumor specimens with a rabbit monoclonal antihuman PD-L1 antibody (clone 28-8) in an automated assay (e.g. p. 124).

Objective responses to the treatment were observed at similar frequencies in patients with PD-L1–positive tumor samples (6 of 13 patients) and PD-L1–negative tumor samples (9 of 22 patients) (p. 130). At the maximum doses that were associated with an acceptable level of adverse events (nivolumab at 1 mg/kg and ipilimumab at 3 mg/kg), 53% of patients had an objective response, all with tumor reduction of 80% or more. A number of patients achieved progression-free survival of over 8 months (Fig. 1A).

Accordingly, Wolchok teaches all the limitations of claims 1, 2, 8, 13, 14, 16-18, 20-22, 25, 27-29, 32, 34 and 36. Claims 46 and 47 are included in the rejection, because the skilled artisan would at once envisage adding another anti-cancer agent to the above treatment regimen, as well as packaging the above combination of antibodies as a kit with instructions for use according to the above treatment regimen.

12. The nonstatutory **double patenting** rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference

claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

13. Claims 2, 8, 21, 22, 25, 27-29, 32, 34, 36 and 46 are rejected on the ground of nonstatutory **double patenting** as being unpatentable over claims 1-22 of commonly assigned U.S. Patent No. 9,856,320, as evidenced by Wolchok et al. (N Engl J Med 2013; 369:122-133; cited on IDS).

Although the claims at issue are not identical, they are not patentably distinct from each other because the instant claims are anticipated by the claims of the Patent. The claims of the '320 Patent are directed to a method of treating a human subject afflicted with cancer, in particular melanoma, comprising administering to the subject an anti-PD-1 antibody such as nivolumab and an anti-CTLA-4 antibody such as ipilimumab (e.g. claims 1-3, 5, 8, 19 and 20). The combination of an anti-PD-1 antibody at 1 mg/kg and anti-CTLA4 antibody at 3 mg/kg is administered every 3 weeks (e.g. claim 5), and can be combined with one of more of chemotherapy, radiation, hormone deprivation, and angiogenesis inhibitors (claim 22).

According to Wolchok (2013), approximately 62% of melanoma tumors are PD-L1-negative; therefore, patients with PD-L1-negative melanoma tumors are inherently present among a population of melanoma patients, and so the instant claims are anticipated by the claims of the '320 Patent.

14. Conclusion: no claim is allowed.

15. Any inquiry concerning this communication or earlier communications from the examiner should be directed to ILIA I OUSPENSKI whose telephone number is (571)272-2920. The examiner can normally be reached 8:30 AM – 5 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel Kolker can be reached at 571-272-3181. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic

Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ILIA I OUSPENSKI/
Primary Examiner, Art Unit 1644

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 8

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG, Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/AHB

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	US1	US-5977318-A	11-02-1999	LINSLEY; Peter S. et al.	
	US2	US-6051227-A	04-18-2000	ALLISON; James Patrick et al.	
	US3	US-6682736-B1	01-27-2004	HANSON; Douglas Charles et al.	
	US4	US-6808710-B1	10-26-2004	WOOD; Clive et al.	
	US5	US-6984720-B1	01-10-2006	KORMAN; Alan J. et al.	
	US6	US-7034121-B2	04-25-2006	CARRENO; Beatriz M. et al.	
	US7	US-7488802-B2	02-10-2009	COLLINS; Mary et al.	
	US8	US-7595048-B2	09-29-2009	HONJO; Tasuku et al.	
	US9	US-7605238-B2	10-20-2009	KORMAN; Alan J. et al.	
	US10	US-7943743-B2	05-17-2011	KORMAN; Alan J. et al.	
	US11	US-8008449-B2	08-30-2011	KORMAN; Alan J. et al.	
	US12	US-8168757-B2	05-01-2012	FINNEFROCK; Adam C. et al.	
	US13	US-8217149-B2	07-10-2012	IRVING; Bryan et al.	
	US14	US-8354509-B2	01-15-2013	CARVEN; Gregory John et al.	
	US15	US-8383796-B2	02-26-2013	KORMAN; Alan J. et al.	
	US16	US-8609089-B2	12-17-2013	LANGERMANN; Solomon et al.	
	US17	US-8686119-B2	04-01-2014	ROTEM-YEHUDAR RINAT et al.	
	US18	US-8779105-B2	07-15-2014	KORMAN; Alan J. et al.	
	US19	US-8900587-B2	12-02-2014	CARVEN; Gregory John et al.	
	US20	US-9084776-B2	07-21-2015	KORMAN; Alan J. et al.	
	US21	US-9102725-B2	08-11-2015	KORMAN; Alan J. et al.	
	US22	US-9273135-B2	03-01-2016	KORMAN; Alan J. et al.	

Examiner Signature		Date Considered	

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Sheet 2 of 8

Complete if Known	
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Filing Date	April 28, 2016
First Named Inventor	YANG, Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/AHB

U. S. PATENT DOCUMENTS

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	US23	US-9358289-B2	06-07-2016	KORMAN; Alan J. et al.	
	US24	US-9387247-B2	07-12-2016	KORMAN; Alan J. et al.	
	US25	US-9492539-B2	11-15-2016	KORMAN; Alan J. et al.	
	US26	US-9492540-B2	11-15-2016	KORMAN; Alan J. et al.	
	US27	US-20060110383-A1	05-25-2006	HONJO; Tasuku et al.	
	US28	US-20120263677-A1	10-18-2012	EAGLE; Craig J. et al.	
	US29	US-20130017199-A1	01-17-2013	LANGERMANN; Solomon	
	US30	US-20130309250-A1	11-21-2013	COGSWELL; John et al.	
	US31	US-20150079109-A1	03-19-2015	LI; Kang et al.	
	US32	US-20160075782-A1	03-17-2016	KORMAN; Alan J. et al.	
	US33	US-20160362495-A1	12-15-2016	KORMAN; Alan J. et al.	

Examiner Signature		Date Considered	

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Sheet 3 of 8

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Art Unit	1644
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	FP1	WO-2006121168-A1	11-16-2006	ONO PHARMACEUTICAL CO., LTD. et al.		☐
	FP2	WO-2007005874-A2	01-11-2007	MEDAREX, INC. *et al.*		☐
	FP3	WO-2007113648-A2	10-11-2007	PFIZER PRODUCTS INC. et al.		☐
	FP4	WO-2012122444-A1	09-13-2012	PROVECTUS PHARMACEUTICALS, INC. et al.		☐
	FP5	WO-2012145493-A1	10-26-2012	AMPLIMMUNE, INC. et al.		☐
	FP6	WO-2013014668-A1	01-31-2013	CURETECH LTD. et al.		☐
	FP7	WO-2013173223-A1	11-21-2013	BRISTOL-MYERS SQUIBB COMPANY		☐

Examiner Signature		Date Considered	

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First Named Inventor	YANG, Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/AHB

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author(in CAPITAL LETTERS),title of the article(when appropriate), title of the item (book,magazine,journal,serial,symposium,catalog,etc.),date,page(s),volume-issue number(s),publisher, city and/or country where published.	T[2]
	NPL1	ASCIERTO, P.A., *et al.*, "Biomarkers for Immunostimulatory Monoclonal Antibodies in Combination Strategies for Melanoma and Other Tumor Types," Clinical Cancer Research 19(5):1009-1020, American Association for Cancer Research, United States (2013).	☐
	NPL2	ASCIERTO, P.A., *et al.*, "The Additional Facet of Immunoscore: Immunoprofiling as a Possible Predictive Tool for Cancer Treatment," Journal of Translational Medicine 11:54, BioMed Central Ltd., England, 4 pages (2013).	☐
	NPL3	BRAHMER, J.R., *et al.*, "Safety and Activity of Anti-PD-L1 Antibody in Patients with Advanced Cancer," The New England Journal of Medicine 366(26):2455-2465, Massachusetts Medical Society, United States (2012).	☐
	NPL4	CONDEELIS, J. and WEISSLEDER, R., "In Vivo Imaging in Cancer," Cold Spring Harbor Perspectives in Biology 2(12):a003848, Cold Spring Harbor Laboratory Press, United States (2010).	☐
	NPL5	DONG, H., *et al.*, "Tumor-associated B7-H1 Promotes T-cell Apoptosis: A Potential Mechanism of Immune Evasion," Nature Medicine 8(8):793-800, Nature Publishing Company, United States (2002).	☐
	NPL6	GADIOT, J., *et al.*, "Overall Survival and PD-L1 Expression in Metastasized Malignant Melanoma," Cancer 117(10):2192-2201, American Cancer Society , United States (2011).	☐
	NPL7	GARBE, C., *et al.*, "Diagnosis and Treatment of Melanoma. European Consensus-based Interdisciplinary Guideline – Update 2012," European Journal of Cancer 48(15):2375-2390, Elsevier Ltd., England (2012).	☐
	NPL8	GenBank, "cytotoxic T-lymphocyte-associated protein 4 [Homo sapiens]," Accession Number AAB59385.1, November 1, 1994, accessed at https://www.ncbi.nlm.nih.gov/protein/AAB59385, accessed on December 6, 2016, 3 pages.	☐

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet 5 of 8

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG, Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/AHB

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author(in CAPITAL LETTERS),title of the article(when appropriate), title of the item (book,magazine,journal,serial,symposium,catalog,etc.),date,page(s),volume-issue number(s),publisher, city and/or country where published.	T[2]
	NPL9	GenBank, "RecName: Full=Programmed cell death 1 ligand 1; Short=PD-L1; Short=PDCD1 ligand 1; Short=Programmed death ligand 1; AltName: Full=B7 homolog 1; Short=B7-H1; AltName: CD_antigen=CD274; Flags: Precursor," Accession Number Q9NZQ7.1, November 2, 2016, accessed at https://www.ncbi.nlm.nih.gov/protein/Q9NZQ7, accessed on December 6, 2016, 11 pages.	☐
	NPL10	GenBank, "Human hPD-1 (hPD-1) mRNA, complete cds," Accession Number U64863.1, October 12, 2005, accessed at https://www.ncbi.nlm.nih.gov/nuccore/U64863, accessed on December 6, 2016, 3 pages.	☐
	NPL11	HAMANISHI, J., *et al.*, "Programmed Cell Death 1 Ligand 1 and Tumor-infiltrating $CD8^+$ T Lymphocytes are Prognostic Factors of Human Ovarian Cancer," Proceedings of the National Academy of Sciences USA 104(9):3360-3365, National Academy of Sciences, United States (2007).	☐
	NPL12	HERBST, R.S., *et al.*, "A Study of MPDL3280A, an Engineered PD-L1 Antibody in Patients with Locally Advanced or Metastatic Tumors," Journal of Clinical Oncology 31(Suppl; Abstr 3000), American Society of Clinical Oncology, United States (2013).	☐
	NPL13	International Search Report and Written Opinion for International Application No. PCT/US2016/029877, European Patent Office, Rijswijk, mailed on July 13, 2016, 16 pages.	☐
	NPL14	KHLEIF, S., *et al.*, "MEDI4736, An Anti-PD-L1 Antibody with Modified Fc Domain: Preclinical Evaluation and Early Clinical Results from a Phase 1 Study in Patients with Advanced Solid Tumors," Abstract 802, in Proceedings from the European Cancer Congress 2013, Amsterdam, The Netherlands (September 27-October 1, 2013).	☐
	NPL15	MAHONEY, K.M., *et al.*, "The Next Immune-Checkpoint Inhibitors: PD-1/PD-L1 Blockade in Melanoma," Clinical Therapeutics 37(4):764-782, Elsevier HS Journals, Inc., United States (March 29, 2015).	☐

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Sheet 6 of 8

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG, Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/AHB

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	NPL16	MARZUKA, A., *et al.*, "Melanoma Treatments: Advances and Mechanisms," Journal of Cellular Physiology 230(11):2626-2633, Wiley Periodicals, Inc., United States (July 27, 2015).	☐
	NPL17	MCCABE, K.E. and WU, A.M., "Positive Progress in ImmunoPET – Not Just a Coincidence," Cancer Biotherapy & Radiopharmaceuticals 25(3):253-261, Mary Ann Liebert, Inc., United States (2010).	☐
	NPL18	National Comprehensive Cancer Network, "NCCN GUIDELINES," nccn.org, accessed at http://www.nccn.org/professionals/physician_gls/f_guidelines.asp#site, accessed on December 8, 2016, 4 pages.	☐
	NPL19	NCI Drug Dictionary, anti-PD-1 Fusion Protein AMP-224, accessed on December 1, 2016, retrieved from the Internet URL: https://www.cancer.gov/publications/dictionaries/cancer-drug?cdrid=700595.	☐
	NPL20	NCI Drug Dictionary, anti-PD-1 monoclonal antibody MEDI0680, accessed on December 1, 2016, retrieved from the Internet URL: https://www.cancer.gov/publications/dictionaries/cancer-drug?cdrid=756047.	☐
	NPL21	NCI Drug Dictionary, pembrolizumab, accessed on December 1, 2016, retrieved from the Internet URL: https://www.cancer.gov/drugdictionary?cancer-drug?cdrid=695789.	☐
	NPL22	OLAFSEN, T., *et al.*, "ImmunoPET imaging of B-Cell Lymphoma Using ^{124}I-Anti-CD20 scFv Dimers (Diabodies)," Protein Engineering, Design & Selection 23(4):243-249, Oxford University Press, England (2010).	☐
	NPL23	OTT, P.A., *et al.*, "CTLA-4 and PD-1/PD-L1 Blockade: New Immunotherapeutic Modalities with Durable Clinical Benefit in Melanoma Patients," Clinical Cancer Research 19(19):5300-5309, American Association of Cancer Research, United States (2013).	☐

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Sheet 7 of 8

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First Named Inventor	YANG, Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/AHB

NON-PATENT LITERATURE DOCUMENTS

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	NPL24	PHILIPS, G.K. and ATKINS, M., "Therapeutic Uses of Anti-PD-1 and Anti-PD-L1 Antibodies," International Immunology 27(1):39-46, The Japanese Society for Immunology, Japan (October 16, 2014).	☐
	NPL25	SIEGEL, R., *et al.*, "Cancer Statistics, 2013," CA: A Cancer Journal for Clinicians 63(1):11-30, American Cancer Society, United States (2013).	☐
	NPL26	SJOBLOM, T., *et al.*, "The Consensus Coding Sequences of Human Breast and Colorectal Cancers," Science 314(5797):268-274, American Association for the Advancement of Science, United States (2006).	☐
	NPL27	TAUBE, J.M., *et al.*, "Colocalization of Inflammatory Response With B7-H1 Expression in Human Melanocytic Lesions Supports an Adaptive Resistance Mechanism of Immune Escape," Science Translational Medicine 4(127):127ra37, American Association for the Advancement of Science, United States (2012).	☐
	NPL28	TOPALIAN, S.L., *et al.*, "Safety, Activity, and Immune Correlates of Anti-PD-1 Antibody in Cancer," The New England Journal of Medicine 366(26):2443-2454, Massachusetts Medical Society, United States (2012).	☐
	NPL29	United States Adopted Name (USAN) Drug Finder, "Pembrolizumab: Statement on a nonproprietary name adopted by the USAN Council (ZZ-165)," published November 27, 2013, accessed at https://searchusan.ama-assn.org/usan/documentDownload?uri=%2Funstructured%2Fbinary%2Fusan%2Fpembrolizumab.pdf, accessed on December 8, 2016, 2 pages.	☐
	NPL30	WANG, C., *et al.*, "*In Vitro* Characterization of the Anti-PD-1 Antibody Nivolumab, BMS-936558, and *In Vivo* Toxicology in Non-Human Primates," Cancer Immunology Research 2(9):846-856, American Association for Cancer Research, United States (May 28, 2014).	☐

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Sheet 8 of 8

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Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/AHB

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	NPL31	WOLCHOK, J.D., *et al.*, "Nivolumab plus Ipilimumab in Advanced Melanoma," The New England Journal of Medicine 369(2):122-133, Massachusetts Medical Society, United States (2013).	☐

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Examiner Signature	/ILIA I OUSPENSKI/	Date Considered	02/27/2018

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☐	L5	(L4 same melanoma$)	527
☐	L4	L3 with (cancer$ or tumor$ or tumour$ or malignan$ or neoplas$ or metasta$ or onco$)	2025
☐	L3	(L1 with L2)	4585
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☐	L1	(pd-1$ or pd1$ or pdhd1$ or cd279$ or (programmed cell death protein 1) or (programmed cell death protein-1) or (programmed death protein 1) or (programmed death protein-1) or (programmed death 1) or (programmed death-1) or anti-pd-1$ or anti-pd1$ or anti-pdhd1$ or anti-cd279$ or ".alpha.pd-1$" or ".alpha.pd1$" or ".alpha.pdhd1$" or ".alpha.cd279$" or pd-1-binding or pd1-binding or pdhd1-binding or cd279-binding or (pd-1 binding) or (pd1 binding) or (pdhd1 binding) or (cd279 binding)) or (nivolumab$ or 5C4 or BMS-936558 or bms936558 or MDX-1106 or mdx1106 or GTPL7335 or gtpl-7335 or ONO-4538 or ono4538 or Opdivo$ or pembrolizumab or Keytruda$ or pidilizumab or CT-011 or CT011 or lambrolizumab or MK-3475 or MK3475 or (SCH 900475) or SCH-900475 or SCH900475 or REGN2810 or REGN-2810 or MEDI0680 or MEDI-0680 or AMP514 or AMP-514 or PDR001 or PDR-001 or SHR-1210 or SHR1210 or TSR-042 or TSR042)	47570

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BIB DATA SHEET

CONFIRMATION NO. 6926

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
15/141,769	04/28/2016 RULE	424	1644	3338.0330001/ELE/C-K

APPLICANTS
Bristol-Myers Squibb Company, Princeton, NJ

INVENTORS
Arvin YANG, Princeton, NJ;

**** CONTINUING DATA ***************************
This appln claims benefit of 62/153,973 04/28/2015

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****
05/06/2016

Foreign Priority claimed ☐ Yes ☑ No 35 USC 119(a-d) conditions met ☐ Yes ☑ No Verified and Acknowledged /ILIA I OUSPENSKI/ Examiner's Signature	☐ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		NJ	6	20	3

ADDRESS

Sterne, Kessler, Goldstein & Fox P.L.L.C.
1100 New York Avenue NW
Washington, DC 20005
UNITED STATES

TITLE

Treatment of PD-L1-Negative Melanoma Using an Anti-PD-1 Antibody and an Anti-CTLA-4 Antibody

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Sheet 1 of 8

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U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	US1	US-5977318-A	11-02-1999	LINSLEY; Peter S. et al.	
	US2	US-6051227-A	04-18-2000	ALLISON; James Patrick et al.	
	US3	US-6682736-B1	01-27-2004	HANSON; Douglas Charles et al.	
	US4	US-6808710-B1	10-26-2004	WOOD; Clive et al.	
	US5	US-6984720-B1	01-10-2006	KORMAN; Alan J. et al.	
	US6	US-7034121-B2	04-25-2006	CARRENO; Beatriz M. et al.	
	US7	US-7488802-B2	02-10-2009	COLLINS; Mary et al.	
	US8	US-7595048-B2	09-29-2009	HONJO; Tasuku et al.	
	US9	US-7605238-B2	10-20-2009	KORMAN; Alan J. et al.	
	US10	US-7943743-B2	05-17-2011	KORMAN; Alan J. et al.	
	US11	US-8008449-B2	08-30-2011	KORMAN; Alan J. et al.	
	US12	US-8168757-B2	05-01-2012	FINNEFROCK; Adam C. et al.	
	US13	US-8217149-B2	07-10-2012	IRVING; Bryan et al.	
	US14	US-8354509-B2	01-15-2013	CARVEN; Gregory John et al.	
	US15	US-8383796-B2	02-26-2013	KORMAN; Alan J. et al.	
	US16	US-8609089-B2	12-17-2013	LANGERMANN; Solomon et al.	
	US17	US-8686119-B2	04-01-2014	ROTEM-YEHUDAR RINAT et al.	
	US18	US-8779105-B2	07-15-2014	KORMAN; Alan J. et al.	
	US19	US-8900587-B2	12-02-2014	CARVEN; Gregory John et al.	
	US20	US-9084776-B2	07-21-2015	KORMAN; Alan J. et al.	
	US21	US-9102725-B2	08-11-2015	KORMAN; Alan J. et al.	
	US22	US-9273135-B2	03-01-2016	KORMAN; Alan J. et al.	

Examiner Signature		Date Considered	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet 2 of 8

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG, Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/AHB

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	US23	US-9358289-B2	06-07-2016	KORMAN; Alan J. et al.	
	US24	US-9387247-B2	07-12-2016	KORMAN; Alan J. et al.	
	US25	US-9492539-B2	11-15-2016	KORMAN; Alan J. et al.	
	US26	US-9492540-B2	11-15-2016	KORMAN; Alan J. et al.	
	US27	US-20060110383-A1	05-25-2006	HONJO; Tasuku et al.	
	US28	US-20120263677-A1	10-18-2012	EAGLE; Craig J. et al.	
	US29	US-20130017199-A1	01-17-2013	LANGERMANN; Solomon	
	US30	US-20130309250-A1	11-21-2013	COGSWELL; John et al.	
	US31	US-20150079109-A1	03-19-2015	LI; Kang et al.	
	US32	US-20160075782-A1	03-17-2016	KORMAN; Alan J. et al.	
	US33	US-20160362495-A1	12-15-2016	KORMAN; Alan J. et al.	

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Sheet 3 of 8

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Filing Date	April 28, 2016
First Named Inventor	YANG, Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/AHB

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	FP1	WO-2006121168-A1	11-16-2006	ONO PHARMACEUTICAL CO., LTD. et al.		☐
	FP2	WO-2007005874-A2	01-11-2007	MEDAREX, INC. *et al.*		☐
	FP3	WO-2007113648-A2	10-11-2007	PFIZER PRODUCTS INC. et al.		☐
	FP4	WO-2012122444-A1	09-13-2012	PROVECTUS PHARMACEUTICALS, INC. et al.		☐
	FP5	WO-2012145493-A1	10-26-2012	AMPLIMMUNE, INC. et al.		☐
	FP6	WO-2013014668-A1	01-31-2013	CURETECH LTD. et al.		☐
	FP7	WO-2013173223-A1	11-21-2013	BRISTOL-MYERS SQUIBB COMPANY		☐

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet 4 of 8

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG, Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/AHB

NON-PATENT LITERATURE DOCUMENTS

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	NPL1	ASCIERTO, P.A., *et al.*, "Biomarkers for Immunostimulatory Monoclonal Antibodies in Combination Strategies for Melanoma and Other Tumor Types," Clinical Cancer Research 19(5):1009-1020, American Association for Cancer Research, United States (2013).	☐
	NPL2	ASCIERTO, P.A., *et al.*, "The Additional Facet of Immunoscore: Immunoprofiling as a Possible Predictive Tool for Cancer Treatment," Journal of Translational Medicine 11:54, BioMed Central Ltd., England, 4 pages (2013).	☐
	NPL3	BRAHMER, J.R., *et al.*, "Safety and Activity of Anti-PD-L1 Antibody in Patients with Advanced Cancer," The New England Journal of Medicine 366(26):2455-2465, Massachusetts Medical Society, United States (2012).	☐
	NPL4	CONDEELIS, J. and WEISSLEDER, R., "In Vivo Imaging in Cancer," Cold Spring Harbor Perspectives in Biology 2(12):a003848, Cold Spring Harbor Laboratory Press, United States (2010).	☐
	NPL5	DONG, H., *et al.*, "Tumor-associated B7-H1 Promotes T-cell Apoptosis: A Potential Mechanism of Immune Evasion," Nature Medicine 8(8):793-800, Nature Publishing Company, United States (2002).	☐
	NPL6	GADIOT, J., *et al.*, "Overall Survival and PD-L1 Expression in Metastasized Malignant Melanoma," Cancer 117(10):2192-2201, American Cancer Society , United States (2011).	☐
	NPL7	GARBE, C., *et al.*, "Diagnosis and Treatment of Melanoma. European Consensus-based Interdisciplinary Guideline – Update 2012," European Journal of Cancer 48(15):2375-2390, Elsevier Ltd., England (2012).	☐
	NPL8	GenBank, "cytotoxic T-lymphocyte-associated protein 4 [Homo sapiens]," Accession Number AAB59385.1, November 1, 1994, accessed at https://www.ncbi.nlm.nih.gov/protein/AAB59385, accessed on December 6, 2016, 3 pages.	☐

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet 5 of 8

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG, Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/AHB

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	NPL9	GenBank, "RecName: Full=Programmed cell death 1 ligand 1; Short=PD-L1; Short=PDCD1 ligand 1; Short=Programmed death ligand 1; AltName: Full=B7 homolog 1; Short=B7-H1; AltName: CD_antigen=CD274; Flags: Precursor," Accession Number Q9NZQ7.1, November 2, 2016, accessed at https://www.ncbi.nlm.nih.gov/protein/Q9NZQ7, accessed on December 6, 2016, 11 pages.	☐
	NPL10	GenBank, "Human hPD-1 (hPD-1) mRNA, complete cds," Accession Number U64863.1, October 12, 2005, accessed at https://www.ncbi.nlm.nih.gov/nuccore/U64863, accessed on December 6, 2016, 3 pages.	☐
	NPL11	HAMANISHI, J., *et al.*, "Programmed Cell Death 1 Ligand 1 and Tumor-infiltrating $CD8^+$ T Lymphocytes are Prognostic Factors of Human Ovarian Cancer," Proceedings of the National Academy of Sciences USA 104(9):3360-3365, National Academy of Sciences, United States (2007).	☐
	NPL12	HERBST, R.S., *et al.*, "A Study of MPDL3280A, an Engineered PD-L1 Antibody in Patients with Locally Advanced or Metastatic Tumors," Journal of Clinical Oncology 31(Suppl; Abstr 3000), American Society of Clinical Oncology, United States (2013).	☐
	NPL13	International Search Report and Written Opinion for International Application No. PCT/US2016/029877, European Patent Office, Rijswijk, mailed on July 13, 2016, 16 pages.	☐
	NPL14	KHLEIF, S., *et al.*, "MEDI4736, An Anti-PD-L1 Antibody with Modified Fc Domain: Preclinical Evaluation and Early Clinical Results from a Phase 1 Study in Patients with Advanced Solid Tumors," Abstract 802, in Proceedings from the European Cancer Congress 2013, Amsterdam, The Netherlands (September 27-October 1, 2013).	☐
	NPL15	MAHONEY, K.M., *et al.*, "The Next Immune-Checkpoint Inhibitors: PD-1/PD-L1 Blockade in Melanoma," Clinical Therapeutics 37(4):764-782, Elsevier HS Journals, Inc., United States (March 29, 2015).	☐

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Sheet 6 of 8

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG, Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/AHB

NON-PATENT LITERATURE DOCUMENTS

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	NPL16	MARZUKA, A., *et al.*, "Melanoma Treatments: Advances and Mechanisms," Journal of Cellular Physiology 230(11):2626-2633, Wiley Periodicals, Inc., United States (July 27, 2015).	☐
	NPL17	MCCABE, K.E. and WU, A.M., "Positive Progress in ImmunoPET – Not Just a Coincidence," Cancer Biotherapy & Radiopharmaceuticals 25(3):253-261, Mary Ann Liebert, Inc., United States (2010).	☐
	NPL18	National Comprehensive Cancer Network, "NCCN GUIDELINES," nccn.org, accessed at http://www.nccn.org/professionals/physician_gls/f_guidelines.asp#site, accessed on December 8, 2016, 4 pages.	☐
	NPL19	NCI Drug Dictionary, anti-PD-1 Fusion Protein AMP-224, accessed on December 1, 2016, retrieved from the Internet URL: https://www.cancer.gov/publications/dictionaries/cancer-drug?cdrid=700595.	☐
	NPL20	NCI Drug Dictionary, anti-PD-1 monoclonal antibody MEDI0680, accessed on December 1, 2016, retrieved from the Internet URL: https://www.cancer.gov/publications/dictionaries/cancer-drug?cdrid=756047.	☐
	NPL21	NCI Drug Dictionary, pembrolizumab, accessed on December 1, 2016, retrieved from the Internet URL: https://www.cancer.gov/drugdictionary?cancer-drug?cdrid=695789.	☐
	NPL22	OLAFSEN, T., *et al.*, "ImmunoPET imaging of B-Cell Lymphoma Using ^{124}I-Anti-CD20 scFv Dimers (Diabodies)," Protein Engineering, Design & Selection 23(4):243-249, Oxford University Press, England (2010).	☐
	NPL23	OTT, P.A., *et al.*, "CTLA-4 and PD-1/PD-L1 Blockade: New Immunotherapeutic Modalities with Durable Clinical Benefit in Melanoma Patients," Clinical Cancer Research 19(19):5300-5309, American Association of Cancer Research, United States (2013).	☐

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Sheet 7 of 8

Complete if Known	
Application Number	15/141,769
Filing Date	April 28, 2016
First Named Inventor	YANG, Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/AHB

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	NPL24	PHILIPS, G.K. and ATKINS, M., "Therapeutic Uses of Anti-PD-1 and Anti-PD-L1 Antibodies," International Immunology 27(1):39-46, The Japanese Society for Immunology, Japan (October 16, 2014).	☐
	NPL25	SIEGEL, R., *et al.*, "Cancer Statistics, 2013," CA: A Cancer Journal for Clinicians 63(1):11-30, American Cancer Society, United States (2013).	☐
	NPL26	SJOBLOM, T., *et al.*, "The Consensus Coding Sequences of Human Breast and Colorectal Cancers," Science 314(5797):268-274, American Association for the Advancement of Science, United States (2006).	☐
	NPL27	TAUBE, J.M., *et al.*, "Colocalization of Inflammatory Response With B7-H1 Expression in Human Melanocytic Lesions Supports an Adaptive Resistance Mechanism of Immune Escape," Science Translational Medicine 4(127):127ra37, American Association for the Advancement of Science, United States (2012).	☐
	NPL28	TOPALIAN, S.L., *et al.*, "Safety, Activity, and Immune Correlates of Anti-PD-1 Antibody in Cancer," The New England Journal of Medicine 366(26):2443-2454, Massachusetts Medical Society, United States (2012).	☐
	NPL29	United States Adopted Name (USAN) Drug Finder, "Pembrolizumab: Statement on a nonproprietary name adopted by the USAN Council (ZZ-165)," published November 27, 2013, accessed at https://searchusan.ama-assn.org/usan/documentDownload?uri=%2Funstructured%2Fbinary%2Fusan%2Fpembrolizumab.pdf, accessed on December 8, 2016, 2 pages.	☐
	NPL30	WANG, C., *et al.*, "*In Vitro* Characterization of the Anti-PD-1 Antibody Nivolumab, BMS-936558, and *In Vivo* Toxicology in Non-Human Primates," Cancer Immunology Research 2(9):846-856, American Association for Cancer Research, United States (May 28, 2014).	☐

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Sheet 8 of 8

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Filing Date	April 28, 2016
First Named Inventor	YANG, Arvin
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0330001/ELE/C-K/AHB

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	NPL31	WOLCHOK, J.D., *et al.*, "Nivolumab plus Ipilimumab in Advanced Melanoma," The New England Journal of Medicine 369(2):122-133, Massachusetts Medical Society, United States (2013).	☐

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor: Arvin YANG	Confirmation No.: 6926
Applicant: Bristol-Myers Squibb Company	Art Unit: 1644
Application No.: 15/141,769	Examiner: OUSPENSKI, Ilia I.
Filing Date: April 28, 2016	Atty. Docket: 3338.0330001/ELE/C-K/AHB

Title: **TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY**

Information Disclosure Statement

Mail Stop Amendment

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Commissioner:

Listed on accompanying IDS Form PTO/SB/08a; PTO/SB/08b equivalent are documents that may be considered material to the patentability of this application as defined in 37 C.F.R. §1.56, and in compliance with the duty of disclosure requirements of 37 C.F.R. §§ 1.97 and 1.98.

Where the publication date of a listed document does not provide a month of publication, the year of publication of the listed document is sufficiently earlier than the effective U.S. filing date and any foreign priority date so that the month of publication is not in issue. Applicant has listed publication dates on the attached IDS Form based on information presently available to the undersigned. However, the listed publication dates should not be construed as an admission that the information was actually published on the date indicated.

Applicant reserves the right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered.

This statement should not be construed as a representation that a search has been made, or that information more material to the examination of the present patent application does not exist. The Examiner is specifically requested not to rely solely on the material submitted herewith.

This is a filing under 37 C.F.R. § 1.97(b). This Information Disclosure Statement is being filed before the mailing date of a first Office Action on the merits. No statement or fee is required.

Copies of documents **FP1-FP7** and **NPL1-NPL31** are submitted. However, in accordance with 37 C.F.R. § 1.98(a)(2)(ii), no copies of U.S. patents and patent application publications cited as documents **US1-US33** on the attached IDS Form are submitted.

It is respectfully requested that the Examiner initial and return a copy of the enclosed IDS Form, and indicate in the official file wrapper of this patent application that the documents have been considered.

The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 19-0036.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Ji-Eun Kim
Attorney for Applicant
Registration No. 68,504

Date: Feb. 22, 2017

1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

5021142_1

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JI-EUN KIM
DIRECTOR
(202) 772-8619
CKIM@SKGF.COM

Sterne Kessler Goldstein Fox
ATTORNEYS AT LAW

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Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

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Re: U.S. Utility Patent Application
Application No. 15/141,769; Filing Date: April 28, 2016
For: **TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY**
Inventor: Arvin YANG
Our Ref: 3338.0330001/ELE/C-K/AHB

Commissioner:

Transmitted herewith for appropriate action are the following documents:

1. Information Disclosure Statement;

2. Forms PTO/SB/08a and PTO/SB/08b equivalent (8 sheets) listing 71 documents (US1-US33, FP1-FP7, and NPL1-NPL31); and

3. Copies of cited documents (FP1-FP7 and NPL1-NPL31).

In the event that extensions of time are necessary to prevent abandonment of this patent application, then such extensions of time are hereby petitioned.

The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 19-0036.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Ji-Eun Kim
Attorney for Applicant
Registration No. 68,504

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(71) Applicants *(for all designated States except US)*: **ONO PHARMACEUTICAL CO., LTD.** [JP/JP]; 1-5, Doshomachi 2-chome, Chuo-ku, Osaka-shi, Osaka 5418526 (JP). **Medarex, Inc.** [US/US]; 707 State Road, Princeton, New Jersey, 08540 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **KORMAN, Alan, J.. SRINIVASAN, Mohan. WANG, Changyu. SELBY, Mark, J.. CHEN, Bing. CARDARELLI, Josephine, M..**

(74) Agents: **NAITO, Teruo** et al.; Shin-ei Patent Office, 7-13, Nishi-Shimbashi 1-chome, Minato-ku, Tokyo 1050003 (JP).

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(54) Title: HUMAN MONOCLONAL ANTIBODIES TO PROGRAMMED DEATH 1(PD-1) AND METHODS FOR TREATING CANCER USING ANTI-PD-1 ANTIBODIES ALONE OR IN COMBINATION WITH OTHER IMMUNOTHERAPEUTICS

(57) Abstract: The present invention provides isolated monoclonal antibodies, particularly human monoclonal antibodies, that specifically bind to PD-1 with high affinity. Nucleic acid molecules encoding the antibodies of the invention, expression vectors, host cells and methods for expressing the antibodies of the invention are also provided. Immunoconjugates, bispecific molecules and pharmaceutical compositions comprising the antibodies of the invention are also provided. The invention also provides methods for detecting PD-1, as well as methods for treating various diseases, including cancer and infectious diseases, using anti-PD-1 antibodies. The present invention further provides methods for using a combination immunotherapy, such as the combination of anti-CTLA-4 and anti-PD-1 antibodies, to treat hyperproliferative disease, such as cancer. The invention also provides methods for altering adverse events related to treatment with such antibodies individually.

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DESCRIPTION

HUMAN MONOCLONAL ANTIBODIES TO PROGRAMMED DEATH 1 (PD-1) AND METHODS FOR TREATING CANCER USING ANTI-PD-1 ANTIBODIES ALONE OR IN COMBINATION WITH OTHER IMMUNOTHERAPEUTICS

Technical Field

The present invention relates generally to immunotherapy in the treatment of human disease and reduction of adverse events related thereto. More specifically, the present invention relates to the use of anti-PD-1 antibodies and the use of combination immunotherapy, including the combination of anti-CTLA-4 and anti-PD-1 antibodies, to treat cancer and/or to decrease the incidence or magnitude of adverse events related to treatment with such antibodies individually.

Background of the Invention

The protein Programmed Death 1 (PD-1) is an inhibitory member of the CD28 family of receptors, that also includes CD28, CTLA-4, ICOS and BTLA. PD-1 is expressed on activated B cells, T cells, and myeloid cells (Agata *et al.*, *supra*; Okazaki *et al.* (2002) *Curr. Opin. Immunol.* 14: 391779-82; Bennett *et al.* (2003) *J Immunol* 170:711-8). The initial members of the family, CD28 and ICOS, were discovered by functional effects on augmenting T cell proliferation following the addition of monoclonal antibodies (Hutloff *et al.* (1999) *Nature* 397:263-266; Hansen *et al.* (1980) *Immunogenics* 10:247-260). PD-1 was discovered through screening for differential expression in apototic cells (Ishida *et al.* (1992) *EMBO J* 11:3887-95). The other members of the family, CTLA-4, and BTLA were discovered through screening for differential expression in cytotoxic T lymphocytes and TH1 cells, respectively. CD28, ICOS and CTLA-4 all have an unpaired cysteine residue allowing for homodimerization. In contrast, PD-1 is suggested to exist as a monomer, lacking the unpaired cysteine residue characteristic in other CD28 family members.

The PD-1 gene is a 55 kDa type I transmembrane protein that is part of the Ig gene superfamily (Agata *et al.* (1996) *Int Immunol* 8:765-72). PD-1 contains a membrane proximal immunoreceptor tyrosine inhibitory motif (ITIM) and a membrane distal tyrosine-based switch motif (ITSM) (Thomas, M.L. (1995) *J Exp Med* 181:1953-6; Vivier, E and Daeron, M (1997) *Immunol Today* 18:286-91). Although structurally similar to CTLA-4, PD-1 lacks the MYPPPY motif that is critical for B7-1 and B7-2 binding. Two ligands for PD-1 have been identified, PD-L1 and PD-L2, that have been shown to downregulate T cell activation upon binding to PD-1 (Freeman *et al.* (2000) *J Exp Med* 192:1027-34; Latchman *et al.* (2001) *Nat Immunol* 2:261-8; Carter *et al.* (2002) *Eur J Immunol* 32:634-43). Both PD-L1 and PD-L2 are B7 homologs that bind to PD-1, but do not bind to other CD28 family members. One ligand for PD-1, PD-L1 is abundant in a variety of human cancers (Dong *et al.*

(2002) *Nat. Med.* 8:787-9). The interaction between PD-1 and PD-L1 results in a decrease in tumor infiltrating lymphocytes, a decrease in T-cell receptor mediated proliferation, and immune evasion by the cancerous cells (Dong *et al.* (2003) *J. Mol. Med.* 81:281-7; Blank *et al.* (2005) *Cancer Immunol. Immunother.* 54:307-314; Konishi *et al.* (2004) *Clin. Cancer Res.* 10:5094-100). Immune suppression can be reversed by inhibiting the local interaction of PD-1 with PD-L1, and the effect is additive when the interaction of PD-1 with PD-L2 is blocked as well (Iwai *et al.* (2002) *Proc. Nat'l. Acad. Sci. USA* 99:12293-7; Brown *et al.* (2003) *J. Immunol.* 170:1257-66).

PD-1 is an inhibitory member of the CD28 family expressed on activated B cells, T cells, and myeloid cells (Agata *et al.*, *supra*; Okazaki *et al.* (2002) *Curr Opin Immunol* 14: 391779-82; Bennett *et al.* (2003) *J Immunol* 170:711-8). PD-1 deficient animals develop various autoimmune phenotypes, including autoimmune cardiomyopathy and a lupus-like syndrome with arthritis and nephritis (Nishimura *et al.* (1999) *Immunity* 11:141-51; Nishimura *et al.* (2001) *Science* 291:319-22). Additionally, PD-1 has been found to play a role in autoimmune encephalomyelitis, systemic lupus erythematosus, graft-versus-host disease (GVHD), type I diabetes, and rheumatoid arthritis (Salama *et al.* (2003) *J Exp Med* 198:71-78; Prokunina and Alarcon-Riquelme (2004) *Hum Mol Genet* 13:R143; Nielsen *et al.* (2004) *Lupus* 13:510). In a murine B cell tumor line, the ITSM of PD-1 was shown to be essential to block BCR-mediated Ca^{2+}-flux and tyrosine phosphorylation of downstream effector molecules (Okazaki *et al.* (2001) *PNAS* 98:13866-71).

Accordingly, agents that recognize PD-1, and methods of using such agents, are desired.

Disclosure of the Invention

The present invention provides isolated monoclonal antibodies, in particular human monoclonal antibodies, that bind to PD-1 and that exhibit numerous desirable properties. These properties include, for example, high affinity binding to human PD-1, but lacking substantial cross-reactivity with either human CD28, CTLA-4 or ICOS. Still further, antibodies of the invention have been shown to modulate immune responses. Accordingly, another aspect of the invention pertains to methods of modulating immune responses using anti-PD-1 antibodies. In particular, the invention provides a method of inhibiting growth of tumor cells *in vivo* using anti-PD-1 antibodies.

In one aspect, the invention pertains to an isolated monoclonal antibody, or an antigen-binding portion thereof, wherein the antibody exhibits at least one of the following properties:

(a) binds to human PD-1 with a K_D of 1×10^{-7} M or less;

(b) does not substantially bind to human CD28, CTLA-4 or ICOS;

(c) increases T-cell proliferation in an Mixed Lymphocyte Reaction (MLR) assay;

(d) increases interferon-gamma production in an MLR assay;

(e) increases IL-2 secretion in an MLR assay;

(f) binds to human PD-1 and cynomolgus monkey PD-1;

(g) inhibits the binding of PD-L1 and/or PD-L2 to PD-1;

(h) stimulates antigen-specific memory responses;

(i) stimulates antibody responses;

(j) inhibits tumor cell growth *in vivo*.

Preferably the antibody is a human antibody, although in alternative embodiments the antibody can be, for example, a murine antibody, a chimeric antibody or humanized antibody.

In more preferred embodiments, the antibody binds to human PD-1 with a K_D of 5 x 10^{-8} M or less, binds to human PD-1 with a K_D of 1 x 10^{-8} M or less, binds to human PD-1 with a K_D of 5 x 10^{-9} M or less, or binds to human PD-1 with a K_D of between 1 x10^{-8} M and 1 x 10^{-10} M.

In another embodiment, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, wherein the antibody cross-competes for binding to PD-1 with a reference antibody comprising:

(a) a human heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6 and 7; and

(b) a human light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13 and 14.

In various embodiments, the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 1; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8; or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 2; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 9; or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 3; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 10; or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 11; or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 5; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 12; or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 13; or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14.

In another aspect, the invention pertains to an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 3-33 gene, wherein the antibody specifically binds PD-1. The invention further provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 4-39 gene, wherein the antibody specifically binds PD-1. The invention further provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L6 gene, wherein the antibody specifically binds PD-1. The invention further provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L15 gene, wherein the antibody specifically binds PD-1.

In a preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising:

(a) a heavy chain variable region of a human V_H 3-33 gene; and

(b) a light chain variable region of a human V_K L6 gene;

wherein the antibody specifically binds to PD-1.

In another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising:

(a) a heavy chain variable region of a human V_H 4-39 gene; and

(b) a light chain variable region of a human V_K L15 gene;

wherein the antibody specifically binds to PD-1.

In another aspect, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, comprising:

a heavy chain variable region that comprises CDR1, CDR2, and CDR3 sequences; and a light chain variable region that comprises CDR1, CDR2, and CDR3 sequences, wherein:

(a) the heavy chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35, and conservative modifications thereof;

(b) the light chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56, and conservative modifications thereof; and

(c) the antibody specifically binds to human PD-1.

Preferably, the heavy chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28, and conservative modifications thereof; and the light chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49, and conservative modifications thereof. Preferably, the heavy chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21, and conservative modifications thereof; and the light chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42, and conservative modifications thereof.

In yet another aspect, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, comprising a heavy chain variable region and a light chain variable region, wherein:

(a) the heavy chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6 and 7;

(b) the light chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13 and 14;

(c) the antibody binds to human PD-1 with a K_D of 1×10^{-7} M or less; and

(d) the antibody does not substantially bind to human CD28, CTLA-4 or ICOS.

In a preferred embodiment, the antibodies additionally comprise at least one of the following properties:

(a) the antibody increases T-cell proliferation in an MLR assay;

(b) the antibody increases interferon-gamma production in an MLR assay; or

(c) the antibody increases IL-2 secretion in an MLR assay.

Additionally or alternatively, the antibody may comprise one or more of the other features listed above.

In preferred embodiments, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, comprising:

(a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21;

(b) a heavy chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28;

(c) a heavy chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35;
(d) a light chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42;
(e) a light chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49; and
(f) a light chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56;
wherein the antibody specifically binds PD-1.

A preferred combination comprises:
(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 15;
(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 22;
(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 29;
(d) a light chain variable region CDR1 comprising SEQ ID NO: 36;
(e) a light chain variable region CDR2 comprising SEQ ID NO: 43; and
(f) a light chain variable region CDR3 comprising SEQ ID NO: 50.

Another preferred combination comprises:
(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 16;
(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 23;
(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 30;
(d) a light chain variable region CDR1 comprising SEQ ID NO: 37;
(e) a light chain variable region CDR2 comprising SEQ ID NO: 44; and
(f) a light chain variable region CDR3 comprising SEQ ID NO: 51.

Another preferred combination comprises:
(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 17;
(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 24;
(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 31;
(d) a light chain variable region CDR1 comprising SEQ ID NO: 38;
(e) a light chain variable region CDR2 comprising SEQ ID NO: 45; and
(f) a light chain variable region CDR3 comprising SEQ ID NO: 52.

Another preferred combination comprises:
(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 18;
(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 25;
(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 32;
(d) a light chain variable region CDR1 comprising SEQ ID NO: 39;
(e) a light chain variable region CDR2 comprising SEQ ID NO: 46; and
(f) a light chain variable region CDR3 comprising SEQ ID NO: 53.

Another preferred combination comprises:
(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 19;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 26;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 33;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 40;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 47; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 54.

Another preferred combination comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 20;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 27;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 34;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 41;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 48; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 55.

Another preferred combination comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 21;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 28;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 35;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 42;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 49; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 56.

Other preferred antibodies of the invention, or antigen-binding portions thereof, comprise:

(a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6 and 7; and

(b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13 and 14;

wherein the antibody specifically binds PD-1.

A preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 1; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 2; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 9.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 3; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 10.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 11.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 5; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 12.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 13.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14.

The antibodies of the invention can be, for example, full-length antibodies, for example of an IgG1 or IgG4 isotype. Alternatively, the antibodies can be antibody fragments, such as Fab or Fab'2 fragments, or single chain antibodies.

The invention also provides an immunoconjugate comprising an antibody of the invention, or antigen-binding portion thereof, linked to a therapeutic agent, such as a cytotoxin or a radioactive isotope. The invention also provides a bispecific molecule comprising an antibody, or antigen-binding portion thereof, of the invention, linked to a second functional moiety having a different binding specificity than said antibody, or antigen-binding portion thereof.

Compositions comprising an antibody, or antigen-binding portion thereof, or immunoconjugate or bispecific molecule of the invention, and a pharmaceutically acceptable carrier, are also provided.

Nucleic acid molecules encoding the antibodies, or antigen-binding portions thereof, of the invention are also encompassed by the invention, as well as expression vectors comprising such nucleic acids and host cells comprising such expression vectors. Moreover, the invention provides a transgenic mouse comprising human immunoglobulin heavy and light chain transgenes, wherein the mouse expresses an antibody of the invention, as well as hybridomas prepared from such a mouse, wherein the hybridoma produces the antibody of the invention.

In yet another aspect, the invention provides a method of modulating an immune response in a subject comprising administering to the subject the antibody, or antigen-binding

portion thereof, of the invention such that the immune response in the subject is modulated. Preferably, the antibody of the invention enhances, stimulates or increases the immune response in the subject.

In a further aspect, the invention provides a method of inhibiting growth of tumor cells in a subject, comprising administering to a subject a therapeutically effective amount of an anti-PD-1 antibody, or antigen-binding portion thereof. The antibodies of the invention are preferred for use in the method although other anti-PD-1 antibodies can be used instead (or in combination with an anti-PD-1 antibody of the invention). For example, a chimeric, humanized or fully human anti-PD-1 antibody can be used in the method of inhibiting tumor growth.

In a further aspect, the invention provides a method of treating an infectious disease in a subject, comprising administering to a subject a therapeutically effective amount of an anti-PD-1 antibody, or antigen-binding portion thereof. The antibodies of the invention are preferred for use in the method although other anti-PD-1 antibodies can be used instead (or in combination with an anti-PD-1 antibody of the invention). For example, a chimeric, humanized or fully human anti-PD-1 antibody can be used in the method of treating an infectious disease.

Still further, the invention provides a method of enhancing an immune response to an antigen in a subject, comprising administering to the subject: (i) the antigen; and (ii) an anti-PD-1 antibody, or antigen-binding portion thereof, such that an immune response to the antigen in the subject is enhanced. The antigen can be, for example, a tumor antigen, a viral antigen, a bacterial antigen or an antigen from a pathogen. The antibodies of the invention are preferred for use in the method although other anti-PD-1 antibodies can be used instead (or in combination with an anti-PD-1 antibody of the invention). For example, a chimeric, humanized or fully human anti-PD-1 antibody can be used in the method of enhancing an immune response to an antigen in a subject.

The invention also provides methods for making "second generation" anti-PD-1 antibodies based on the sequences of the anti-PD-1 antibodies provided herein. For example, the invention provides a method for preparing an anti-PD-1 antibody comprising:

(a) providing: (i) a heavy chain variable region antibody sequence comprising a CDR1 sequence that is selected from the group consisting of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21, and/or a CDR2 sequence that is selected from the group consisting of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28; and/or a CDR3 sequence that is selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35; or (ii) a light chain variable region antibody sequence comprising a CDR1 sequence that is selected from the group consisting of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42, and/or a CDR2 sequence that is selected from the group consisting of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49, and/or a CDR3 sequence that is selected from the group consisting of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56;

(b) altering at least one amino acid residue within at least one variable region antibody sequence, said sequence being selected from the heavy chain variable region antibody sequence and the light chain variable region antibody sequence, to create at least one altered antibody sequence; and

(c) expressing the altered antibody sequence as a protein.

Other features and advantages of the instant invention will be apparent from the following detailed description and examples which should not be construed as limiting. The contents of all references, GenBank entries, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

Brief Description of the Drawings

Figure 1A shows the nucleotide sequence (SEQ ID NO: 57) and amino acid sequence (SEQ ID NO: 1) of the heavy chain variable region of the 17D8 human monoclonal antibody. The CDR1 (SEQ ID NO: 15), CDR2 (SEQ ID NO: 22) and CDR3 (SEQ ID NO: 29) regions are delineated and the V, D and J germline derivations are indicated.

Figure 1B shows the nucleotide sequence (SEQ ID NO: 64) and amino acid sequence (SEQ ID NO: 8) of the light chain variable region of the 17D8 human monoclonal antibody. The CDR1 (SEQ ID NO: 36), CDR2 (SEQ ID NO: 43) and CDR3 (SEQ ID NO: 50) regions are delineated and the V and J germline derivations are indicated.

Figure 2A shows the nucleotide sequence (SEQ ID NO: 58) and amino acid sequence (SEQ ID NO: 2) of the heavy chain variable region of the 2D3 human monoclonal antibody. The CDR1 (SEQ ID NO: 16), CDR2 (SEQ ID NO: 23) and CDR3 (SEQ ID NO: 30) regions are delineated and the V and J germline derivations are indicated.

Figure 2B shows the nucleotide sequence (SEQ ID NO: 65) and amino acid sequence (SEQ ID NO: 9) of the light chain variable region of the 2D3 human monoclonal antibody. The CDR1 (SEQ ID NO: 37), CDR2 (SEQ ID NO: 44) and CDR3 (SEQ ID NO: 51) regions are delineated and the V and J germline derivations are indicated.

Figure 3A shows the nucleotide sequence (SEQ ID NO: 59) and amino acid sequence (SEQ ID NO: 3) of the heavy chain variable region of the 4H1 human monoclonal antibody. The CDR1 (SEQ ID NO: 17), CDR2 (SEQ ID NO: 24) and CDR3 (SEQ ID NO: 31) regions are delineated and the V and J germline derivations are indicated.

Figure 3B shows the nucleotide sequence (SEQ ID NO: 66) and amino acid sequence (SEQ ID NO: 10) of the light chain variable region of the 4H1 human monoclonal antibody. The CDR1 (SEQ ID NO: 38), CDR2 (SEQ ID NO: 45) and CDR3 (SEQ ID NO: 52) regions are delineated and the V and J germline derivations are indicated.

Figure 4A shows the nucleotide sequence (SEQ ID NO: 60) and amino acid sequence (SEQ ID NO: 4) of the heavy chain variable region of the 5C4 human monoclonal antibody. The CDR1 (SEQ ID NO: 18), CDR2 (SEQ ID NO: 25) and CDR3 (SEQ ID NO: 32) regions are delineated and the V and J germline derivations are indicated.

Figure 4B shows the nucleotide sequence (SEQ ID NO: 67) and amino acid sequence (SEQ ID NO: 11) of the light chain variable region of the 5C4 human monoclonal antibody. The CDR1 (SEQ ID NO: 39), CDR2 (SEQ ID NO: 46) and CDR3 (SEQ ID NO: 53) regions are delineated and the V and J germline derivations are indicated.

Figure 5A shows the nucleotide sequence (SEQ ID NO: 61) and amino acid sequence (SEQ ID NO: 5) of the heavy chain variable region of the 4A11 human monoclonal antibody. The CDR1 (SEQ ID NO: 19), CDR2 (SEQ ID NO: 26) and CDR3 (SEQ ID NO: 33) regions are delineated and the V and J germline derivations are indicated.

Figure 5B shows the nucleotide sequence (SEQ ID NO: 68) and amino acid sequence (SEQ ID NO: 12) of the light chain variable region of the 4A11 human monoclonal antibody. The CDR1 (SEQ ID NO: 40), CDR2 (SEQ ID NO: 47) and CDR3 (SEQ ID NO: 54) regions are delineated and the V and J germline derivations are indicated.

Figure 6A shows the nucleotide sequence (SEQ ID NO: 62) and amino acid sequence (SEQ ID NO: 6) of the heavy chain variable region of the 7D3 human monoclonal antibody. The CDR1 (SEQ ID NO: 20), CDR2 (SEQ ID NO: 27) and CDR3 (SEQ ID NO: 34) regions are delineated and the V and J germline derivations are indicated.

Figure 6B shows the nucleotide sequence (SEQ ID NO: 69) and amino acid sequence (SEQ ID NO: 13) of the light chain variable region of the 7D3 human monoclonal antibody. The CDR1 (SEQ ID NO: 41), CDR2 (SEQ ID NO: 48) and CDR3 (SEQ ID NO: 55) regions are delineated and the V and J germline derivations are indicated.

Figure 7A shows the nucleotide sequence (SEQ ID NO: 63) and amino acid sequence (SEQ ID NO: 7) of the heavy chain variable region of the 5F4 human monoclonal antibody. The CDR1 (SEQ ID NO: 21), CDR2 (SEQ ID NO: 28) and CDR3 (SEQ ID NO: 35) regions are delineated and the V and J germline derivations are indicated.

Figure 7B shows the nucleotide sequence (SEQ ID NO: 70) and amino acid sequence (SEQ ID NO: 14) of the light chain variable region of the 5F4 human monoclonal antibody. The CDR1 (SEQ ID NO: 42), CDR2 (SEQ ID NO: 49) and CDR3 (SEQ ID NO: 56) regions are delineated and the V and J germline derivations are indicated.

Figure 8 shows the alignment of the amino acid sequence of the heavy chain variable region of 17D8, 2D3, 4H1, 5C4 and 7D3 with the human germline V_H 3-33 amino acid sequence (SEQ ID NO: 71).

Figure 9 shows the alignment of the amino acid sequence of the light chain variable region of 17D8, 2D3 and 7D3 with the human germline V_k L6 amino acid sequence (SEQ ID NO: 73).

Figure 10 shows the alignment of the amino acid sequence of the light chain variable region of 4H1 and 5C4 with the human germline V_k L6 amino acid sequence (SEQ ID NO: 73).

Figure 11 shows the alignment of the amino acid sequence of the heavy chain variable region of 4A11 and 5F4 with the human germline V_H 4-39 amino acid sequence (SEQ ID NO: 72).

Figure 12 shows the alignment of the amino acid sequence of the light chain variable region of 4A11 and 5F4 with the human germline V_k L15 amino acid sequence (SEQ ID NO: 74).

Figures 13A-13B show the results of flow cytometry experiments demonstrating that the human monoclonal antibodies 5C4 and 4H1, directed against human PD-1, binds the cell surface of CHO cells transfected with full-length human PD-1. Figure 13A shows to the flow cytometry plot for 5C4. Figure 13B shows the flow cytometry plot for 4H1. Thin line represents the binding to CHO cells and solid line represents the binding to CHO hPD-1 cells.

Figure 14 shows a graph demonstrating that the human monoclonal antibodies 17D8, 2D3, 4H1, 5C4, and 4A11, directed against human PD-1, bind specifically to PD-1, and not to other members of the CD28 family.

Figures 15A-15C show the results of flow cytometry experiments demonstrating that the human monoclonal antibodies 4H1 and 5C4, directed against human PD-1, binds to PD-1 on the cell surface. Figure 15A shows binding to activated human T-cells. Figure 15B shows the binding to cynomolgous monkey T-cells. Figure 15C shows the binding to CHO transfected cells expressing PD-1.

Figures 16A-16C show the results of experiments demonstrating that human monoclonal antibodies against human PD-1 promote T-cell proliferation, IFN-gamma secretion and IL-2 secretion in a mixed lymphocyte reaction assay. Figure 16A is a bar graph showing concentration dependent T-cell proliferation; Figure 16B is a bar graph showing concentration dependent IFN-gamma secretion; Figure 16C is a bar graph showing concentration dependent IL-2 secretion.

Figures 17A-17B show the results of flow cytometry experiments demonstrating that human monoclonal antibodies against human PD-1 block the binding of PD-L1 and PD-L2 to CHO transfected cells expressing PD-1. Figure 17A is a graph showing inhibition of binding of PD-L1; Figure 17B is a graph showing inhibition of binding of PD-L2.

Figure 18 shows the results of flow cytometry experiments demonstrating that human monoclonal antibodies against human PD-1 do not promote T-cell apoptosis.

Figure 19 shows the results of experiments demonstrating that anti-PD-1 HuMabs have a concentration dependent effect on IFN gamma secretion by PBMCs from CMV-positive donors when PBMCs were stimulated with a CMV lysate and anti-PD-1.

Figure 20 shows the results of tumor growth experiments in a mouse model system demonstrating that treatment *in vivo* of mouse tumors with anti-PD-1 antibodies inhibits the growth of tumors.

Figures 21A to 21D show the tumor volume over time in individual mice that were implanted with MC38 colon tumor cells (PD-L1$^-$) and on the same day treated with one of the

following therapies: (A) mouse IgG (control), (B) anti-CTLA-4 antibody, (C) anti-PD-1 antibody, and (D) anti-CTLA-4 antibody and anti-PD-1 antibody. The mice received subsequent antibody treatments on days 3, 6 and 10 as described in Example 13 and tumor volume was monitored over 60 days.

Figure 22 shows the mean tumor volume of the mice shown in Figure 21.

Figure 23 shows the median tumor volume of the mice shown in Figure 21.

Figures 24A to 24D show the tumor volume over time in individual mice that were implanted with MC38 colon tumor cells (PD-L1$^-$) and one week later treated with one of the following therapies: (A) mouse IgG (control), (B) anti-CTLA-4 antibody, (C) anti-PD-1 antibody, and (D) anti-CTLA-4 antibody and anti-PD-1 antibody. The tumor volume on the first day of treatment was about 315 mm^3. The mice received subsequent antibody treatments on days 3, 6 and 10 as described in Example 14.

Figure 25 shows the mean tumor volume of the mice shown in Figure 24.

Figure 26 shows the median tumor volume of the mice shown in Figure 24.

Figure 27 shows the mean tumor volume over time in individual mice that were implanted with MC38 colon tumor cells (PD-L1$^-$) (day -7) and then treated on days 0, 3, 6 and 10 post-implantation (as described in Example 15) with one of the following therapies: (A) mouse IgG as a control (20 mg/kg, X_{20}) (B) anti-PD-1 antibody (10 mg/kg) and mouse IgG (10 mg/kg) ($P_{10}X_{10}$), (C) anti-CTLA-4 antibody (10 mg/kg) and mouse IgG (10 mg/kg) ($C_{10}X_{10}$), (D) anti-CTLA-4 antibody and anti-PD-1 antibody (10 mg/kg each) ($C_{10}P_{10}$), (E) anti-CTLA-4 antibody and anti-PD-1 antibody (3 mg/kg each) (C_3P_3), and (F) anti-CTLA-4 antibody and anti-PD-1 antibody (1 mg/kg each) (C_1P_1). Two groups of mice were treated with each antibody sequentially as follows: (G) anti-CTLA-4 antibody (10 mg/kg, day 0), anti-CTLA-4 antibody (10 mg/kg, day 3), anti-PD-1 antibody (10 mg/kg, day 6), and anti-PD-1 antibody (10 mg/kg, day 10) ($C_{10}C_{10}P_{10}P_{10}$); and (H) anti-PD-1 antibody (10 mg/kg, day 0), anti-PD-1 antibody (10 mg/kg, day 3), anti-CTLA-4 antibody (10 mg/kg, day 6), and anti-CTLA-4 antibody (10 mg/kg, day 10) (10 mg/kg, day 10) ($P_{10}P_{10}C_{10}C_{10}$).

Figure 28 shows the mean tumor volume of the mice shown in Figure 27.

Figure 29 shows the median tumor volume of the mice shown in Figure 27.

Figures 30A to 30F show the tumor volume over time in individual mice that were implanted with SA1/N fibrosarcoma cells (PD-L1$^-$) and one day later treated with one of the following therapies: (A) PBS (vehicle control), (B) mouse IgG (antibody control, 10 mg/kg), (C) anti-PD-1 antibody (10 mg/kg), (D) anti-CTLA-4 antibody (10 mg/kg), (E) anti-CTLA-4 antibody (0.2 mg/kg), and (F) anti-PD-1 antibody (10 mg/kg) and anti-CTLA-4 antibody (0.2 mg/kg). The mice received subsequent antibody treatments on days 4, 7 and 11 as described in Example 16 and tumor volume was monitored over 41 days.

Figure 31 shows the mean tumor volume of the mice shown in Figure 29.

Figure 32 shows the median tumor volume of the mice shown in Figure 29.

Figures 33A to 33J show the tumor volume over time in individual mice that were implanted with SA1/N fibrosarcoma cells (PD-L1$^-$) and then treated on days 7, 10, 13 and 17 post-implantation (as described in Example 17) with one of the following therapies: (A) PBS (vehicle control), (B) mouse IgG (antibody control, 10 mg/kg), (C) anti-CTLA-4 antibody (0.25 mg/kg), (D) anti-CTLA-4 antibody (0.5 mg/kg), (E) anti-CTLA-4 antibody (5 mg/kg), (F) anti-PD-1 antibody (3 mg/kg), (G) anti-PD-1 antibody (10 mg/kg), (H) anti-PD-1 antibody (10 mg/kg) and anti-CTLA-4 antibody (0.25 mg/kg), (I) anti-PD-1 antibody (10 mg/kg) and anti-CTLA-4 antibody (0.5 mg/kg), and (F) anti-PD-1 antibody (3 mg/kg) and anti-CTLA-4 antibody (0.5 mg/kg). The tumor volume on the first day of treatment was about 110 mm^3.

Figure 34 shows the mean tumor volume of the mice shown in Figure 33.

Figure 35 shows the median tumor volume of the mice shown in Figure 33.

Figures 36A and 36B show the tumor volume over time in individual mice that were implanted with SA1/N fibrosarcoma cells (PD-L1$^-$) and then treated on days 10, 13, 16 and 19 post-implantation (as described in Example 17) with one of the following therapies: (A) mouse IgG (antibody control, 10 mg/kg) or (B) anti-PD-1 antibody (10 mg/kg) and anti-CTLA-4 antibody (1 mg/kg). The tumor volume on the first day of treatment was about 250 mm^3.

Figure 37 shows the mean tumor volume of the mice shown in Figure 36.

Figure 38 shows the median tumor volume of the mice shown in Figure 36.

Figure 39 shows the mean and median percent tumor inhibition calculated from the tumor volumes shown in Figures 33 and 36.

Figures 40A to 40D show the tumor volume in BALB/c mice that were implanted subcutaneously with RENCA renal adenocarcinoma cells (PD-L1$^+$) (Murphy and Hrushesky (1973) *J. Nat'l. Cancer Res. 50*:1013-1025) (day -12) and then treated intraperitoneally on days 0, 3, 6 and 9 post-implantation with one of the following therapies: (A) mouse IgG (antibody control, 20 mg/kg), (B) anti-PD-1 antibody (10 mg/kg), (C) anti-CTLA-4 antibody (10 mg/kg), and (D) anti-PD-1 antibody (10 mg/kg) in combination with anti-CTLA-4 antibody (10 mg/kg). The tumor volume on the first day of treatment was about 115 mm^3.

Figure 41 shows binding of mouse PD-L2-Fc fusion protein to mouse PD-1 (mPD-1) is blocked by anti-mPD-1 antibody 4H2 in a dose dependent manner. The binding is detected by measuring fluorescence of FITC-labeled donkey-anti-rat IgG by ELISA. The greater the MFI (mean fluorescence intensity) the greater the binding.

Figure 42 shows binding curves of anti-mPD-1 antibodies to immobilized mPD-1-Fc fusion protein by ELISA.

Figure 43 shows the binding curve of rat anti-mPD-1 antibody 4H2.B3 to mPD-1-expressing CHO cells. Binding was detected with donkey-anti-rat IgG, FITC conjugated and measured by FACS (MFI).

Figure 44 shows the binding curve of mPD-L1-hFc fusion protein to mPD-1-expressing CHO cells in the presence of increasing concentrations of anti-mPD-1 antibody

4H2.B3. Binding was detected with goat–anti–human IgG, FITC conjugated and measured by FACS (MFI).

Figure 45 shows the binding curves of rat anti-mPD-1 antibody 4H2.B3 to mPD-1-expressing CHO cells as compared to chimeric rat:mouse anti-mPD-1 antibody 4H2.

Figure 46 shows the binding curves of mPD-L1-hFc fusion protein to mPD-1-expressing CHO cells in the presence of increasing concentrations of either rat anti-mPD-1 antibody 4H2.B3 or chimeric rat:mouse anti-mPD-1 antibody 4H2.

Figure 47 shows the mean tumor volume of tumor-free mice previously treated with anti-PD1 antibody and re-challenged with SA1/N fibrosarcoma cells (PD-L1$^-$). Also shown is the mean tumor volume of naïve mice (control, not previously challenged or treated) implanted with SA1/N fibrosarcoma cells.

Figure 48 shows the tumor volume over time in individual mice, which survived tumor-free following implantation of MC38 colon tumor cells (PD-L1$^-$) and treatment with anti-PD1 antibody or a combination of anti-PD1 antibody with anti-CTLA-4 antibody), re-challenged with 10x more MC38 colon tumor cells than the initial treatment. Also shown is the mean tumor volume of naïve mice (control, not previously challenged or treated) implanted with MC38 colon tumor cells.

Figure 49 shows the mean tumor volume of the mice shown in Figure 48.

Figure 50 shows the mean tumor volume over time in individual mice that were implanted with CT26 colon tumor cells.

Figures 51A-B shows the results of experiments demonstrating that human monoclonal antibodies against human PD-1 promote T-cell proliferation and IFN-gamma secretion in cultures containing T regulatory cells. Figure 50A is a bar graph showing concentration dependent T-cell proliferation using HuMAb 5C4; Figure 50B is a bar graph showing concentration dependent IFN-gamma secretion using HuMAb 5C4.

Figures 52A-B shows the results of experiments demonstrating that human monoclonal antibodies against human PD-1 promote T-cell proliferation and IFN-gamma secretion in cultures containing activated T cells. Figure 51A is a bar graph showing concentration dependent T-cell proliferation using HuMAb 5C4; Figure 51B is a bar graph showing concentration dependent IFN-gamma secretion using HuMAb 5C4.

Figure 53 shows the results of an antibody dependent cellular cytotoxicity (ADCC) assay demonstrating that human monoclonal anti-PD-1 antibodies kill human activated T cells in an ADCC concentration-dependent manner in relation to the Fc region of the anti-PD-1 antibody.

Figure 54 shows the results of a complement dependent cytotoxicity (CDC) assay demonstrating that human monoclonal anti-PD-1 antibodies do not kill human activated T cells in a CDC concentration-dependent manner.

Best Mode for Carrying Out the Invention

In one aspect, the present invention relates to isolated monoclonal antibodies, particularly human monoclonal antibodies, that bind specifically to PD-1. In certain embodiments, the antibodies of the invention exhibit one or more desirable functional properties, such as high affinity binding to PD-1, lack of cross-reactivity to other CD28 family members, the ability to stimulate T cell proliferation, IFN-γ and/or IL-2 secretion in mixed lymphocyte reactions, the ability to inhibit binding of one or more PD-1 ligands (*e.g.*, PD-L1 and/or PD-L2), the ability to cross-react with cynomolgus monkey PD-1, the ability to stimulate antigen-specific memory responses, the ability to stimulate antibody responses and/or the ability to inhibit growth of tumor cells *in vivo*. Additionally or alternatively, the antibodies of the invention are derived from particular heavy and light chain germline sequences and/or comprise particular structural features such as CDR regions comprising particular amino acid sequences. In another aspect, the invention relates to the combined use of monoclonal antibodies that bind specifically to PD-1 and monoclonal antibodies that bind specifically to CTLA-4.

The invention provides, for example, isolated antibodies, methods of making such antibodies, immunoconjugates and bispecific molecules comprising such antibodies and pharmaceutical compositions containing the antibodies, immunconjugates or bispecific molecules of the invention.

In another aspect, the invention pertains to methods of inhibiting growth of tumor cells in a subject using anti-PD-1 antibodies. As demonstrated herein, anti-PD-1 antibodies are capable of inhibiting tumor cell growth *in vivo*. The invention also relates to methods of using the antibodies to modify an immune response, as well as to treat diseases such as cancer or infectious disease, or to stimulate a protective autoimmune response or to stimulate antigen-specific immune responses (e.g., by coadministration of anti-PD-1 with an antigen of interest).

In order that the present invention may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

The terms "Programmed Death 1," "Programmed Cell Death 1," "Protein PD-1," "PD-1," PD1," "PDCD1," "hPD-1" and "hPD-I" are used interchangeably, and include variants, isoforms, species homologs of human PD-1, and analogs having at least one common epitope with PD-1. The complete PD-1 sequence can be found under GenBank Accession No. U64863.

The terms "cytotoxic T lymphocyte-associated antigen-4," "CTLA-4," "CTLA4," "CTLA-4 antigen" and "CD152" (*see, e.g.*, Murata, *Am. J. Pathol.* (1999) 155:453-460) are used interchangeably, and include variants, isoforms, species homologs of human CTLA-4, and analogs having at least one common epitope with CTLA-4 (*see, e.g.*, Balzano (1992) *Int. J. Cancer Suppl.* 7:28-32). The complete CTLA-4 nucleic acid sequence can be found under GenBank Accession No. L15006.

The term "immune response" refers to the action of, for example, lymphocytes, antigen presenting cells, phagocytic cells, granulocytes, and soluble macromolecules produced by the above cells or the liver (including antibodies, cytokines, and complement) that results in selective damage to, destruction of, or elimination from the human body of invading pathogens, cells or tissues infected with pathogens, cancerous cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

A "signal transduction pathway" refers to the biochemical relationship between a variety of signal transduction molecules that play a role in the transmission of a signal from one portion of a cell to another portion of a cell. As used herein, the phrase "cell surface receptor" includes, for example, molecules and complexes of molecules capable of receiving a signal and the transmission of such a signal across the plasma membrane of a cell. An example of a "cell surface receptor" of the present invention is the PD-1 receptor.

The term "antibody" as referred to herein includes whole antibodies and any antigen-binding fragment (*i.e.*, "antigen-binding portion") or single chains thereof. An "antibody" refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen-binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, C_{H1}, C_{H2} and C_{H3}. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system.

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, PD-1). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_{H1} domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_{H1} domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a

V_H domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); *see e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds PD-1 is substantially free of antibodies that specifically bind antigens other than PD-1). An isolated antibody that specifically binds PD-1 may, however, have cross-reactivity to other antigens, such as PD-1 molecules from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

The term "human antibody", as used herein, is intended to include antibodies having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "human monoclonal antibody" refers to antibodies displaying a single binding specificity which have variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. In one embodiment, the human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic nonhuman animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

The term "recombinant human antibody", as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies

isolated from an animal (*e.g.*, a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom (described further below), (b) antibodies isolated from a host cell transformed to express the human antibody, *e.g.*, from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial human antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable regions in which the framework and CDR regions are derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

As used herein, "isotype" refers to the antibody class (*e.g.*, IgM or IgGl) that is encoded by the heavy chain constant region genes.

The phrases "an antibody recognizing an antigen" and "an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen."

The term "human antibody derivatives" refers to any modified form of the human antibody, *e.g.*, a conjugate of the antibody and another agent or antibody.

The term "humanized antibody" is intended to refer to antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Additional framework region modifications may be made within the human framework sequences.

The term "chimeric antibody" is intended to refer to antibodies in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

As used herein, an antibody that "specifically binds to human PD-1" is intended to refer to an antibody that binds to human PD-1 with a K_D of 1×10^{-7} M or less, more preferably 5×10^{-8} M or less, more preferably 1×10^{-8} M or less, more preferably 5×10^{-9} M or less.

The term "K_{assoc}" or "K_a", as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term "K_{dis}" or "K_d," as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term "K_D", as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of K_d to K_a (*i.e*,. K_d/K_a) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art. A preferred method for determining the K_D of an antibody is by using surface plasmon resonance, preferably using a biosensor system such as a Biacore® system.

As used herein, the term "high affinity" for an IgG antibody refers to an antibody having a K_D of 10^{-8} M or less, more preferably 10^{-9} M or less and even more preferably 10^{-10} M or less for a target antigen. However, "high affinity" binding can vary for other antibody isotypes. For example, "high affinity" binding for an IgM isotype refers to an antibody having a K_D of 10^{-7} M or less, more preferably 10^{-8} M or less, even more preferably 10^{-9} M or less.

The term "treatment" or "therapy" refers to administering an active agent with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect a condition (*e.g.*, a disease), the symptoms of the condition, or to prevent or delay the onset of the symptoms, complications, biochemical indicia of a disease, or otherwise arrest or inhibit further development of the disease, condition, or disorder in a statistically significant manner.

An "adverse event" (AE) as used herein is any unfavorable and generally unintended, even undesirable, sign (including an abnormal laboratory finding), symptom, or disease associated with the use of a medical treatment. For example, an adverse event may be associated with activation of the immune system or expansion of immune system cells (*e.g.*, T cells) in response to a treatment. A medical treatment may have one or more associated AEs and each AE may have the same or different level of severity. Reference to methods capable of "altering adverse events" means a treatment regime that decreases the incidence and/or severity of one or more AEs associated with the use of a different treatment regime.

As used herein, "hyperproliferative disease" refers to conditions wherein cell growth is increased over normal levels. For example, hyperproliferative diseases or disorders include malignant diseases (*e.g.*, esophageal cancer, colon cancer, biliary cancer) and non-malignant diseases (*e.g.*, atherosclerosis, benign hyperplasia, benign prostatic hypertrophy).

As used herein, "subtherapeutic dose" means a dose of a therapeutic compound (*e.g.*, an antibody) that is lower than the usual or typical dose of the therapeutic compound when administered alone for the treatment of a hyperproliferative disease (*e.g.*, cancer). For example, a subtherapeutic dose of CTLA-4 antibody is a single dose of the antibody at less than about 3 mg/kg, *i.e.*, the known dose of anti-CTLA-4 antibody.

The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the indefinite articles "a" or "an" should be understood to refer to "one or more" of any recited or enumerated component.

As used herein, "about" or "comprising essentially of" mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" or "comprising essentially of" can mean within 1 or more than 1 standard deviation per the practice in the art. Alternatively, "about" or "comprising essentially of" can mean a range of up to 20%. Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values are provided in the application and claims,

unless otherwise stated, the meaning of "about" or "comprising essentially of" should be assumed to be within an acceptable error range for that particular value.

As described herein, any concentration range, percentage range, ratio range or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated.

As used herein, the term "subject" includes any human or nonhuman animal. The term "nonhuman animal" includes all vertebrates, *e.g.*, mammals and non-mammals, such as nonhuman primates, sheep, dogs, cats, horses, cows chickens, amphibians, reptiles, *etc.* Except when noted, the terms "patient" or "subject" are used interchangeably.

Various aspects of the invention are described in further detail in the following subsections.

Anti-PD-1 Antibodies

The antibodies of the invention are characterized by particular functional features or properties of the antibodies. For example, the antibodies bind specifically to PD-1 (e.g., bind to human PD-1 and may cross-react with PD-1 from other species, such as cynomolgus monkey). Preferably, an antibody of the invention binds to PD-1 with high affinity, for example with a K_D of 1×10^{-7} M or less. The anti-PD-1 antibodies of the invention preferably exhibit one or more of the following characteristics:

(a) binds to human PD-1 with a K_D of 1×10^{-7} M or less;

(b) does not substantially bind to human CD28, CTLA-4 or ICOS;

(c) increases T-cell proliferation in an Mixed Lymphocyte Reaction (MLR) assay;

(d) increases interferon-gamma production in an MLR assay;

(e) increases IL-2 secretion in an MLR assay;

(f) binds to human PD-1 and cynomolgus monkey PD-1;

(g) inhibits the binding of PD-L1 and/or PD-L2 to PD-1;

(h) stimulates antigen-specific memory responses;

(i) stimulates antibody responses;

(j) inhibits tumor cell growth *in vivo*.

Preferably, the antibody binds to human PD-1 with a K_D of 5×10^{-8} M or less, binds to human PD-1 with a K_D of 1×10^{-8} M or less, binds to human PD-1 with a K_D of 5×10^{-9} M or less, or binds to human PD-1 with a K_D of between 1×10^{-8} M and 1×10^{-10} M or less.

An antibody of the invention may exhibit any combination of the above-listed features, such as two, three, four, five or more of the above-listed features.

Standard assays to evaluate the binding ability of the antibodies toward PD-1 are known in the art, including for example, ELISAs, Western blots and RIAs. The binding kinetics (*e.g.*, binding affinity) of the antibodies also can be assessed by standard assays known in the art, such as by Biacore analysis. Suitable assays for evaluating any of the above-described characteristics are described in detail in the Examples.

Monoclonal Antibodies 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4

Preferred antibodies of the invention are the human monoclonal antibodies 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4isolated and structurally characterized as described in Examples 1 and 2. The V_H amino acid sequences of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 1, 2, 3, 4, 5, 6 and 7, respectively. The V_L amino acid sequences of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 8, 9, 10, 11, 12, 13 and 14, respectively.

Given that each of these antibodies can bind to PD-1, the V_H and V_L sequences can be "mixed and matched" to create other anti-PD-1 binding molecules of the invention. PD-1 binding of such "mixed and matched" antibodies can be tested using the binding assays described above and in the Examples (*e.g.*, ELISAs). Preferably, when V_H and V_L chains are mixed and matched, a V_H sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_H sequence. Likewise, preferably a V_L sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_L sequence.

Accordingly, in one aspect, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof comprising:

(a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6 and 7; and

(b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13 and 14;

wherein the antibody specifically binds PD-1, preferably human PD-1.

Preferred heavy and light chain combinations include:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 1; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 2; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 9; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 3; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 10; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 11; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 5; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 12; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 13; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14.

In another aspect, the invention provides antibodies that comprise the heavy chain and light chain CDR1s, CDR2s and CDR3s of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4, or combinations thereof. The amino acid sequences of the V_H CDR1s of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21, respectively. The amino acid sequences of the V_H CDR2s of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28, respectively. The amino acid sequences of the V_H CDR3s of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35, respectively. The amino acid sequences of the V_k CDR1s of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42, respectively. The amino acid sequences of the V_k CDR2s of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49, respectively. The amino acid sequences of the V_k CDR3s of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56, respectively. The CDR regions are delineated using the Kabat system (Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242).

Given that each of these antibodies can bind to PD-1 and that antigen-binding specificity is provided primarily by the CDR1, CDR2, and CDR3 regions, the V_H CDR1, CDR2, and CDR3 sequences and V_k CDR1, CDR2, and CDR3 sequences can be "mixed and matched" (*i.e.*, CDRs from different antibodies can be mixed and match, although each antibody must contain a V_H CDR1, CDR2, and CDR3 and a V_k CDR1, CDR2, and CDR3) to create other anti-PD-1 binding molecules of the invention. PD-1 binding of such "mixed and matched" antibodies can be tested using the binding assays described above and in the Examples (*e.g.*, ELISAs, Biacore analysis). Preferably, when V_H CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular V_H sequence is replaced with a structurally similar CDR sequence(s). Likewise, when V_k CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular V_k sequence preferably is replaced with a structurally similar CDR sequence(s). It will be readily apparent to the ordinarily skilled artisan that novel V_H and V_L sequences can be created by substituting one or more V_H and/or V_L CDR region sequences with structurally similar sequences from the CDR sequences disclosed herein for monoclonal antibodies antibodies 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4.

Accordingly, in another aspect, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof comprising:

(a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21;

(b) a heavy chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28;

(c) a heavy chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35;

(d) a light chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42;

(e) a light chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49; and

(f) a light chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56;

wherein the antibody specifically binds PD-1, preferably human PD-1.

In a preferred embodiment, the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 15;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 22;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 29;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 36;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 43; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 50.

In another preferred embodiment, the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 16;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 23;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 30;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 37;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 44; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 51.

In another preferred embodiment, the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 17;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 24;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 31;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 38;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 45; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 52.

In another preferred embodiment, the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 18;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 25;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 32;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 39;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 46; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 53.

In another preferred embodiment, the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 19;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 26;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 33;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 40;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 47; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 54.

Antibodies Having Particular Germline Sequences

In certain embodiments, an antibody of the invention comprises a heavy chain variable region from a particular germline heavy chain immunoglobulin gene and/or a light chain variable region from a particular germline light chain immunoglobulin gene.

For example, in a preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 3-33 gene, wherein the antibody specifically binds PD-1, preferably human PD-1. In another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 4-39 gene, wherein the antibody specifically binds PD-1, preferably human PD-1. In yet another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L6 gene, wherein the antibody specifically binds PD-1, preferably human PD-1. In yet another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L15 gene, wherein the antibody specifically binds PD-1, preferably human PD-1. In yet another preferred embodiment, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, wherein the antibody:

(a) comprises a heavy chain variable region that is the product of or derived from a human V_H 3-33 or 4-39 gene (which gene encodes the amino acid sequence set forth in SEQ ID NO: 71 or 73, respectively);

(b) comprises a light chain variable region that is the product of or derived from a human V_K L6 or L15 gene (which gene encodes the amino acid sequence set forth in SEQ ID NO: 72 or 74, respectively); and

(c) specifically binds to PD-1.

Examples of antibodies having V_H and V_K of V_H 3-33 and V_K L6, respectively, are 17D8, 2D3, 4H1, 5C4, and 7D3. Examples of antibodies having V_H and V_K of V_H 4-39 and V_K L15, respectively are 4A11 and 5F4.

As used herein, a human antibody comprises heavy or light chain variable regions that is "the product of" or "derived from" a particular germline sequence if the variable regions of the antibody are obtained from a system that uses human germline immunoglobulin genes. Such systems include immunizing a transgenic mouse carrying human immunoglobulin genes with the antigen of interest or screening a human immunoglobulin gene library displayed on phage with the antigen of interest. A human antibody that is "the product of" or "derived from" a human germline immunoglobulin sequence can be identified as such by comparing the amino acid sequence of the human antibody to the amino acid sequences of human germline immunoglobulins and selecting the human germline immunoglobulin sequence that is closest in sequence (*i.e.*, greatest % identity) to the sequence of the human antibody. A human antibody that is "the product of" or "derived from" a particular human germline immunoglobulin sequence may contain amino acid differences as compared to the germline sequence, due to, for example, naturally-occurring somatic mutations or intentional introduction of site-directed mutation. However, a selected human antibody typically is at least 90% identical in amino acids sequence to an amino acid sequence encoded by a human germline immunoglobulin gene and contains amino acid residues that identify the human antibody as being human when compared to the germline immunoglobulin amino acid sequences of other species (*e.g.*, murine germline sequences). In certain cases, a human antibody may be at least 95%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, a human antibody derived from a particular human germline sequence will display no more than 10 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene. In certain cases, the human antibody may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

Homologous Antibodies

In yet another embodiment, an antibody of the invention comprises heavy and light chain variable regions comprising amino acid sequences that are homologous to the amino acid sequences of the preferred antibodies described herein, and wherein the antibodies retain the desired functional properties of the anti-PD-1 antibodies of the invention.

For example, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, comprising a heavy chain variable region and a light chain variable region, wherein:

(a) the heavy chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6 and 7;

(b) the light chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13 and 14; and

the antibody exhibits one or more of the following properties:

(c) the antibody binds to human PD-1 with a K_D of 1 x 10^{-7} M or less;

(d) the antibody does not substantially bind to human CD28, CTLA-4 or ICOS;

(e) the antibody increases T-cell proliferation in an MLR assay;

(f) the antibody increases interferon-gamma production in an MLR assay;

(g) the antibody increases Il-2 secretion in an MLR assay;

(h) the antibody binds to human PD-1 and cynomolgus monkey PD-1;

(i) the antibody inhibits the binding of PD-L1 and/or PD-L2 to PD-1;

(j) the antibody stimulates antigen-specific memory responses;

(k) the antibody stimulates antibody responses;

(l) the antibody inhibits tumor cell growth *in vivo*.

In other embodiments, the V_H and/or V_L amino acid sequences may be 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to the sequences set forth above. An antibody having V_H and V_L regions having high (*i.e.*, 80% or greater) homology to the V_H and V_L regions of the sequences set forth above, can be obtained by mutagenesis (*e.g.*, site-directed or PCR-mediated mutagenesis) of nucleic acid molecules encoding SEQ ID NOs: 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67 , 68, 69 and 70, followed by testing of the encoded altered antibody for retained function (*i.e.*, the functions set forth in (c) through (l) above) using the functional assays described herein.

As used herein, the percent homology between two amino acid sequences is equivalent to the percent identity between the two sequences. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

The percent identity between two amino acid sequences can be determined using the algorithm of E. Meyers and W. Miller (*Comput. Appl. Biosci.*, 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (*J. Mol. Biol.* 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

Additionally or alternatively, the protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the XBLAST program (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST protein searches

can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to the antibody molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. (See www.ncbi.nlm.nih.gov).

Antibodies with Conservative Modifications

In certain embodiments, an antibody of the invention comprises a heavy chain variable region comprising CDR1, CDR2 and CDR3 sequences and a light chain variable region comprising CDR1, CDR2 and CDR3 sequences, wherein one or more of these CDR sequences comprise specified amino acid sequences based on the preferred antibodies described herein (*e.g.*, 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4), or conservative modifications thereof, and wherein the antibodies retain the desired functional properties of the anti-PD-1 antibodies of the invention. Accordingly, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, comprising a heavy chain variable region comprising CDR1, CDR2, and CDR3 sequences and a light chain variable region comprising CDR1, CDR2, and CDR3 sequences, wherein:

(a) the heavy chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35, and conservative modifications thereof;

(b) the light chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequence of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56, and conservative modifications thereof; and

the antibody exhibits one or more of the following properties:

(c) the antibody binds to human PD-1 with a K_D of 1 x 10^{-7} M or less;

(d) the antibody does not substantially bind to human CD28, CTLA-4 or ICOS;

(e) the antibody increases T-cell proliferation in an MLR assay;

(f) the antibody increases interferon-gamma production in an MLR assay;

(g) the antibody increases Il-2 secretion in an MLR assay;

(h) the antibody binds to human PD-1 and cynomolgus monkey PD-1;

(i) the antibody inhibits the binding of PD-L1 and/or PD-L2 to PD-1;

(j) the antibody stimulates antigen-specific memory responses;

(k) the antibody stimulates antibody responses;

(l) the antibody inhibits tumor cell growth *in vivo*.

In a preferred embodiment, the heavy chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28, and conservative modifications thereof; and the light chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49, and

conservative modifications thereof. In another preferred embodiment, the heavy chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21, and conservative modifications thereof; and the light chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42, and conservative modifications thereof.

As used herein, the term "conservative sequence modifications" is intended to refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into an antibody of the invention by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within the CDR regions of an antibody of the invention can be replaced with other amino acid residues from the same side chain family and the altered antibody can be tested for retained function (*i.e.*, the functions set forth in (c) through (l) above) using the functional assays described herein.

Antibodies that Bind to the Same Epitope as Anti-PD-1 Antibodies of the Invention

In another embodiment, the invention provides antibodies that bind to the same epitope on human PD-1 as any of the PD-1 monoclonal antibodies of the invention (*i.e.*, antibodies that have the ability to cross-compete for binding to PD-1 with any of the monoclonal antibodies of the invention). In preferred embodiments, the reference antibody for cross-competition studies can be the monoclonal antibody 17D8 (having V_H and V_L sequences as shown in SEQ ID NOs: 1 and 8, respectively), or the monoclonal antibody 2D3 (having V_H and V_L sequences as shown in SEQ ID NOs: 2 and 9, respectively), or the monoclonal antibody 4H1 (having V_H and V_L sequences as shown in SEQ ID NOs: 3 and 10, respectively), or the monoclonal antibody 5C4 (having V_H and V_L sequences as shown in SEQ ID NOs: 4 and 11, respectively), or the monoclonal antibody 4A11 (having V_H and V_L sequences as shown in SEQ ID NOs: 5 and 12, or the monoclonal antibody 7D3 (having V_H and V_L sequences as shown in SEQ ID NOs: 6 and 13, or the monoclonal antibody 5F4 (having V_H and V_L sequences as shown in SEQ ID NOs: 7 and 14, respectively). Such cross-competing antibodies can be identified based on their ability to cross-compete with 17D8, 2D3, 4H1, 5C4,

4A11, 7D3 or 5F4 in standard PD-1 binding assays. For example, BIAcore analysis, ELISA assays or flow cytometry may be used to demonstrate cross-competition with the antibodies of the current invention. The ability of a test antibody to inhibit the binding of, for example, 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4, to human PD-1 demonstrates that the test antibody can compete with 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4 for binding to human PD-1 and thus binds to the same epitope on human PD-1 as 17D8, 2D3, 4H1, 5C4, or 4A11. In a preferred embodiment, the antibody that binds to the same epitope on human PD-1 as 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4 is a human monoclonal antibody. Such human monoclonal antibodies can be prepared and isolated as described in the Examples.

Engineered and Modified Antibodies

An antibody of the invention further can be prepared using an antibody having one or more of the V_H and/or V_L sequences disclosed herein as starting material to engineer a modified antibody, which modified antibody may have altered properties from the starting antibody. An antibody can be engineered by modifying one or more residues within one or both variable regions (*i.e.*, V_H and/or V_L), for example within one or more CDR regions and/or within one or more framework regions. Additionally or alternatively, an antibody can be engineered by modifying residues within the constant region(s), for example to alter the effector function(s) of the antibody.

One type of variable region engineering that can be performed is CDR grafting. Antibodies interact with target antigens predominantly through amino acid residues that are located in the six heavy and light chain complementarity determining regions (CDRs). For this reason, the amino acid sequences within CDRs are more diverse between individual antibodies than sequences outside of CDRs. Because CDR sequences are responsible for most antibody-antigen interactions, it is possible to express recombinant antibodies that mimic the properties of specific naturally occurring antibodies by constructing expression vectors that include CDR sequences from the specific naturally occurring antibody grafted onto framework sequences from a different antibody with different properties (see, *e.g.*, Riechmann, L. *et al.* (1998) *Nature* 332:323-327; Jones, P. *et al.* (1986) *Nature* 321:522-525; Queen, C. *et al.* (1989) *Proc. Natl. Acad. See. U.S.A.* 86:10029-10033; U.S. Patent No. 5,225,539 to Winter, and U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen *et al.*)

Accordingly, another embodiment of the invention pertains to an isolated monoclonal antibody, or antigen-binding portion thereof, comprising a heavy chain variable region comprising CDR1, CDR2, and CDR3 sequences comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21, SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28, and SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35, respectively, and a light chain variable region comprising CDR1, CDR2, and CDR3 sequences comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42, SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49, and SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56, respectively. Thus, such antibodies contain the V_H and V_L CDR sequences of

monoclonal antibodies 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4 yet may contain different framework sequences from these antibodies.

Such framework sequences can be obtained from public DNA databases or published references that include germline antibody gene sequences. For example, germline DNA sequences for human heavy and light chain variable region genes can be found in the "VBase" human germline sequence database (available on the Internet at www.mrc-cpe.cam.ac.uk/vbase), as well as in Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Tomlinson, I. M., *et al.* (1992) "The Repertoire of Human Germline V_H Sequences Reveals about Fifty Groups of V_H Segments with Different Hypervariable Loops" *J. Mol. Biol.* 227:776-798; and Cox, J. P. L. *et al.* (1994) "A Directory of Human Germ-line V_H Segments Reveals a Strong Bias in their Usage" *Eur. J. Immunol.* 24:827-836; the contents of each of which are expressly incorporated herein by reference. As another example, the germline DNA sequences for human heavy and light chain variable region genes can be found in the GenBank database. For example, the following heavy chain germline sequences found in the HCo7 HuMAb mouse are available in the accompanying GenBank accession numbers: 1-69 (NG_0010109, NT_024637 and BC070333), 3-33 (NG_0010109 and NT_024637) and 3-7 (NG_0010109 and NT_024637). As another example, the following heavy chain germline sequences found in the HCo12 HuMAb mouse are available in the accompanying GenBank accession numbers: 1-69 (NG_0010109, NT_024637 and BC070333), 5-51 (NG_0010109 and NT_024637), 4-34 (NG_0010109 and NT_024637), 3-30.3 (AJ556644) and 3-23 (AJ406678).

Preferred framework sequences for use in the antibodies of the invention are those that are structurally similar to the framework sequences used by selected antibodies of the invention, *e.g.*, similar to the V_H 3-33 framework sequences (SEQ ID NO: 71) and/or the V_H 4-39 framework sequences (SEQ ID NO: 73) and/or the V_K L6 framework sequences (SEQ ID NO: 72) and/or the V_K L15 framework sequences (SEQ ID NO: 74) used by preferred monoclonal antibodies of the invention. The V_H CDR1, CDR2, and CDR3 sequences, and the V_K CDR1, CDR2, and CDR3 sequences, can be grafted onto framework regions that have the identical sequence as that found in the germline immunoglobulin gene from which the framework sequence derive, or the CDR sequences can be grafted onto framework regions that contain one or more mutations as compared to the germline sequences. For example, it has been found that in certain instances it is beneficial to mutate residues within the framework regions to maintain or enhance the antigen binding ability of the antibody (see *e.g.*, U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen *et al*).

Another type of variable region modification is to mutate amino acid residues within the V_H and/or V_K CDR1, CDR2 and/or CDR3 regions to thereby improve one or more binding properties (*e.g.*, affinity) of the antibody of interest. Site-directed mutagenesis or PCR-mediated mutagenesis can be performed to introduce the mutation(s) and the effect on

antibody binding, or other functional property of interest, can be evaluated in *in vitro* or *in vivo* assays as described herein and provided in the Examples. Preferably conservative modifications (as discussed above) are introduced. The mutations may be amino acid substitutions, additions or deletions, but are preferably substitutions. Moreover, typically no more than one, two, three, four or five residues within a CDR region are altered.

Accordingly, in another embodiment, the invention provides isolated anti-PD-1 monoclonal antibodies, or antigen-binding portions thereof, comprising a heavy chain variable region comprising: (a) a V_H CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21; (b) a V_H CDR2 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28; (c) a V_H CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35; (d) a V_K CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42; (e) a V_K CDR2 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49; and (f) a V_K CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56.

Engineered antibodies of the invention include those in which modifications have been made to framework residues within V_H and/or V_K, *e.g.* to improve the properties of the antibody. Typically such framework modifications are made to decrease the immunogenicity of the antibody. For example, one approach is to "backmutate" one or more framework residues to the corresponding germline sequence. More specifically, an antibody that has undergone somatic mutation may contain framework residues that differ from the germline sequence from which the antibody is derived. Such residues can be identified by comparing the antibody framework sequences to the germline sequences from which the antibody is derived.

For example, Table 1 below shows a number of amino acid changes in the framework regions of the anti-PD-1 antibodies 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 that differ from

the heavy chain parent germline sequence. To return one or more of the amino acid residues in the framework region sequences to their germline configuration, the somatic mutations can be "backmutated" to the germline sequence by, for example, site-directed mutagenesis or PCR-mediated mutagenesis.

Amino acid changes may occur in the framework regions of anti-PD-1 antibodies that differ from the light chain parent germline sequence. For example, for 17D8, amino acid residue #47 (within FR2) of V_K is an isoleucine whereas this residue in the corresponding V_K L6 germline sequence is a leucine. To return the framework region sequences to their germline configuration, the somatic mutations can be "backmutated" to the germline sequence by, for example, site-directed mutagenesis or PCR-mediated mutagenesis (*e.g.*, residue #47 (residue #13 of FR2) of the V_K of 17D8 can be "backmutated" from isoleucine to leucine).

As another example, for 4A11, amino acid residue #20 (within FR1) of V_K is a serine whereas this residue in the corresponding V_K L15 germline sequence is a threonine. To return the framework region sequences to their germline configuration, for example, residue #20 of the V_K of 4A11 can be "backmutated" from serine to threonine. Such "backmutated" antibodies are also intended to be encompassed by the invention.

The alignment of V_H regions for 17D8, 2D3, 4H1, 5C4 and 7D3, against the parent germline V_H 3-33 sequence is shown in Figure 8. The alignment of V_H regions for 4A11 and 5F4 against the parent germline V_H 4-39 sequence is shown in Figure 11.

Table 1. Modifications to antibodies 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 from the heavy chain germline configuration.

Anti-PD-1 Ab	Amino acid position	Amino acid of antibody	Original amino acid of germline configuration
17D8	10	D	G
	16	G	R
	27	V	F
	28	A	T
	78	M	T
	93	M	V
2D3	10	D	G
	27	L	F
	30	T	S
	85	N	S
	98	T	R
4H1	3	Y	Q
	84	T	N
	88	V	A
	98	S	R
5C4	21	D	S
	23	K	A
	27	I	F
	80	F	Y
	98	T	R
4A11	29	L	I
	79	Q	H
	98	V	A
7D3	23	T	A
	24	T	A
	27	I	F
	70	L	I
	74	D	N
	97	V	A
	98	T	R
5F4	23	S	T
	29	L	I
	51	A	G
	77	R	K

Another type of framework modification involves mutating one or more residues within the framework region, or even within one or more CDR regions, to remove T cell epitopes to thereby reduce the potential immunogenicity of the antibody. This approach is also referred to as "deimmunization" and is described in further detail in U.S. Patent Publication No. 20030153043 by Carr *et al.*

In addition or alternative to modifications made within the framework or CDR regions, antibodies of the invention may be engineered to include modifications within the Fc region, typically to alter one or more functional properties of the antibody, such as serum half-life,

complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity. Furthermore, an antibody of the invention may be chemically modified (*e.g.*, one or more chemical moieties can be attached to the antibody) or be modified to alter its glycosylation, again to alter one or more functional properties of the antibody. Each of these embodiments is described in further detail below. The numbering of residues in the Fc region is that of the EU index of Kabat.

In one embodiment, the hinge region of CH1 is modified such that the number of cysteine residues in the hinge region is altered, *e.g.*, increased or decreased. This approach is described further in U.S. Patent No. 5,677,425 by Bodmer *et al.* The number of cysteine residues in the hinge region of CH1 is altered to, for example, facilitate assembly of the light and heavy chains or to increase or decrease the stability of the antibody.

In another embodiment, the Fc hinge region of an antibody is mutated to decrease the biological half life of the antibody. More specifically, one or more amino acid mutations are introduced into the CH2-CH3 domain interface region of the Fc-hinge fragment such that the antibody has impaired Staphylococcyl protein A (SpA) binding relative to native Fc-hinge domain SpA binding. This approach is described in further detail in U.S. Patent No. 6,165,745 by Ward *et al.*

In another embodiment, the antibody is modified to increase its biological half life. Various approaches are possible. For example, one or more of the following mutations can be introduced: T252L, T254S, T256F, as described in U.S. Patent No. 6,277,375 to Ward. Alternatively, to increase the biological half life, the antibody can be altered within the CH1 or CL region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Patent Nos. 5,869,046 and 6,121,022 by Presta *et al.*

In yet other embodiments, the Fc region is altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector function(s) of the antibody. For example, one or more amino acids selected from amino acid residues 234, 235, 236, 237, 297, 318, 320 and 322 can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in U.S. Patent Nos. 5,624,821 and 5,648,260, both by Winter *et al.*

In another example, one or more amino acids selected from amino acid residues 329, 331 and 322 can be replaced with a different amino acid residue such that the antibody has altered C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in further detail in U.S. Patent Nos. 6,194,551 by Idusogie *et al.*

In another example, one or more amino acid residues within amino acid positions 231 and 239 are altered to thereby alter the ability of the antibody to fix complement. This approach is described further in PCT Publication WO 94/29351 by Bodmer *et al.*

In yet another example, the Fc region is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fcγ receptor by modifying one or more amino acids at the following positions: 238, 239, 248, 249, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 301, 303, 305, 307, 309, 312, 315, 320, 322, 324, 326, 327, 329, 330, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 378, 382, 388, 389, 398, 414, 416, 419, 430, 434, 435, 437, 438 or 439. This approach is described further in PCT Publication WO 00/42072 by Presta. Moreover, the binding sites on human IgG1 for FcγR1, FcγRII, FcγRIII and FcRn have been mapped and variants with improved binding have been described (see Shields, R.L. *et al.* (2001) *J. Biol. Chem.* 276:6591-6604). Specific mutations at positions 256, 290, 298, 333, 334 and 339 were shown to improve binding to FcγRIII. Additionally, the following combination mutants were shown to improve FcγRIII binding: T256A/S298A, S298A/E333A, S298A/K224A and S298A/E333A/K334A.

In still another embodiment, the glycosylation of an antibody is modified. For example, an aglycoslated antibody can be made (*i.e.*, the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. Such an approach is described in further detail in U.S. Patent Nos. 5,714,350 and 6,350,861 by Co *et al.*

Additionally or alternatively, an antibody can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNac structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation. For example, the cell lines Ms704, Ms705, and Ms709 lack the fucosyltransferase gene, FUT8 (alpha (1,6) fucosyltransferase), such that antibodies expressed in the Ms704, Ms705, and Ms709 cell lines lack fucose on their carbohydrates. The Ms704, Ms705, and Ms709 $FUT8^{-/-}$ cell lines were created by the targeted disruption of the FUT8 gene in CHO/DG44 cells using two replacement vectors (see U.S. Patent Publication No. 20040110704 by Yamane *et al.* and Yamane-Ohnuki *et al.* (2004) *Biotechnol Bioeng* 87:614-22). As another example, EP 1,176,195 by Hanai *et al.* describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, such that antibodies expressed in such a cell line exhibit

hypofucosylation by reducing or eliminating the alpha 1,6 bond-related enzyme. Hanai *et al.* also describe cell lines which have a low enzyme activity for adding fucose to the N-acetylglucosamine that binds to the Fc region of the antibody or does not have the enzyme activity, for example the rat myeloma cell line YB2/0 (ATCC CRL 1662). PCT Publication WO 03/035835 by Presta describes a variant CHO cell line, Lec13 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields, R.L. *et al.* (2002) *J. Biol. Chem.* 277:26733-26740). PCT Publication WO 99/54342 by Umana *et al.* describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (*e.g.*, beta(1,4)-N-acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNac structures which results in increased ADCC activity of the antibodies (see also Umana *et al.* (1999) *Nat. Biotech.* 17:176-180). Alternatively, the fucose residues of the antibody may be cleaved off using a fucosidase enzyme. For example, the fucosidase alpha-L-fucosidase removes fucosyl residues from antibodies (Tarentino, A.L. *et al.* (1975) *Biochem.* 14:5516-23).

Another modification of the antibodies herein that is contemplated by the invention is pegylation. An antibody can be pegylated to, for example, increase the biological (*e.g.*, serum) half life of the antibody. To pegylate an antibody, the antibody, or fragment thereof, typically is reacted with polyethylene glycol (PEG), such as a reactive ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the antibody or antibody fragment. Preferably, the pegylation is carried out via an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive water-soluble polymer). As used herein, the term "polyethylene glycol" is intended to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono (C1-C10) alkoxy- or aryloxy-polyethylene glycol or polyethylene glycol-maleimide. In certain embodiments, the antibody to be pegylated is an aglycosylated antibody. Methods for pegylating proteins are known in the art and can be applied to the antibodies of the invention. See for example, EP 0 154 316 by Nishimura *et al.* and EP 0 401 384 by Ishikawa *et al.*

Methods of Engineering Antibodies

As discussed above, the anti-PD-1 antibodies having V_H and V_K sequences disclosed herein can be used to create new anti-PD-1 antibodies by modifying the VH and/or V_K sequences, or the constant region(s) attached thereto. Thus, in another aspect of the invention, the structural features of an anti-PD-1 antibody of the invention, *e.g.* 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4, are used to create structurally related anti-PD-1 antibodies that retain at least one functional property of the antibodies of the invention, such as binding to human PD-1. For example, one or more CDR regions of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4, or mutations thereof, can be combined recombinantly with known framework regions and/or other CDRs to create additional, recombinantly-engineered, anti-PD-1 antibodies of the

invention, as discussed above. Other types of modifications include those described in the previous section. The starting material for the engineering method is one or more of the V_H and/or V_K sequences provided herein, or one or more CDR regions thereof. To create the engineered antibody, it is not necessary to actually prepare (*i.e.*, express as a protein) an antibody having one or more of the V_H and/or V_K sequences provided herein, or one or more CDR regions thereof. Rather, the information contained in the sequence(s) is used as the starting material to create a "second generation" sequence(s) derived from the original sequence(s) and then the "second generation" sequence(s) is prepared and expressed as a protein.

Accordingly, in another embodiment, the invention provides a method for preparing an anti-PD-1 antibody comprising:

(a) providing: (i) a heavy chain variable region antibody sequence comprising a CDR1 sequence selected from the group consisting of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21, a CDR2 sequence selected from the group consisting of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28, and/or a CDR3 sequence selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35; and/or (ii) a light chain variable region antibody sequence comprising a CDR1 sequence selected from the group consisting of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42, a CDR2 sequence selected from the group consisting of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49, and/or a CDR3 sequence selected from the group consisting of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56;

(b) altering at least one amino acid residue within the heavy chain variable region antibody sequence and/or the light chain variable region antibody sequence to create at least one altered antibody sequence; and

(c) expressing the altered antibody sequence as a protein.

Standard molecular biology techniques can be used to prepare and express the altered antibody sequence.

Preferably, the antibody encoded by the altered antibody sequence(s) is one that retains one, some or all of the functional properties of the anti-PD-1 antibodies described herein, which functional properties include, but are not limited to:

(a) the antibody binds to human PD-1 with a K_D of 1×10^{-7} M or less;

(b) the antibody does not substantially bind to human CD28, CTLA-4 or ICOS;

(c) the antibody increases T-cell proliferation in an MLR assay;

(d) the antibody increases interferon-gamma production in an MLR assay;

(e) the antibody increases Il-2 secretion in an MLR assay;

(f) the antibody binds to human PD-1 and cynomolgus monkey PD-1;

(g) the antibody inhibits the binding of PD-L1 and/or PD-L2 to PD-1;

(h) the antibody stimulates antigen-specific memory responses;

(i) the antibody stimulates antibody responses;

(j) the antibody inhibits tumor cell growth *in vivo*.

The functional properties of the altered antibodies can be assessed using standard assays available in the art and/or described herein, such as those set forth in the Examples (*e.g.*, flow cytometry, binding assays).

In certain embodiments of the methods of engineering antibodies of the invention, mutations can be introduced randomly or selectively along all or part of an anti-PD-1 antibody coding sequence and the resulting modified anti-PD-1 antibodies can be screened for binding activity and/or other functional properties as described herein. Mutational methods have been described in the art. For example, PCT Publication WO 02/092780 by Short describes methods for creating and screening antibody mutations using saturation mutagenesis, synthetic ligation assembly, or a combination thereof. Alternatively, PCT Publication WO 03/074679 by Lazar *et al.* describes methods of using computational screening methods to optimize physiochemical properties of antibodies.

Nucleic Acid Molecules Encoding Antibodies of the Invention

Another aspect of the invention pertains to nucleic acid molecules that encode the antibodies of the invention. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid is "isolated" or "rendered substantially pure" when purified away from other cellular components or other contaminants, *e.g.*, other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis and others well known in the art. *See*, F. Ausubel, *et al.*, ed. (1987) Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York. A nucleic acid of the invention can be, for example, DNA or RNA and may or may not contain intronic sequences. In a preferred embodiment, the nucleic acid is a cDNA molecule.

Nucleic acids of the invention can be obtained using standard molecular biology techniques. For antibodies expressed by hybridomas (*e.g.*, hybridomas prepared from transgenic mice carrying human immunoglobulin genes as described further below), cDNAs encoding the light and heavy chains of the antibody made by the hybridoma can be obtained by standard PCR amplification or cDNA cloning techniques. For antibodies obtained from an immunoglobulin gene library (*e.g.*, using phage display techniques), nucleic acid encoding the antibody can be recovered from the library.

Preferred nucleic acids molecules of the invention are those encoding the VH and VL sequences of the 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4 monoclonal antibodies. DNA sequences encoding the VH sequences of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 57, 58, 59, 60, 61, 62 and 63, respectively. DNA sequences encoding the VL sequences of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 64, 65, 66, 67, 68, 69 and 70, respectively.

Once DNA fragments encoding VH and VL segments are obtained, these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes

or to a scFv gene. In these manipulations, a VL- or VH-encoding DNA fragment is operatively linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term "operatively linked", as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

The isolated DNA encoding the VH region can be converted to a full-length heavy chain gene by operatively linking the VH-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are known in the art (see *e.g.*, Kabat, E. A., el al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region, but most preferably is an IgG1 or IgG4 constant region. For a Fab fragment heavy chain gene, the VH-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region.

The isolated DNA encoding the VL region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the VL-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art (see *e.g.*, Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region, but most preferably is a kappa constant region.

To create a scFv gene, the VH- and VL-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, *e.g.*, encoding the amino acid sequence $(Gly_4\text{-}Ser)_3$, such that the VH and VL sequences can be expressed as a contiguous single-chain protein, with the VL and VH regions joined by the flexible linker (see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883; McCafferty *et al.*, (1990) *Nature* 348:552-554).

Production of Monoclonal Antibodies of the Invention

Monoclonal antibodies (mAbs) of the present invention can be produced by a variety of techniques, including conventional monoclonal antibody methodology *e.g.*, the standard somatic cell hybridization technique of Kohler and Milstein (1975) *Nature* 256: 495. Although somatic cell hybridization procedures are preferred, in principle, other techniques for producing monoclonal antibody can be employed *e.g.*, viral or oncogenic transformation of B lymphocytes.

The preferred animal system for preparing hybridomas is the murine system. Hybridoma production in the mouse is a very well-established procedure. Immunization protocols and techniques for isolation of immunized splenocytes for fusion are known in the art. Fusion partners (*e.g.*, murine myeloma cells) and fusion procedures are also known.

Chimeric or humanized antibodies of the present invention can be prepared based on the sequence of a murine monoclonal antibody prepared as described above. DNA encoding the heavy and light chain immunoglobulins can be obtained from the murine hybridoma of interest and engineered to contain non-murine (*e.g.*, human) immunoglobulin sequences using standard molecular biology techniques. For example, to create a chimeric antibody, the murine variable regions can be linked to human constant regions using methods known in the art (see *e.g.*, U.S. Patent No. 4,816,567 to Cabilly *et al.*). To create a humanized antibody, the murine CDR regions can be inserted into a human framework using methods known in the art (see *e.g.*, U.S. Patent No. 5,225,539 to Winter, and U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen *et al.*).

In a preferred embodiment, the antibodies of the invention are human monoclonal antibodies. Such human monoclonal antibodies directed against PD-1 can be generated using transgenic or transchromosomic mice carrying parts of the human immune system rather than the mouse system. These transgenic and transchromosomic mice include mice referred to herein as HuMAb mice and KM mice™, respectively, and are collectively referred to herein as "human Ig mice."

The HuMAb mouse® (Medarex, Inc.) contains human immunoglobulin gene miniloci that encode unrearranged human heavy (μ and γ) and κ light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (see *e.g.*, Lonberg, *et al.* (1994) Nature 368(6474): 856-859). Accordingly, the mice exhibit reduced expression of mouse IgM or κ, and in response to immunization, the introduced human heavy and light chain transgenes undergo class switching and somatic mutation to generate high affinity human IgGκ monoclonal (Lonberg, N. *et al.* (1994), *supra*; reviewed in Lonberg, N. (1994) *Handbook of Experimental Pharmacology* 113:49-101; Lonberg, N. and Huszar, D. (1995) *Intern. Rev. Immunol.* 13: 65-93, and Harding, F. and Lonberg, N. (1995) *Ann. N.Y. Acad. Sci.* 764:536-546). The preparation and use of HuMab mice, and the genomic modifications carried by such mice, is further described in Taylor, L. *et al.* (1992) *Nucleic Acids Research* 20:6287-6295; Chen, J. *et al.* (1993) *International Immunology* 5: 647-656; Tuaillon *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:3720-3724; Choi *et al.* (1993) *Nature Genetics* 4:117-123; Chen, J. *et al.* (1993) *EMBO J.* 12: 821-830; Tuaillon *et al.* (1994) *J. Immunol.* 152:2912-2920; Taylor, L. *et al.* (1994) *International Immunology* 6: 579-591; and Fishwild, D. *et al.* (1996) *Nature Biotechnology* 14: 845-851, the contents of all of which are hereby specifically incorporated by reference in their entirety. See further, U.S. Patent Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661,016; 5,814,318; 5,874,299; and 5,770,429; all to Lonberg and Kay; U.S. Patent No. 5,545,807 to Surani *et al.*;

PCT Publication Nos. WO 92/03918, WO 93/12227, WO 94/25585, WO 97/13852, WO 98/24884 and WO 99/45962, all to Lonberg and Kay; and PCT Publication No. WO 01/14424 to Korman *et al.*

In another embodiment, human antibodies of the invention can be raised using a mouse that carries human immunoglobulin sequences on transgenes and transchomosomes, such as a mouse that carries a human heavy chain transgene and a human light chain transchromosome. Such mice, referred to herein as "KM mice™", are described in detail in PCT Publication WO 02/43478 to Ishida *et al.*

Still further, alternative transgenic animal systems expressing human immunoglobulin genes are available in the art and can be used to raise anti-PD-1 antibodies of the invention. For example, an alternative transgenic system referred to as the Xenomouse (Abgenix, Inc.) can be used; such mice are described in, for example, U.S. Patent Nos. 5,939,598; 6,075,181; 6,114,598; 6, 150,584 and 6,162,963 to Kucherlapati *et al.*

Moreover, alternative transchromosomic animal systems expressing human immunoglobulin genes are available in the art and can be used to raise anti-PD-1 antibodies of the invention. For example, mice carrying both a human heavy chain transchromosome and a human light chain transchromosome, referred to as "TC mice" can be used; such mice are described in Tomizuka *et al.* (2000) *Proc. Natl. Acad. Sci. USA* 97:722-727. Furthermore, cows carrying human heavy and light chain transchromosomes have been described in the art (Kuroiwa *et al.* (2002) *Nature Biotechnology* 20:889-894) and can be used to raise anti-PD-1 antibodies of the invention.

Human monoclonal antibodies of the invention can also be prepared using phage display methods for screening libraries of human immunoglobulin genes. Such phage display methods for isolating human antibodies are established in the art. See for example: U.S. Patent Nos. 5,223,409; 5,403,484; and 5,571,698 to Ladner *et al.*; U.S. Patent Nos. 5,427,908 and 5,580,717 to Dower *et al.*; U.S. Patent Nos. 5,969,108 and 6,172,197 to McCafferty *et al.*; and U.S. Patent Nos. 5,885,793; 6,521,404; 6,544,731; 6,555,313; 6,582,915 and 6,593,081 to Griffiths *et al.*

Human monoclonal antibodies of the invention can also be prepared using SCID mice into which human immune cells have been reconstituted such that a human antibody response can be generated upon immunization. Such mice are described in, for example, U.S. Patent Nos. 5,476,996 and 5,698,767 to Wilson *et al.*

Immunization of Human Ig Mice

When human Ig mice are used to raise human antibodies of the invention, such mice can be immunized with a purified or enriched preparation of PD-1 antigen and/or recombinant PD-1, or an PD-1 fusion protein, as described by Lonberg, N. *et al.* (1994) *Nature* 368(6474): 856-859; Fishwild, D. *et al.* (1996) *Nature Biotechnology* 14: 845-851; and PCT Publication WO 98/24884 and WO 01/14424. Preferably, the mice will be 6-16 weeks of age upon the

first infusion. For example, a purified or recombinant preparation (5-50 μg) of PD-1 antigen can be used to immunize the human Ig mice intraperitoneally.

Detailed procedures to generate fully human monoclonal antibodies to PD-1 are described in Example 1 below. Cumulative experience with various antigens has shown that the transgenic mice respond when initially immunized intraperitoneally (IP) with antigen in complete Freund's adjuvant, followed by every other week IP immunizations (up to a total of 6) with antigen in incomplete Freund's adjuvant. However, adjuvants other than Freund's are also found to be effective. In addition, whole cells in the absence of adjuvant are found to be highly immunogenic. The immune response can be monitored over the course of the immunization protocol with plasma samples being obtained by retroorbital bleeds. The plasma can be screened by ELISA (as described below), and mice with sufficient titers of anti-PD-1 human immunoglobulin can be used for fusions. Mice can be boosted intravenously with antigen 3 days before sacrifice and removal of the spleen. It is expected that 2-3 fusions for each immunization may need to be performed. Between 6 and 24 mice are typically immunized for each antigen. Usually both HCo7 and HCo12 strains are used. In addition, both HCo7 and HCo12 transgene can be bred together into a single mouse having two different human heavy chain transgenes (HCo7/HCo12). Alternatively or additionally, the KM mouse™ strain can be used, as described in Example 1.

Generation of Hybridomas Producing Human Monoclonal Antibodies of the Invention

To generate hybridomas producing human monoclonal antibodies of the invention, splenocytes and/or lymph node cells from immunized mice can be isolated and fused to an appropriate immortalized cell line, such as a mouse myeloma cell line. The resulting hybridomas can be screened for the production of antigen-specific antibodies. For example, single cell suspensions of splenic lymphocytes from immunized mice can be fused to one-sixth the number of P3X63-Ag8.653 nonsecreting mouse myeloma cells (ATCC, CRL 1580) with 50% PEG. Alternatively, the single cell suspensions of splenic lymphocytes from immunized mice can be fused using an electric field based electrofusion method, using a Cyto Pulse large chamber cell fusion electroporator (Cyto Pulse Sciences, Inc., Glen Burnie, MD). Cells are plated at approximately 2 x 10^5 in flat bottom microtiter plate, followed by a two week incubation in selective medium containing 20% fetal Clone Serum, 18% "653" conditioned media, 5% origen (IGEN), 4 mM L-glutamine, 1 mM sodium pyruvate, 5mM HEPES, 0.055 mM 2-mercaptoethanol, 50 units/ml penicillin, 50 mg/ml streptomycin, 50 mg/ml gentamycin and 1X HAT (Sigma; the HAT is added 24 hours after the fusion). After approximately two weeks, cells can be cultured in medium in which the HAT is replaced with HT. Individual wells can then be screened by ELISA for human monoclonal IgM and IgG antibodies. Once extensive hybridoma growth occurs, medium can be observed usually after 10-14 days. The antibody secreting hybridomas can be replated, screened again, and if still positive for human IgG, the monoclonal antibodies can be subcloned at least twice by limiting

dilution. The stable subclones can then be cultured *in vitro* to generate small amounts of antibody in tissue culture medium for characterization.

To purify human monoclonal antibodies, selected hybridomas can be grown in two-liter spinner-flasks for monoclonal antibody purification. Supernatants can be filtered and concentrated before affinity chromatography with protein A-sepharose (Pharmacia, Piscataway, N.J.). Eluted IgG can be checked by gel electrophoresis and high performance liquid chromatography to ensure purity. The buffer solution can be exchanged into PBS, and the concentration can be determined by OD_{280} using 1.43 extinction coefficient. The monoclonal antibodies can be aliquoted and stored at -80 °C.

Generation of Transfectomas Producing Monoclonal Antibodies of the Invention

Antibodies of the invention also can be produced in a host cell transfectoma using, for example, a combination of recombinant DNA techniques and gene transfection methods as is well known in the art (*e.g.*, Morrison, S. (1985) *Science* 229:1202).

For example, to express the antibodies, or antibody fragments thereof, DNAs encoding partial or full-length light and heavy chains, can be obtained by standard molecular biology techniques (*e.g.*, PCR amplification or cDNA cloning using a hybridoma that expresses the antibody of interest) and the DNAs can be inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vector or, more typically, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (*e.g.*, ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). The light and heavy chain variable regions of the antibodies described herein can be used to create full-length antibody genes of any antibody isotype by inserting them into expression vectors already encoding heavy chain constant and light chain constant regions of the desired isotype such that the V_H segment is operatively linked to the C_H segment(s) within the vector and the V_K segment is operatively linked to the C_L segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (*i.e.*, a signal peptide from a non-immunoglobulin protein).

In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that control the expression of the antibody chain genes in

a host cell. The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel (Gene Expression Technology. Methods in Enzymology 185, Academic Press, San Diego, CA (1990)). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences, may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, *etc.* Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV), Simian Virus 40 (SV40), adenovirus, (*e.g.*, the adenovirus major late promoter (AdMLP) and polyoma. Alternatively, nonviral regulatory sequences may be used, such as the ubiquitin promoter or β-globin promoter. Still further, regulatory elements composed of sequences from different sources, such as the SRα promoter system, which contains sequences from the SV40 early promoter and the long terminal repeat of human T cell leukemia virus type 1 (Takebe, Y. *et al.* (1988) *Mol. Cell. Biol.* 8:466-472).

In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (*e.g.*, origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see, *e.g.*, U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel *et al.*). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr- host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is theoretically possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, and most preferably mammalian host cells, is the most preferred because such eukaryotic cells, and in particular mammalian cells, are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. Prokaryotic expression of antibody genes has been reported to be ineffective for production of high yields of active antibody (Boss, M. A. and Wood, C. R. (1985) *Immunology Today* 6:12-13).

Preferred mammalian host cells for expressing the recombinant antibodies of the

invention include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in R. J. Kaufman and P. A. Sharp (1982) *Mol. Biol. 159*:601-621), NSO myeloma cells, COS cells and SP2 cells. In particular, for use with NSO myeloma cells, another preferred expression system is the GS gene expression system disclosed in WO 87/04462, WO 89/01036 and EP 338,841. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Characterization of Antibody Binding to Antigen

Antibodies of the invention can be tested for binding to PD-1 by, for example, standard ELISA. Briefly, microtiter plates are coated with purified PD-1 at 0.25 μg/ml in PBS, and then blocked with 5% bovine serum albumin in PBS. Dilutions of antibody (*e.g.*, dilutions of plasma from PD-1-immunized mice) are added to each well and incubated for 1-2 hours at 37°C. The plates are washed with PBS/Tween and then incubated with secondary reagent (*e.g.*, for human antibodies, a goat-anti-human IgG Fc-specific polyclonal reagent) conjugated to alkaline phosphatase for 1 hour at 37°C. After washing, the plates are developed with pNPP substrate (1 mg/ml), and analyzed at OD of 405-650. Preferably, mice which develop the highest titers will be used for fusions.

An ELISA assay as described above can also be used to screen for hybridomas that show positive reactivity with PD-1 immunogen. Hybridomas that bind with high avidity to PD-1 are subcloned and further characterized. One clone from each hybridoma, which retains the reactivity of the parent cells (by ELISA), can be chosen for making a 5-10 vial cell bank stored at -140 °C, and for antibody purification.

To purify anti-PD-1 antibodies, selected hybridomas can be grown in two-liter spinner-flasks for monoclonal antibody purification. Supernatants can be filtered and concentrated before affinity chromatography with protein A-sepharose (Pharmacia, Piscataway, NJ). Eluted IgG can be checked by gel electrophoresis and high performance liquid chromatography to ensure purity. The buffer solution can be exchanged into PBS, and the concentration can be determined by OD_{280} using 1.43 extinction coefficient. The monoclonal antibodies can be aliquoted and stored at -80 °C.

To determine if the selected anti-PD-1 monoclonal antibodies bind to unique epitopes, each antibody can be biotinylated using commercially available reagents (Pierce, Rockford, IL). Competition studies using unlabeled monoclonal antibodies and biotinylated monoclonal antibodies can be performed using PD-1 coated-ELISA plates as described above. Biotinylated mAb binding can be detected with a strep-avidin-alkaline phosphatase probe.

To determine the isotype of purified antibodies, isotype ELISAs can be performed using reagents specific for antibodies of a particular isotype. For example, to determine the isotype of a human monoclonal antibody, wells of microtiter plates can be coated with 1 μg/ml of anti-human immunoglobulin overnight at 4 °C. After blocking with 1% BSA, the plates are reacted with 1 μg /ml or less of test monoclonal antibodies or purified isotype controls, at ambient temperature for one to two hours. The wells can then be reacted with either human IgG1 or human IgM-specific alkaline phosphatase-conjugated probes. Plates are developed and analyzed as described above.

Anti-PD-1 human IgGs can be further tested for reactivity with PD-1 antigen by Western blotting. Briefly, PD-1 can be prepared and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. After electrophoresis, the separated antigens are transferred to nitrocellulose membranes, blocked with 10% fetal calf serum, and probed with the monoclonal antibodies to be tested. Human IgG binding can be detected using anti-human IgG alkaline phosphatase and developed with BCIP/NBT substrate tablets (Sigma Chem. Co., St. Louis, Mo.).

Immunoconjugates

In another aspect, the present invention features an anti-PD-1 antibody, or a fragment thereof, conjugated to a therapeutic moiety, such as a cytotoxin, a drug (*e.g.*, an immunosuppressant) or a radiotoxin. Such conjugates are referred to herein as "immunoconjugates". Immunoconjugates that include one or more cytotoxins are referred to as "immunotoxins." A cytotoxin or cytotoxic agent includes any agent that is detrimental to (*e.g.*, kills) cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents also include, for example, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

Other preferred examples of therapeutic cytotoxins that can be conjugated to an antibody of the invention include duocarmycins, calicheamicins, maytansines and auristatins, and derivatives thereof. An example of a calicheamicin antibody conjugate is commercially available (Mylotarg™; Wyeth-Ayerst).

Cytoxins can be conjugated to antibodies of the invention using linker technology available in the art. Examples of linker types that have been used to conjugate a cytotoxin to

an antibody include, but are not limited to, hydrazones, thioethers, esters, disulfides and peptide-containing linkers. A linker can be chosen that is, for example, susceptible to cleavage by low pH within the lysosomal compartment or susceptible to cleavage by proteases, such as proteases preferentially expressed in tumor tissue such as cathepsins (*e.g.*, cathepsins B, C, D).

For further discussion of types of cytotoxins, linkers and methods for conjugating therapeutic agents to antibodies, see also Saito, G. *et al.* (2003) *Adv. Drug Deliv. Rev.* 55:199-215; Trail, P.A. *et al.* (2003) *Cancer Immunol. Immunother.* 52:328-337; Payne, G. (2003) *Cancer Cell* 3:207-212; Allen, T.M. (2002) *Nat. Rev. Cancer* 2:750-763; Pastan, I. and Kreitman, R. J. (2002) *Curr. Opin. Investig. Drugs* 3:1089-1091; Senter, P.D. and Springer, C.J. (2001) *Adv. Drug Deliv. Rev.* 53:247-264.

Antibodies of the present invention also can be conjugated to a radioactive isotope to generate cytotoxic radiopharmaceuticals, also referred to as radioimmunoconjugates. Examples of radioactive isotopes that can be conjugated to antibodies for use diagnostically or therapeutically include, but are not limited to, iodine^{131}, indium^{111}, yttrium^{90} and lutetium^{177}. Method for preparing radioimmunconjugates are established in the art. Examples of radioimmunoconjugates are commercially available, including Zevalin™ (IDEC Pharmaceuticals) and Bexxar™ (Corixa Pharmaceuticals), and similar methods can be used to prepare radioimmunoconjugates using the antibodies of the invention.

The antibody conjugates of the invention can be used to modify a given biological response, and the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, an enzymatically active toxin, or active fragment thereof, such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor or interferon-γ; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*,

"The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982).

Bispecific Molecules

In another aspect, the present invention features bispecific molecules comprising an anti-PD-1 antibody, or a fragment thereof, of the invention. An antibody of the invention, or antigen-binding portions thereof, can be derivatized or linked to another functional molecule, *e.g.*, another peptide or protein (*e.g.*, another antibody or ligand for a receptor) to generate a bispecific molecule that binds to at least two different binding sites or target molecules. The antibody of the invention may in fact be derivatized or linkd to more than one other functional molecule to generate multispecific molecules that bind to more than two different binding sites and/or target molecules; such multispecific molecules are also intended to be encompassed by the term "bispecific molecule" as used herein. To create a bispecific molecule of the invention, an antibody of the invention can be functionally linked (*e.g.*, by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other binding molecules, such as another antibody, antibody fragment, peptide or binding mimetic, such that a bispecific molecule results.

Accordingly, the present invention includes bispecific molecules comprising at least one first binding specificity for PD-1 and a second binding specificity for a second target epitope. In a particular embodiment of the invention, the second target epitope is an Fc receptor, *e.g.*, human FcγRI (CD64) or a human Fcα receptor (CD89). Therefore, the invention includes bispecific molecules capable of binding both to FcγR or FcαR expressing effector cells (*e.g.*, monocytes, macrophages or polymorphonuclear cells (PMNs)), and to target cells expressing PD-1. These bispecific molecules target PD-1 expressing cells to effector cell and trigger Fc receptor-mediated effector cell activities, such as phagocytosis of an PD-1 expressing cells, antibody dependent cell-mediated cytotoxicity (ADCC), cytokine release, or generation of superoxide anion.

In an embodiment of the invention in which the bispecific molecule is multispecific, the molecule can further include a third binding specificity, in addition to an anti-Fc binding specificity and an anti-PD-1 binding specificity. In one embodiment, the third binding specificity is an anti-enhancement factor (EF) portion, *e.g.*, a molecule which binds to a surface protein involved in cytotoxic activity and thereby increases the immune response against the target cell. The "anti-enhancement factor portion" can be an antibody, functional antibody fragment or a ligand that binds to a given molecule, *e.g.*, an antigen or a receptor, and thereby results in an enhancement of the effect of the binding determinants for the F_c receptor or target cell antigen. The "anti-enhancement factor portion" can bind an F_c receptor or a target cell antigen. Alternatively, the anti-enhancement factor portion can bind to an entity that is different from the entity to which the first and second binding specificities bind. For example, the anti-enhancement factor portion can bind a cytotoxic T-cell (*e.g.* via CD2, CD3,

CD8, CD28, CD4, CD40, ICAM-1 or other immune cell that results in an increased immune response against the target cell).

In one embodiment, the bispecific molecules of the invention comprise as a binding specificity at least one antibody, or an antibody fragment thereof, including, *e.g.*, an Fab, Fab', $F(ab')_2$, Fv, or a single chain Fv. The antibody may also be a light chain or heavy chain dimer, or any minimal fragment thereof such as a Fv or a single chain construct as described in Ladner *et al.* U.S. Patent No. 4,946,778, the contents of which is expressly incorporated by reference.

In one embodiment, the binding specificity for an Fcγ receptor is provided by a monoclonal antibody, the binding of which is not blocked by human immunoglobulin G (IgG). As used herein, the term "IgG receptor" refers to any of the eight γ-chain genes located on chromosome 1. These genes encode a total of twelve transmembrane or soluble receptor isoforms which are grouped into three Fcγ receptor classes: FcγRI (CD64), FcγRII(CD32), and FcγRIII (CD16). In one preferred embodiment, the Fcγ receptor a human high affinity FcγRI. The human FcγRI is a 72 kDa molecule, which shows high affinity for monomeric IgG (10^8 - $10^9 M^{-1}$).

The production and characterization of certain preferred anti-Fcγ monoclonal antibodies are described by Fanger *et al.* in PCT Publication WO 88/00052 and in U.S. Patent No. 4,954,617, the teachings of which are fully incorporated by reference herein. These antibodies bind to an epitope of FcγRI, FcγRII or FcγRIII at a site which is distinct from the Fc γ binding site of the receptor and, thus, their binding is not blocked substantially by physiological levels of IgG. Specific anti-FcγRI antibodies useful in this invention are mAb 22, mAb 32, mAb 44, mAb 62 and mAb 197. The hybridoma producing mAb 32 is available from the American Type Culture Collection, ATCC Accession No. HB9469. In other embodiments, the anti-Fcγ receptor antibody is a humanized form of monoclonal antibody 22 (H22). The production and characterization of the H22 antibody is described in Graziano, R.F. *et al.* (1995) *J. Immunol* 155 (10): 4996-5002 and PCT Publication WO 94/10332. The H22 antibody producing cell line was deposited at the American Type Culture Collection under the designation HA022CL1 and has the accession no. CRL 11177.

In still other preferred embodiments, the binding specificity for an Fc receptor is provided by an antibody that binds to a human IgA receptor, *e.g.*, an Fc-alpha receptor (FcαRI (CD89)), the binding of which is preferably not blocked by human immunoglobulin A (IgA). The term "IgA receptor" is intended to include the gene product of one α-gene (FcαRI) located on chromosome 19. This gene is known to encode several alternatively spliced transmembrane isoforms of 55 to 110 kDa. FcαRI (CD89) is constitutively expressed on monocytes/macrophages, eosinophilic and neutrophilic granulocytes, but not on non-effector cell populations. FcαRI has medium affinity ($\approx 5 \times 10^7 M^{-1}$) for both IgA1 and IgA2, which is increased upon exposure to cytokines such as G-CSF or GM-CSF (Morton, H.C. *et al.* (1996) *Critical Reviews in Immunology* 16:423-440). Four FcαRI-specific monoclonal

antibodies, identified as A3, A59, A62 and A77, which bind FcαRI outside the IgA ligand binding domain, have been described (Monteiro, R.C. *et al.* (1992) *J. Immunol.* 148:1764).

FcαRI and FcγRI are preferred trigger receptors for use in the bispecific molecules of the invention because they are (1) expressed primarily on immune effector cells, *e.g.*, monocytes, PMNs, macrophages and dendritic cells; (2) expressed at high levels (*e.g.*, 5,000-100,000 per cell); (3) mediators of cytotoxic activities (*e.g.*, ADCC, phagocytosis); (4) mediate enhanced antigen presentation of antigens, including self-antigens, targeted to them.

While human monoclonal antibodies are preferred, other antibodies which can be employed in the bispecific molecules of the invention are murine, chimeric and humanized monoclonal antibodies.

The bispecific molecules of the present invention can be prepared by conjugating the constituent binding specificities, *e.g.*, the anti-FcR and anti-PD-1 binding specificities, using methods known in the art. For example, each binding specificity of the bispecific molecule can be generated separately and then conjugated to one another. When the binding specificities are proteins or peptides, a variety of coupling or cross-linking agents can be used for covalent conjugation. Examples of cross-linking agents include protein A, carbodiimide, N-succinimidyl-S-acetyl-thioacetate (SATA), 5, 5'-dithiobis(2-nitrobenzoic acid) (DTNB), o-phenylenedimaleimide (oPDM), N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), and sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohaxane-1-carboxylate (sulfo-SMCC) (see *e.g.*, Karpovsky *et al.* (1984) *J. Exp. Med.* 160:1686; Liu, MA *et al.* (1985) *Proc. Natl. Acad. Sci. USA* 82:8648). Other methods include those described in Paulus (1985) Behring Ins. Mitt. No. 78, 118-132; Brennan *et al.* (1985) *Science* 229:81-83), and Glennie *et al.* (1987) *J. Immunol.* 139: 2367-2375). Preferred conjugating agents are SATA and sulfo-SMCC, both available from Pierce Chemical Co. (Rockford, IL).

When the binding specificities are antibodies, they can be conjugated via sulfhydryl bonding of the C-terminus hinge regions of the two heavy chains. In a particularly preferred embodiment, the hinge region is modified to contain an odd number of sulfhydryl residues, preferably one, prior to conjugation.

Alternatively, both binding specificities can be encoded in the same vector and expressed and assembled in the same host cell. This method is particularly useful where the bispecific molecule is a mAb x mAb, mAb x Fab, Fab x $F(ab')_2$ or ligand x Fab fusion protein. A bispecific molecule of the invention can be a single chain molecule comprising one single chain antibody and a binding determinant, or a single chain bispecific molecule comprising two binding determinants. Bispecific molecules may comprise at least two single chain molecules. Methods for preparing bispecific molecules are described for example in U.S. Patent Number 5,260,203; U.S. Patent Number 5,455,030; U.S. Patent Number 4,881,175; U.S. Patent Number 5,132,405; U.S. Patent Number 5,091,513; U.S. Patent Number 5,476,786; U.S. Patent Number 5,013,653; U.S. Patent Number 5,258,498; and U.S. Patent Number 5,482,858.

Binding of the bispecific molecules to their specific targets can be confirmed by, for example, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), FACS analysis, bioassay (*e.g.*, growth inhibition), or Western Blot assay. Each of these assays generally detects the presence of protein-antibody complexes of particular interest by employing a labeled reagent (*e.g.*, an antibody) specific for the complex of interest. For example, the FcR-antibody complexes can be detected using *e.g.*, an enzyme-linked antibody or antibody fragment which recognizes and specifically binds to the antibody-FcR complexes. Alternatively, the complexes can be detected using any of a variety of other immunoassays. For example, the antibody can be radioactively labeled and used in a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by such means as the use of a γ counter or a scintillation counter or by autoradiography.

Pharmaceutical Compositions

In another aspect, the present invention provides a composition, *e.g.*, a pharmaceutical composition, containing one or a combination of monoclonal antibodies, or antigen-binding portion(s) thereof, of the present invention, formulated together with a pharmaceutically acceptable carrier. Such compositions may include one or a combination of (*e.g.*, two or more different) antibodies, or immunoconjugates or bispecific molecules of the invention. For example, a pharmaceutical composition of the invention can comprise a combination of antibodies (or immunoconjugates or bispecifics) that bind to different epitopes on the target antigen or that have complementary activities.

Pharmaceutical compositions of the invention also can be administered in combination therapy, *i.e.*, combined with other agents. For example, the combination therapy can include an anti-PD-1 antibody of the present invention combined with at least one other anti-inflammatory or immunosuppressant agent. Examples of therapeutic agents that can be used in combination therapy are described in greater detail below in the section on uses of the antibodies of the invention.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (*e.g.*, by injection or infusion). Depending on the route of administration, the active compound, *i.e.*, antibody, immunoconjuage, or bispecific molecule, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

The pharmaceutical compounds of the invention may include one or more pharmaceutically acceptable salts. A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any

undesired toxicological effects (see *e.g.*, Berge, S.M., *et al.* (1977) *J. Pharm. Sci.* 66:1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N, N'-dibenzylethylenediamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

A pharmaceutical composition of the invention also may include a pharmaceutically acceptable anti-oxidant. Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, *supra*, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 0.01 per cent to about ninety-nine percent of active ingredient, preferably from about 0.1 per cent to about 70 per cent, most preferably from about 1 per cent to about 30 per cent of active ingredient in combination with a pharmaceutically acceptable carrier.

Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active

compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

For administration of the antibody, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight. For example dosages can be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg body weight, 5 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. An exemplary treatment regime entails administration once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months. Preferred dosage regimens for an anti-PD-1 antibody of the invention include 1 mg/kg body weight or 3 mg/kg body weight via intravenous administration, with the antibody being given using one of the following dosing schedules: (i) every four weeks for six dosages, then every three months; (ii) every three weeks; (iii) 3 mg/kg body weight once followed by 1 mg/kg body weight every three weeks.

In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated. Antibody is usually administered on multiple occasions. Intervals between single dosages can be, for example, weekly, monthly, every three months or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody to the target antigen in the patient. In some methods, dosage is adjusted to achieve a plasma antibody concentration of about 1-1000 μg /ml and in some methods about 25-300 μg /ml.

Alternatively, antibody can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, human antibodies show the longest half life, followed by humanized antibodies, chimeric antibodies, and nonhuman antibodies. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular

compositions of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A "therapeutically effective dosage" of an anti-PD-1 antibody of the invention preferably results in a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. For example, for the treatment of tumors, a "therapeutically effective dosage" preferably inhibits cell growth or tumor growth by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to inhibit tumor growth can be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit, such inhibition *in vitro* by assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound can decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

In another aspect, the instant disclosure provides a pharmaceutical kit of parts comprising an anti-PD-1 antibody and an anti-CTLA-4 antibody, as described herein. The kit may also further comprise instructions for use in the treatment of a hyperproliferative disease (such as cancer as described herein). In another embodiment, the anti-PD-1 and anti-CTLA-4 antibodies may be co-packaged in unit dosage form.

In certain embodiments, two or more monoclonal antibodies with different binding specificities (*e.g.*, anti-PD-1 and anti-CTLA-4) are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated. Antibody can be administered as a single dose or more commonly can be administered on multiple occasions. Intervals between single dosages can be, for example, weekly, monthly, every three months or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody to the target antigen in the patient. In some methods, dosage is adjusted to achieve a plasma antibody concentration of about 1-1000 μg /ml and in some methods about 25-300 μg/ml.

A composition of the present invention can be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Preferred routes of administration for antibodies of the invention include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other

parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

Alternatively, an antibody of the invention can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically.

The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. *See, e.g., Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

Therapeutic compositions can be administered with medical devices known in the art. For example, in a preferred embodiment, a therapeutic composition of the invention can be administered with a needleless hypodermic injection device, such as the devices disclosed in U.S. Patent Nos. 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824; or 4,596,556. Examples of well-known implants and modules useful in the present invention include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,486,194, which discloses a therapeutic device for administering medicants through the skin; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Patent No. 4,475,196, which discloses an osmotic drug delivery system. These patents are incorporated herein by reference. Many other such implants, delivery systems, and modules are known to those skilled in the art.

In certain embodiments, the human monoclonal antibodies of the invention can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the invention cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, *e.g.*, U.S. Patents 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (*see, e.g.*, V.V.

Ranade (1989) *J. Clin. Pharmacol.* 29:685). Exemplary targeting moieties include folate or biotin (see, *e.g.*, U.S. Patent 5,416,016 to Low *et al.*); mannosides (Umezawa *et al.*, (1988) *Biochem. Biophys. Res. Commun.* 153:1038); antibodies (P.G. Bloeman *et al.* (1995) *FEBS Lett.* 357:140; M. Owais *et al.* (1995) *Antimicrob. Agents Chemother.* 39:180); surfactant protein A receptor (Briscoe *et al.* (1995) *Am. J. Physiol.* 1233:134); p120 (Schreier *et al.* (1994) *J. Biol. Chem.* 269:9090); see also K. Keinanen; M.L. Laukkanen (1994) *FEBS Lett.* 346:123; J.J. Killion; I.J. Fidler (1994) *Immunomethods* 4:273.

Uses and Methods of the Invention

The antibodies, antibody compositions and methods of the present invention have numerous *in vitro* and *in vivo* utilities involving, for example, detection of PD-1 or enhancement of immune response by blockade of PD-1. In a preferred embodiment, the antibodies of the present invention are human antibodies. For example, these molecules can be administered to cells in culture, *in vitro* or *ex vivo*, or to human subjects, *e.g.*, *in vivo*, to enhance immunity in a variety of situations. Accordingly, in one aspect, the invention provides a method of modifying an immune response in a subject comprising administering to the subject the antibody, or antigen-binding portion thereof, of the invention such that the immune response in the subject is modified. Preferably, the response is enhanced, stimulated or up-regulated.

As used herein, the term "subject" is intended to include human and non-human animals. Non-human animals includes all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, sheep, dogs, cats, cows, horses, chickens, amphibians, and reptiles, although mammals are preferred, such as non-human primates, sheep, dogs, cats, cows and horses. Preferred subjects include human patients in need of enhancement of an immune response. The methods are particularly suitable for treating human patients having a disorder that can be treated by augmenting the T-cell mediated immune response. In a particular embodiment, the methods are particularly suitable for treatment of cancer cells *in vivo*. To achieve antigen-specific enhancement of immunity, the anti-PD-1 antibodies can be administered together with an antigen of interest. When antibodies to PD-1 are administered together with another agent, the two can be administered in either order or simultaneously.

The invention further provides methods for detecting the presence of human PD-1 antigen in a sample, or measuring the amount of human PD-1 antigen, comprising contacting the sample, and a control sample, with a human monoclonal antibody, or an antigen-binding portion thereof, which specifically binds to human PD-1, under conditions that allow for formation of a complex between the antibody or portion thereof and human PD-1. The formation of a complex is then detected, wherein a difference complex formation between the sample compared to the control sample is indicative the presence of human PD-1 antigen in the sample.

Given the specific binding of the antibodies of the invention for PD-1, compared to CD28, ICOS and CTLA-4, the antibodies of the invention can be used to specifically detect

PD-1 expression on the surface of cells and, moreover, can be used to purify PD-1 via immunoaffinity purification.

Cancer

Blockade of PD-1 by antibodies can enhance the immune response to cancerous cells in the patient. The ligand for PD-1, PD-L1, is not expressed in normal human cells, but is abundant in a variety of human cancers (Dong *et al.* (2002) *Nat Med* 8:787-9). The interaction between PD-1 and PD-L1 results in a decrease in tumor infiltrating lymphocytes, a decrease in T-cell receptor mediated proliferation, and immune evasion by the cancerous cells (Dong *et al.* (2003) *J Mol Med* 81:281-7; Blank *et al.* (2005) *Cancer Immunol. Immunother.* 54:307-314; Konishi *et al.* (2004) *Clin. Cancer Res.* 10:5094-100). Immune suppression can be reversed by inhibiting the local interaction of PD-1 to PD-L1 and the effect is additive when the interaction of PD-1 to PD-L2 is blocked as well (Iwai *et al.* (2002) *PNAS* 99:12293-7; Brown *et al.* (2003) *J. Immunol.* 170:1257-66). While previous studies have shown that T-cell proliferation can be restored by inhibiting the interaction of PD-1 to PD-L1, there have been no reports of a direct effect on cancer tumor growth *in vivo* by blocking the PD-1/PD-L1 interaction. In one aspect, the present invention relates to treatment of a subject *in vivo* using an anti-PD-1 antibody such that growth of cancerous tumors is inhibited. An anti-PD-1 antibody may be used alone to inhibit the growth of cancerous tumors. Alternatively, an anti-PD-1 antibody may be used in conjunction with other immunogenic agents, standard cancer treatments, or other antibodies, as described below.

Accordingly, in one embodiment, the invention provides a method of inhibiting growth of tumor cells in a subject, comprising administering to the subject a therapeutically effective amount of an anti-PD-1 antibody, or antigen-binding portion thereof. Preferably, the antibody is a human anti-PD-1 antibody (such as any of the human anti-human PD-1 antibodies described herein). Additionally or alternatively, the antibody may be a chimeric or humanized anti-PD-1 antibody.

Preferred cancers whose growth may be inhibited using the antibodies of the invention include cancers typically responsive to immunotherapy. Non-limiting examples of preferred cancers for treatment include melanoma (*e.g.*, metastatic malignant melanoma), renal cancer (*e.g.* clear cell carcinoma), prostate cancer (*e.g.* hormone refractory prostate adenocarcinoma), breast cancer, colon cancer and lung cancer (*e.g.* non-small cell lung cancer). Additionally, the invention includes refractory or recurrent malignancies whose growth may be inhibited using the antibodies of the invention.

Examples of other cancers that may be treated using the methods of the invention include bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of

the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers. The present invention is also useful for treatment of metastatic cancers, especially metastatic cancers that express PD-L1 (Iwai *et al.* (2005) *Int. Immunol.* 17:133-144).

Optionally, antibodies to PD-1 can be combined with an immunogenic agent, such as cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), cells, and cells transfected with genes encoding immune stimulating cytokines (He et al (2004) *J. Immunol.* 173:4919-28). Non-limiting examples of tumor vaccines that can be used include peptides of melanoma antigens, such as peptides of gp100, MAGE antigens, Trp-2, MART1 and/or tyrosinase, or tumor cells transfected to express the cytokine GM-CSF (discussed further below).

In humans, some tumors have been shown to be immunogenic such as melanomas. It is anticipated that by raising the threshold of T cell activation by PD-1 blockade, we may expect to activate tumor responses in the host.

PD-1 blockade is likely to be most effective when combined with a vaccination protocol. Many experimental strategies for vaccination against tumors have been devised (see Rosenberg, S., 2000, Development of Cancer Vaccines, ASCO Educational Book Spring: 60-62; Logothetis, C., 2000, ASCO Educational Book Spring: 300-302; Khayat, D. 2000, ASCO Educational Book Spring: 414-428; Foon, K. 2000, ASCO Educational Book Spring: 730-738; see also Restifo, N. and Sznol, M., Cancer Vaccines, Ch. 61, pp. 3023-3043 in DeVita, V. *et al.* (eds.), 1997, Cancer: Principles and Practice of Oncology. Fifth Edition). In one of these strategies, a vaccine is prepared using autologous or allogeneic tumor cells. These cellular vaccines have been shown to be most effective when the tumor cells are transduced to express GM-CSF. GM-CSF has been shown to be a potent activator of antigen presentation for tumor vaccination (Dranoff *et al.* (1993) *Proc. Natl. Acad. Sci U.S.A.* 90: 3539-43).

The study of gene expression and large scale gene expression patterns in various tumors has led to the definition of so called tumor specific antigens (Rosenberg, SA (1999) Immunity 10: 281-7). In many cases, these tumor specific antigens are differentiation antigens expressed in the tumors and in the cell from which the tumor arose, for example melanocyte antigens gp100, MAGE antigens, and Trp-2. More importantly, many of these antigens can be shown to be the targets of tumor specific T cells found in the host. PD-1 blockade may be used in conjunction with a collection of recombinant proteins and/or peptides

expressed in a tumor in order to generate an immune response to these proteins. These proteins are normally viewed by the immune system as self antigens and are therefore tolerant to them. The tumor antigen may also include the protein telomerase, which is required for the synthesis of telomeres of chromosomes and which is expressed in more than 85% of human cancers and in only a limited number of somatic tissues (Kim, N *et al.* (1994) *Science* 266: 2011-2013). (These somatic tissues may be protected from immune attack by various means). Tumor antigen may also be "neo-antigens" expressed in cancer cells because of somatic mutations that alter protein sequence or create fusion proteins between two unrelated sequences (ie. bcr-abl in the Philadelphia chromosome), or idiotype from B cell tumors.

Other tumor vaccines may include the proteins from viruses implicated in human cancers such a Human Papilloma Viruses (HPV), Hepatitis Viruses (HBV and HCV) and Kaposi's Herpes Sarcoma Virus (KHSV). Another form of tumor specific antigen which may be used in conjunction with PD-1 blockade is purified heat shock proteins (HSP) isolated from the tumor tissue itself. These heat shock proteins contain fragments of proteins from the tumor cells and these HSPs are highly efficient at delivery to antigen presenting cells for eliciting tumor immunity (Suot, R & Srivastava, P (1995) *Science* 269:1585-1588; Tamura, Y. *et al.* (1997) *Science* 278:117-120).

Dendritic cells (DC) are potent antigen presenting cells that can be used to prime antigen-specific responses. DC's can be produced *ex vivo* and loaded with various protein and peptide antigens as well as tumor cell extracts (Nestle, F. *et al.* (1998) *Nature Medicine* 4: 328-332). DCs may also be transduced by genetic means to express these tumor antigens as well. DCs have also been fused directly to tumor cells for the purposes of immunization (Kugler, A. *et al.* (2000) *Nature Medicine* 6:332-336). As a method of vaccination, DC immunization may be effectively combined with PD-1 blockade to activate more potent anti-tumor responses.

PD-1 blockade may also be combined with standard cancer treatments. PD-1 blockade may be effectively combined with chemotherapeutic regimes. In these instances, it may be possible to reduce the dose of chemotherapeutic reagent administered (Mokyr, M. *et al.* (1998) *Cancer Research* 58: 5301-5304). An example of such a combination is an anti-PD-1 antibody in combination with decarbazine for the treatment of melanoma. Another example of such a combination is an anti-PD-1 antibody in combination with interleukin-2 (IL-2) for the treatment of melanoma. The scientific rationale behind the combined use of PD-1 blockade and chemotherapy is that cell death, that is a consequence of the cytotoxic action of most chemotherapeutic compounds, should result in increased levels of tumor antigen in the antigen presentation pathway. Other combination therapies that may result in synergy with PD-1 blockade through cell death are radiation, surgery, and hormone deprivation. Each of these protocols creates a source of tumor antigen in the host. Angiogenesis inhibitors may also be combined with PD-1 blockade. Inhibition of angiogenesis leads to tumor cell death which may feed tumor antigen into host antigen presentation pathways.

PD-1 blocking antibodies can also be used in combination with bispecific antibodies that target Fc alpha or Fc gamma receptor-expressing effectors cells to tumor cells (see, *e.g.*, U.S. Pat. Nos. 5,922,845 and 5,837,243). Bispecific antibodies can be used to target two separate antigens. For example anti-Fc receptor/anti tumor antigen (*e.g.*, Her-2/neu) bispecific antibodies have been used to target macrophages to sites of tumor. This targeting may more effectively activate tumor specific responses. The T cell arm of these responses would by augmented by the use of PD-1 blockade. Alternatively, antigen may be delivered directly to DCs by the use of bispecific antibodies which bind to tumor antigen and a dendritic cell specific cell surface marker.

Tumors evade host immune surveillance by a large variety of mechanisms. Many of these mechanisms may be overcome by the inactivation of proteins which are expressed by the tumors and which are immunosuppressive. These include among others TGF-beta (Kehrl, J. *et al.* (1986) *J. Exp. Med.* 163: 1037-1050), IL-10 (Howard, M. & O'Garra, A. (1992) *Immunology Today* 13: 198-200), and Fas ligand (Hahne, M. *et al.* (1996) *Science* 274: 1363-1365). Antibodies to each of these entities may be used in combination with anti-PD-1 to counteract the effects of the immunosuppressive agent and favor tumor immune responses by the host.

Other antibodies which may be used to activate host immune responsiveness can be used in combination with anti-PD-1. These include molecules on the surface of dendritic cells which activate DC function and antigen presentation. Anti-CD40 antibodies are able to substitute effectively for T cell helper activity (Ridge, J. *et al.* (1998) Nature 393: 474-478) and can be used in conjuction with PD-1 antibodies (Ito, N. *et al.* (2000) *Immunobiology* 201 (5) 527-40). Activating antibodies to T cell costimulatory molecules such as CTLA-4 (e.g., US Patent No. 5,811,097), OX-40 (Weinberg, A. *et al.* (2000) *Immunol* 164: 2160-2169), 4-1BB (Melero, I. *et al.* (1997) *Nature Medicine* 3: 682-685 (1997), and ICOS (Hutloff, A. *et al.* (1999) *Nature* 397: 262-266) may also provide for increased levels of T cell activation.

Bone marrow transplantation is currently being used to treat a variety of tumors of hematopoietic origin. While graft versus host disease is a consequence of this treatment, therapeutic benefit may be obtained from graft vs. tumor responses. PD-1 blockade can be used to increase the effectiveness of the donor engrafted tumor specific T cells.

There are also several experimental treatment protocols that involve *ex vivo* activation and expansion of antigen specific T cells and adoptive transfer of these cells into recipients in order to antigen-specific T cells against tumor (Greenberg, R. & Riddell, S. (1999) *Science* 285: 546-51). These methods may also be used to activate T cell responses to infectious agents such as CMV. *Ex vivo* activation in the presence of anti-PD-1 antibodies may be expected to increase the frequency and activity of the adoptively transferred T cells.

Infectious Diseases

Other methods of the invention are used to treat patients that have been exposed to particular toxins or pathogens. Accordingly, another aspect of the invention provides a

method of treating an infectious disease in a subject comprising administering to the subject an anti-PD-1 antibody, or antigen-binding portion thereof, such that the subject is treated for the infectious disease. Preferably, the antibody is a human anti-human PD-1 antibody (such as any of the human anti-PD-1 antibodies described herein). Additionally or alternatively, the antibody can be a chimeric or humanized antibody.

Similar to its application to tumors as discussed above, antibody mediated PD-1 blockade can be used alone, or as an adjuvant, in combination with vaccines, to stimulate the immune response to pathogens, toxins, and self-antigens. Examples of pathogens for which this therapeutic approach may be particularly useful, include pathogens for which there is currently no effective vaccine, or pathogens for which conventional vaccines are less than completely effective. These include, but are not limited to HIV, Hepatitis (A, B, & C), Influenza, Herpes, Giardia, Malaria, Leishmania, Staphylococcus aureus, Pseudomonas Aeruginosa. PD-1 blockade is particularly useful against established infections by agents such as HIV that present altered antigens over the course of the infections. These novel epitopes are recognized as foreign at the time of anti-human PD-1 administration, thus provoking a strong T cell response that is not dampened by negative signals through PD-1.

Some examples of pathogenic viruses causing infections treatable by methods of the invention include HIV, hepatitis (A, B, or C), herpes virus (*e.g.*, VZV, HSV-1, HAV-6, HSV-II, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus.

Some examples of pathogenic bacteria causing infections treatable by methods of the invention include chlamydia, rickettsial bacteria, mycobacteria, staphylococci, streptococci, pneumonococci, meningococci and conococci, klebsiella, proteus, serratia, pseudomonas, legionella, diphtheria, salmonella, bacilli, cholera, tetanus, botulism, anthrax, plague, leptospirosis, and Lymes disease bacteria.

Some examples of pathogenic fungi causing infections treatable by methods of the invention include Candida (albicans, krusei, glabrata, tropicalis, etc.), Cryptococcus neoformans, Aspergillus (fumigatus, niger, etc.), Genus Mucorales (mucor, absidia, rhizophus), Sporothrix schenkii, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Coccidioides immitis and Histoplasma capsulatum.

Some examples of pathogenic parasites causing infections treatable by methods of the invention include Entamoeba histolytica, Balantidium coli, Naegleriafowleri, Acanthamoeba sp., Giardia lambia, Cryptosporidium sp., Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondi, and Nippostrongylus brasiliensis.

In all of the above methods, PD-1 blockade can be combined with other forms of immunotherapy such as cytokine treatment (*e.g.*, interferons, GM-CSF, G-CSF, IL-2), or bispecific antibody therapy, which provides for enhanced presentation of tumor antigens (see, *e.g.*, Holliger (1993) *Proc. Natl. Acad. Sci.* USA 90:6444-6448; Poljak (1994) *Structure* 2:1121-1123).

Autoimmune reactions

Anti-PD-1 antibodies may provoke and amplify autoimmune responses. Indeed, induction of anti-tumor responses using tumor cell and peptide vaccines reveals that many anti-tumor responses involve anti-self reactivities (depigmentation observed in anti-CTLA-4 + GM-CSF-modified B16 melanoma in van Elsas *et al.* supra; depigmentation in Trp-2 vaccinated mice (Overwijk, W. *et al.* (1999) *Proc. Natl. Acad. Sci. U.S.A.* 96: 2982-2987); autoimmune prostatitis evoked by TRAMP tumor cell vaccines (Hurwitz, A. (2000) *supra*), melanoma peptide antigen vaccination and vitilago observed in human clinical trials (Rosenberg, SA and White, DE (1996) *J. Immunother Emphasis Tumor Immunol* 19 (1): 81-4).

Therefore, it is possible to consider using anti-PD-1 blockade in conjunction with various self proteins in order to devise vaccination protocols to efficiently generate immune responses against these self proteins for disease treatment. For example, Alzheimers disease involves inappropriate accumulation of Aβ peptide in amyloid deposits in the brain; antibody responses against amyloid are able to clear these amyloid deposits (Schenk *et al.*, (1999) *Nature* 400: 173-177).

Other self proteins may also be used as targets such as IgE for the treatment of allergy and asthma, and TNFα for rhematoid arthritis. Finally, antibody responses to various hormones may be induced by the use of anti-PD-1 antibody. Neutralizing antibody responses to reproductive hormones may be used for contraception. Neutralizing antibody response to hormones and other soluble factors that are required for the growth of particular tumors may also be considered as possible vaccination targets.

Analogous methods as described above for the use of anti-PD-1 antibody can be used for induction of therapeutic autoimmune responses to treat patients having an inappropriate accumulation of other self-antigens, such as amyloid deposits, including Aβ in Alzheimer's disease, cytokines such as TNFα, and IgE.

Vaccines

Anti-PD-1 antibodies may be used to stimulate antigen-specific immune responses by coadministration of an anti-PD-1 antibody with an antigen of interest (*e.g.*, a vaccine). Accordingly, in another aspect the invention provides a method of enhancing an immune response to an antigen in a subject, comprising administering to the subject: (i) the antigen; and (ii) an anti-PD-1 antibody, or antigen-binding portion thereof, such that an immune response to the antigen in the subject is enhanced. Preferably, the antibody is a human anti-human PD-1 antibody (such as any of the human anti-PD-1 antibodies described herein). Additionally or alternatively, the antibody can be a chimeric or humanized antibody. The

antigen can be, for example, a tumor antigen, a viral antigen, a bacterial antigen or an antigen from a pathogen. Non-limiting examples of such antigens include those discussed in the sections above, such as the tumor antigens (or tumor vaccines) discussed above, or antigens from the viruses, bacteria or other pathogens described above.

Suitable routes of administering the antibody compositions (*e.g.*, human monoclonal antibodies, multispecific and bispecific molecules and immunoconjugates) of the invention *in vivo* and *in vitro* are well known in the art and can be selected by those of ordinary skill. For example, the antibody compositions can be administered by injection (*e.g.*, intravenous or subcutaneous). Suitable dosages of the molecules used will depend on the age and weight of the subject and the concentration and/or formulation of the antibody composition.

As previously described, human anti-PD-1 antibodies of the invention can be co-administered with one or other more therapeutic agents, *e.g.*, a cytotoxic agent, a radiotoxic agent or an immunosuppressive agent. The antibody can be linked to the agent (as an immunocomplex) or can be administered separate from the agent. In the latter case (separate administration), the antibody can be administered before, after or concurrently with the agent or can be co-administered with other known therapies, *e.g.*, an anti-cancer therapy, *e.g.*, radiation. Such therapeutic agents include, among others, anti-neoplastic agents such as doxorubicin (adriamycin), cisplatin bleomycin sulfate, carmustine, chlorambucil, decarbazine and cyclophosphamide hydroxyurea which, by themselves, are only effective at levels which are toxic or subtoxic to a patient. Cisplatin is intravenously administered as a 100 mg/dose once every four weeks and adriamycin is intravenously administered as a 60-75 mg/ml dose once every 21 days. Co-administration of the human anti-PD-1 antibodies, or antigen binding fragments thereof, of the present invention with chemotherapeutic agents provides two anti-cancer agents which operate via different mechanisms which yield a cytotoxic effect to human tumor cells. Such co-administration can solve problems due to development of resistance to drugs or a change in the antigenicity of the tumor cells which would render them unreactive with the antibody.

Also within the scope of the present invention are kits comprising the antibody compositions of the invention (*e.g.*, human antibodies, bispecific or multispecific molecules, or immunoconjugates) and instructions for use. The kit can further contain a least one additional reagent, or one or more additional human antibodies of the invention (e.g., a human antibody having a complementary activity which binds to an epitope in PD-1 antigen distinct from the first human antibody). Kits typically include a label indicating the intended use of the contents of the kit. The term label includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit.

Combination therapy

The present invention is based, in part, on the following experimental data. Mouse tumor models (MC38 colon cancer and SA1/N fibrosarcoma) were used to examine the *in vivo* effect of treating a tumor by combining immunostimulatory therapeutic antibodies -

anti-CTLA-4 and anti-PD-1. The immunotherapeutic combination was provided either simultaneous with the implant of tumor cells (Examples 14 and 17) or after the tumor cells were implanted for a time sufficient to become an established tumor (Examples 15, 16 and 18). Regardless of the timing of antibody treatment, it was found that anti-CTLA-4 antibody treatment alone and anti-PD-1 antibody (chimeric antibody in which a rat anti-mouse PD-1 was modified with a mouse immunoglobulin Fc region, *see* Example 1) treatment alone had a modest effect on reducing tumor growth in the MC38 tumor model (*see, e.g.*, Figures 21, 24 and 27). The anti-CTLA-4 antibody alone was quite effective in the SA1/N tumor model (*see* Figure 30D), which required a lower anti-CTLA-4 antibody dose for the combination studies in this model. Nonetheless, the combination treatment of anti-CTLA-4 antibody and anti-PD-1 antibody showed an unexpected, significantly greater effect on reducing tumor growth as compared to treatment with either antibody alone (*see, e.g.*, Figures 21D, 24D, 30F and 33H-J). In addition, the results of Examples 14, 16 and 18 show that the combination treatment of anti-CTLA-4 antibody and anti-PD-1 antibody had a significant (synergistic) effect on tumor growth even at sub-optimal therapeutic doses as compared to treatment with either antibody alone (*i.e.*, the combination therapy was surprisingly more effective at subtherapeutic doses than either monotherapy). Without wishing to be bound by theory, it is possible that by raising the threshold of T cell activation by PD-1 and CTLA-4 blockade, anti-tumor responses may be activated in a host.

In one embodiment, the present invention provides a method for treating a hyperproliferative disease, comprising administering a PD-1 antibody and a CTLA-4 antibody to a subject. In further embodiments, the anti-PD-1 antibody is administered at a subtherapeutic dose, the anti-CTLA-4 antibody is administered at a subtherapeutic dose, or both are administered at a subtherapeutic dose. In another embodiment, the present invention provides a method for altering an adverse event associated with treatment of a hyperproliferative disease with an immunostimulatory agent, comprising administering an anti-PD-1 antibody and a subtherapeutic dose of anti-CTLA-4 antibody to a subject. In certain embodiments, the subject is human. In certain embodiments, the anti-CTLA-4 antibody is human sequence monoclonal antibody 10D1 and the anti-PD-1 antibody is human sequence monoclonal antibody, such as 17D8, 2D3, 4H1, 5C4 and 4A11. Human sequence monoclonal antibodies 17D8, 2D3, 4H1, 5C4 and 4A11 have been isolated and structurally characterized, as described in U.S. Provisional Patent No. 60/679,466.

The anti-CTLA-4 antibody and anti-PD-1 monoclonal antibodies (mAbs) and the human sequence antibodies of the invention can be produced by a variety of techniques, including conventional monoclonal antibody methodology, *e.g.*, the standard somatic cell hybridization technique of Kohler and Milstein (1975) *Nature* 256:495. Any technique for producing monoclonal antibody can be employed, *e.g.*, viral or oncogenic transformation of B lymphocytes. One animal system for preparing hybridomas is the murine system. Hybridoma production in the mouse is a very well-established procedure. Immunization

protocols and techniques for isolation of immunized splenocytes for fusion are known in the art. Fusion partners (*e.g.*, murine myeloma cells) and fusion procedures are also known (*see, e.g.*, Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor New York).

Anti-CTLA-4 antibodies of the instant invention can bind to an epitope on human CTLA-4 so as to inhibit CTLA-4 from interacting with a human B7 counterreceptor. Because interaction of human CTLA-4 with human B7 transduces a signal leading to inactivation of T-cells bearing the human CTLA-4 receptor, antagonism of the interaction effectively induces, augments or prolongs the activation of T cells bearing the human CTLA-4 receptor, thereby prolonging or augmenting an immune response. Anti-CTLA-4 antibodies are described in U.S. Patent Nos. 5,811,097; 5,855,887; 6,051,227; in PCT Application Publication Nos. WO 01/14424 and WO 00/37504; and in U.S. Patent Publication No. 2002/0039581. Each of these references is specifically incorporated herein by reference for purposes of description of anti-CTLA-4 antibodies. An exemplary clinical anti-CTLA-4 antibody is human monoclonal antibody 10D1 as disclosed in WO 01/14424 and U.S. Patent Application No. 09/644,668. Antibody 10D1 has been administered in single and multiple doses, alone or in combination with a vaccine, chemotherapy, or interleukin-2 to more than 500 patients diagnosed with metastatic melanoma, prostate cancer, lymphoma, renal cell cancer, breast cancer, ovarian cancer, and HIV. Other anti-CTLA-4 antibodies encompassed by the methods of the present invention include, for example, those disclosed in: WO 98/42752; WO 00/37504; U.S. Patent No. 6,207,156; Hurwitz *et al.* (1998) *Proc. Natl. Acad. Sci. USA* 95(17):10067-10071; Camacho *et al.* (2004) *J. Clin. Oncology* 22(145): Abstract No. 2505 (antibody CP-675206); and Mokyr *et al.* (1998) *Cancer Res.* 58:5301-5304. In certain embodiments, the methods of the instant invention comprise use of an anti-CTLA-4 antibody that is a human sequence antibody, preferably a monoclonal antibody and in another embodiment is monoclonal antibody 10D1.

In certain embodiments, the anti-CTLA-4 antibody binds to human CTLA-4 with a K_D of 5×10^{-8} M or less, binds to human CTLA-4 with a K_D of 1×10^{-8} M or less, binds to human CTLA-4 with a K_D of 5×10^{-9} M or less, or binds to human CTLA-4 with a K_D of between 1×10^{-8} M and 1×10^{-10} M or less.

The combination of antibodies is useful for enhancement of an immune response against a hyperproliferative disease by blockade of PD-1 and CTLA-4. In a preferred embodiment, the antibodies of the present invention are human antibodies. For example, these molecules can be administered to cells in culture, *in vitro* or *ex vivo*, or to human subjects, *e.g.*, *in vivo*, to enhance immunity in a variety of situations. Accordingly, in one aspect, the invention provides a method of modifying an immune response in a subject comprising administering to the subject an antibody combination, or a combination of antigen-binding portions thereof, of the invention such that the immune response in the subject is modified. Preferably, the response is enhanced, stimulated or up-regulated. In another

embodiment, the instant disclosure provides a method of altering adverse events associated with treatment of a hyperproliferative disease with an immunostimulatory therapeutic agent, comprising administering an anti-PD-1 antibody and a subtherapeutic dose of anti-CTLA-4 antibody to a subject.

Blockade of PD-1 and CTLA-4 by antibodies can enhance the immune response to cancerous cells in the patient. Cancers whose growth may be inhibited using the antibodies of the instant disclosure include cancers typically responsive to immunotherapy. Representative examples of cancers for treatment with the combination therapy of the instant disclosure include melanoma (*e.g.*, metastatic malignant melanoma), renal cancer, prostate cancer, breast cancer, colon cancer and lung cancer. Examples of other cancers that may be treated using the methods of the instant disclosure include bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers. The present invention is also useful for treatment of metastatic cancers.

In certain embodiments, the combination of therapeutic antibodies discussed herein may be administered concurrently as a single composition in a pharmaceutically acceptable carrier, or concurrently as separate compositions with each antibody in a pharmaceutically acceptable carrier. In another embodiment, the combination of therapeutic antibodies can be administered sequentially. For example, an anti-CTLA-4 antibody and an anti-PD-1 antibody can be administered sequentially, such as anti-CTLA-4 being administered first and anti-PD-1 second, or anti-PD-1 being administered first and anti-CTLA-4 second. Furthermore, if more than one dose of the combination therapy is administered sequentially, the order of the sequential administration can be reversed or kept in the same order at each time point of administration, sequential administrations may be combined with concurrent administrations, or any combination thereof. For example, the first administration of a combination anti-CTLA-4 antibody and anti-PD-1 antibody may be concurrent, the second administration may be sequential with anti-CTLA-4 first and anti-PD-1 second, and the third

administration may be sequential with anti-PD-1 first and anti-CTLA-4 second, etc. Another representative dosing scheme may involve a first administration that is sequential with anti-PD-1 first and anti-CTLA-4 second, and subsequent administrations may be concurrent.

Optionally, the combination of anti-PD-1 and anti-CTLA-4 antibodies can be further combined with an immunogenic agent, such as cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), cells, and cells transfected with genes encoding immune stimulating cytokines (He *et al.* (2004) *J. Immunol.* 173:4919-28). Non-limiting examples of tumor vaccines that can be used include peptides of melanoma antigens, such as peptides of gp100, MAGE antigens, Trp-2, MART1 and/or tyrosinase, or tumor cells transfected to express the cytokine GM-CSF (discussed further below).

A combined PD-1 and CTLA-4 blockade can be further combined with a vaccination protocol. Many experimental strategies for vaccination against tumors have been devised (*see* Rosenberg, S. (2000) Development of Cancer Vaccines, ASCO Educational Book Spring: 60-62; Logothetis, C., 2000, ASCO Educational Book Spring: 300-302; Khayat, D. (2000) ASCO Educational Book Spring: 414-428; Foon, K. (2000) ASCO Educational Book Spring: 730-738; see also Restifo and Sznol, Cancer Vaccines, Ch. 61, pp. 3023-3043 in DeVita *et al.* (eds.), 1997, Cancer: Principles and Practice of Oncology. Fifth Edition). In one of these strategies, a vaccine is prepared using autologous or allogeneic tumor cells. These cellular vaccines have been shown to be most effective when the tumor cells are transduced to express GM-CSF. GM-CSF has been shown to be a potent activator of antigen presentation for tumor vaccination (Dranoff *et al.* (1993) *Proc. Natl. Acad. Sci U.S.A.* 90: 3539-43).

The study of gene expression and large scale gene expression patterns in various tumors has led to the definition of so called tumor specific antigens (Rosenberg (1999) Immunity 10:281-7). In many cases, these tumor specific antigens are differentiation antigens expressed in the tumors and in the cell from which the tumor arose, for example melanocyte antigens gp100, MAGE antigens, and Trp-2. More importantly, many of these antigens can be shown to be the targets of tumor specific T cells found in the host. In certain embodiments, a combined PD-1 and CTLA-4 blockade using the antibody compositions described herein may be used in conjunction with a collection of recombinant proteins and/or peptides expressed in a tumor in order to generate an immune response to these proteins. These proteins are normally viewed by the immune system as self-antigens and are, therefore, tolerant to them. The tumor antigen may also include the protein telomerase, which is required for the synthesis of telomeres of chromosomes and which is expressed in more than 85% of human cancers and in only a limited number of somatic tissues (Kim *et al.* (1994) *Science* 266: 2011-2013). (These somatic tissues may be protected from immune attack by various means). Tumor antigen may also be "neo-antigens" expressed in cancer cells because of somatic mutations that alter protein sequence or create fusion proteins between two

unrelated sequences (*i.e.*, bcr-abl in the Philadelphia chromosome), or idiotype from B cell tumors.

Other tumor vaccines may include the proteins from viruses implicated in human cancers such a Human Papilloma Viruses (HPV), Hepatitis Viruses (HBV and HCV) and Kaposi's Herpes Sarcoma Virus (KHSV). Another form of tumor specific antigen which may be used in conjunction with PD-1 blockade is purified heat shock proteins (HSP) isolated from the tumor tissue itself. These heat shock proteins contain fragments of proteins from the tumor cells and these HSPs are highly efficient at delivery to antigen presenting cells for eliciting tumor immunity (Suot & Srivastava (1995) *Science* 269:1585-1588; Tamura *et al.* (1997) *Science* 278:117-120).

Dendritic cells (DC) are potent antigen presenting cells that can be used to prime antigen-specific responses. DC's can be produced *ex vivo* and loaded with various protein and peptide antigens as well as tumor cell extracts (Nestle *et al.* (1998) *Nature Medicine* 4: 328-332). DCs may also be transduced by genetic means to express these tumor antigens as well. DCs have also been fused directly to tumor cells for the purposes of immunization (Kugler *et al.* (2000) *Nature Medicine* 6:332-336). As a method of vaccination, DC immunization may be effectively further combined with a combined PD-1 and CTLA-4 blockade to activate more potent anti-tumor responses.

A combined PD-1 and CTLA-4 blockade may also be further combined with standard cancer treatments. For example, a combined PD-1 and CTLA-4 blockade may be effectively combined with chemotherapeutic regimes. In these instances, as is observed with the combination of anti-PD-1 and anti-CTLA-4 antibodies, it may be possible to reduce the dose of other chemotherapeutic reagent administered with the combination of the instant disclosure (Mokyr *et al.* (1998) *Cancer Research* 58: 5301-5304). An example of such a combination is a combination of anti-PD-1 and anti-CTLA-4 antibodies further in combination with decarbazine for the treatment of melanoma. Another example is a combination of anti-PD-1 and anti-CTLA-4 antibodies further in combination with interleukin-2 (IL-2) for the treatment of melanoma. The scientific rationale behind the combined use of PD-1 and CTLA-4 blockade with chemotherapy is that cell death, which is a consequence of the cytotoxic action of most chemotherapeutic compounds, should result in increased levels of tumor antigen in the antigen presentation pathway. Other combination therapies that may result in synergy with a combined PD-1 and CTLA-4 blockade through cell death include radiation, surgery, or hormone deprivation. Each of these protocols creates a source of tumor antigen in the host. Angiogenesis inhibitors may also be combined with a combined PD-1 and CTLA-4 blockade. Inhibition of angiogenesis leads to tumor cell death, which may also be a source of tumor antigen to be fed into host antigen presentation pathways.

A combination of PD-1 and CTLA-4 blocking antibodies can also be used in combination with bispecific antibodies that target Fcα or Fcγ receptor-expressing effector cells to tumor cells (see, *e.g.*, U.S. Pat. Nos. 5,922,845 and 5,837,243). Bispecific antibodies can

be used to target two separate antigens. For example anti-Fc receptor/anti tumor antigen (*e.g.*, Her-2/neu) bispecific antibodies have been used to target macrophages to sites of tumor. This targeting may more effectively activate tumor specific responses. The T cell arm of these responses would by augmented by the use of a combined PD-1 and CTLA-4 blockade. Alternatively, antigen may be delivered directly to DCs by the use of bispecific antibodies which bind to tumor antigen and a dendritic cell specific cell surface marker.

In another example, a combination of anti-PD-1 and anti-CTLA-4 antibodies can be used in conjunction with anti–neoplastic antibodies, such as Rituxan® (rituximab), Herceptin® (trastuzumab), Bexxar® (tositumomab), Zevalin® (ibritumomab), Campath® (alemtuzumab), Lymphocide® (eprtuzumab), Avastin® (bevacizumab), and Tarceva® (erlotinib), and the like. By way of example and not wishing to be bound by theory, treatment with an anti–cancer antibody or an anti–cancer antibody conjugated to a toxin can lead to cancer cell death (*e.g.*, tumor cells) which would potentiate an immune response mediated by CTLA-4 or PD-1. In an exemplary embodiment, a treatment of a hyperproliferative disease (*e.g.*, a cancer tumor) may include an anti-cancer antibody in combination with anti-PD-1 and anti-CTLA-4 antibodies, concurrently or sequentially or any combination thereof, which may potentiate an anti–tumor immune responses by the host.

Tumors evade host immune surveillance by a large variety of mechanisms. Many of these mechanisms may be overcome by the inactivation of proteins, which are expressed by the tumors and which are immunosuppressive. These include, among others, TGF-β (Kehrl, J. *et al.* (1986) *J. Exp. Med.* 163: 1037-1050), IL-10 (Howard, M. & O'Garra, A. (1992) *Immunology Today* 13: 198-200), and Fas ligand (Hahne, M. *et al.* (1996) *Science* 274: 1363-1365). In another example, antibodies to each of these entities may be further combined with an anti-PD-1 and anti-CTLA-4 combination to counteract the effects of immunosuppressive agents and favor anti-tumor immune responses by the host.

Other antibodies that may be used to activate host immune responsiveness can be further used in combination with an anti-PD-1 and anti-CTLA-4 combination. These include molecules on the surface of dendritic cells that activate DC function and antigen presentation. Anti-CD40 antibodies are able to substitute effectively for T cell helper activity (Ridge, J. *et al.* (1998) Nature 393: 474-478) and can be used in conjunction with an anti-PD-1 and anti-CTLA-4 combination (Ito, N. *et al.* (2000) *Immunobiology* 201 (5) 527-40). Activating antibodies to T cell costimulatory molecules, such as OX-40 (Weinberg, A. *et al.* (2000) *Immunol* 164: 2160-2169), 4-1BB (Melero, I. *et al.* (1997) *Nature Medicine* 3: 682-685 (1997), and ICOS (Hutloff, A. *et al.* (1999) *Nature* 397: 262-266) may also provide for increased levels of T cell activation.

Bone marrow transplantation is currently being used to treat a variety of tumors of hematopoietic origin. While graft versus host disease is a consequence of this treatment, therapeutic benefit may be obtained from graft vs. tumor responses. A combined PD-1 and

CTLA-4 blockade can be used to increase the effectiveness of the donor engrafted tumor specific T cells.

There are also several experimental treatment protocols that involve *ex vivo* activation and expansion of antigen specific T cells and adoptive transfer of these cells into recipients in order to antigen-specific T cells against tumor (Greenberg, R. & Riddell, S. (1999) *Science* 285: 546-51). These methods may also be used to activate T cell responses to infectious agents such as CMV. *Ex vivo* activation in the presence of anti-PD-1 and anti-CTLA-4 antibodies may be expected to increase the frequency and activity of the adoptively transferred T cells.

As set forth herein, organs can exhibit immune-related adverse events following immunostimulatory therapeutic antibody therapy, such as the GI tract (diarrhea and colitis) and the skin (rash and pruritis) after treatment with anti-CTLA-4 antibody. For example, non-colonic gastrointestinal immune-related adverse events have also been observed in the esophagus (esophagitis), duodenum (duodenitis), and ileum (ileitis) after anti-CTLA-4 antibody treatment.

In certain embodiments, the present invention provides a method for altering an adverse event associated with treatment of a hyperproliferative disease with an immunostimulatory agent, comprising administering a anti-PD-1 antibody and a subtherapeutic dose of anti-CTLA-4 antibody to a subject. For example, the methods of the present invention provide for a method of reducing the incidence of immunostimulatory therapeutic antibody-induced colitis or diarrhea by administering a non-absorbable steroid to the patient. Because any patient who will receive an immunostimulatory therapeutic antibody is at risk for developing colitis or diarrhea induced by such an antibody, this entire patient population is suitable for therapy according to the methods of the present invention. Although steroids have been administered to treat inflammatory bowel disease (IBD) and prevent exacerbations of IBD, they have not been used to prevent (decrease the incidence of) IBD in patients who have not been diagnosed with IBD. The significant side effects associated with steroids, even non-absorbable steroids, have discouraged prophylactic use.

In further embodiments, a combination PD-1 and CTLA-4 blockade (*i.e.*, immunostimulatory therapeutic antibodies anti-PD-1 and anti-CTLA-4) can be further combined with the use of any non-absorbable steroid. As used herein, a "non-absorbable steroid" is a glucocorticoid that exhibits extensive first pass metabolism such that, following metabolism in the liver, the bioavailability of the steroid is low, *i.e.*, less than about 20%. In one embodiment of the invention, the non-absorbable steroid is budesonide. Budesonide is a locally-acting glucocorticosteroid, which is extensively metabolized, primarily by the liver, following oral administration. ENTOCORT EC® (Astra-Zeneca) is a pH- and time-dependent oral formulation of budesonide developed to optimize drug delivery to the ileum and throughout the colon. ENTOCORT EC® is approved in the U.S. for the treatment of mild to moderate Crohn's disease involving the ileum and/or ascending colon. The usual oral

dosage of ENTOCORT EC® for the treatment of Crohn's disease is 6 to 9 mg/day. ENTOCORT EC® is released in the intestines before being absorbed and retained in the gut mucosa. Once it passes through the gut mucosa target tissue, ENTOCORT EC® is extensively metabolized by the cytochrome P450 system in the liver to metabolites with negligible glucocorticoid activity. Therefore, the bioavailability is low (about 10%). The low bioavailability of budesonide results in an improved therapeutic ratio compared to other glucocorticoids with less extensive first-pass metabolism. Budesonide results in fewer adverse effects, including less hypothalamic-pituitary suppression, than systemically-acting corticosteroids. However, chronic administration of ENTOCORT EC® can result in systemic glucocorticoid effects such as hypercorticism and adrenal suppression. *See* PDR 58th ed. 2004; 608-610.

In still further embodiments, a combination PD-1 and CTLA-4 blockade (*i.e.*, immunostimulatory therapeutic antibodies anti-PD-1 and anti-CTLA-4) in conjunction with a non-absorbable steroid can be further combined with a salicylate. Salicylates include 5-ASA agents such as, for example: sulfasalazine (AZULFIDINE®, Pharmacia & UpJohn); olsalazine (DIPENTUM®, Pharmacia & UpJohn); balsalazide (COLAZAL®, Salix Pharmaceuticals, Inc.); and mesalamine (ASACOL®, Procter & Gamble Pharmaceuticals; PENTASA®, Shire US; CANASA®, Axcan Scandipharm, Inc.; ROWASA®, Solvay).

In accordance with the methods of the present invention, a salicylate administered in combination with anti-PD-1 and anti-CTLA-4 antibodies and a non-absorbable steroid can includes any overlapping or sequential administration of the salicylate and the non-absorbable steroid for the purpose of decreasing the incidence of colitis induced by the immunostimulatory antibodies. Thus, for example, methods for reducing the incidence of colitis induced by the immunostimulatory antibodies according to the present invention encompass administering a salicylate and a non-absorbable concurrently or sequentially (*e.g.*, a salicylate is administered 6 hours after a non-absorbable steroid), or any combination thereof. Further, according to the present invention, a salicylate and a non-absorbable steroid can be administered by the same route (*e.g.*, both are administered orally) or by different routes (e.g., a salicylate is administered orally and a non-absorbable steroid is administered rectally), which may differ from the route(s) used to administer the anti-PD-1 and anti-CTLA-4 antibodies.

The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of all figures and all references, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

Examples

Example 1: Generation of Human Monoclonal Antibodies Against PD-1

Antigen

Immunization protocols utilized as antigen both (i) a recombinant fusion protein comprising the extracellular portion of PD-1 and (ii) membrane bound full-length PD-1. Both antigens were generated by recombinant transfection methods in a CHO cell line.

Transgenic HuMab and KM mice™

Fully human monoclonal antibodies to PD-1 were prepared using the HCo7 strain of HuMab transgenic mice and the KM strain of transgenic transchromosomic mice, each of which express human antibody genes. In each of these mouse strains, the endogenous mouse kappa light chain gene has been homozygously disrupted as described in Chen *et al.* (1993) *EMBO J.* 12:811-820 and the endogenous mouse heavy chain gene has been homozygously disrupted as described in Example 1 of PCT Publication WO 01/09187. Each of these mouse strains carries a human kappa light chain transgene, KCo5, as described in Fishwild *et al.* (1996) *Nature Biotechnology* 14:845-851. The HCo7 strain carries the HCo7 human heavy chain transgene as described in U.S. Patent Nos. 5,545,806; 5,625,825; and 5,545,807. The KM strain contains the SC20 transchromosome as described in PCT Publication WO 02/43478.

HuMab and KM Immunizations:

To generate fully human monoclonal antibodies to PD-1, HuMab mice and KM mice™ were immunized with purified recombinant PD-1 fusion protein and PD-1-transfected CHO cells as antigen. General immunization schemes for HuMab mice are described in Lonberg, N. *et al* (1994*) Nature* 368(6474): 856-859; Fishwild, D. *et al.* (1996) *Nature Biotechnology* 14: 845-851 and PCT Publication WO 98/24884. The mice were 6-16 weeks of age upon the first infusion of antigen. A purified recombinant preparation (5-50 μg) of PD-1 fusion protein antigen and 5 -10x10^6 cells were used to immunize the HuMab mice and KM mice™ intraperitonealy, subcutaneously (Sc) or via footpad injection.

Transgenic mice were immunized twice with antigen in complete Freund's adjuvant or Ribi adjuvant IP, followed by 3-21 days IP (up to a total of 11 immunizations) with the antigen in incomplete Freund's or Ribi adjuvant. The immune response was monitored by retroorbital bleeds. The plasma was screened by ELISA (as described below), and mice with sufficient titers of anti-PD-1 human immunogolobulin were used for fusions. Mice were boosted intravenously with antigen 3 days before sacrifice and removal of the spleen. Typically, 10-35 fusions for each antigen were performed. Several dozen mice were immunized for each antigen.

Selection of HuMab or KM Mice™ Producing Anti-PD-1 Antibodies:

To select HuMab or KM mice™ producing antibodies that bound PD-1, sera from immunized mice were tested by ELISA as described by Fishwild, D. *et al.* (1996). Briefly, microtiter plates were coated with purified recombinant PD-1 fusion protein from transfected CHO cells at 1-2 μg /ml in PBS, 100 μl/wells incubated 4 °C overnight then blocked with 200

µl/well of 5% fetal bovine serum in PBS/Tween (0.05%). Dilutions of sera from PD-1-immunized mice were added to each well and incubated for 1-2 hours at ambient temperature. The plates were washed with PBS/Tween and then incubated with a goat-anti-human IgG polyclonal antibody conjugated with horseradish peroxidase (HRP) for 1 hour at room temperature. After washing, the plates were developed with ABTS substrate (Sigma, A-1888, 0.22 mg/ml) and analyzed by spectrophotometer at OD 415-495. Mice that developed the highest titers of anti-PD-1 antibodies were used for fusions. Fusions were performed as described below and hybridoma supernatants were tested for anti-PD-1 activity by ELISA.

Generation of Hybridomas Producing Human Monoclonal Antibodies to PD-1:

The mouse splenocytes, isolated from the HuMab or KM mice, were fused to a mouse myeloma cell line either using PEG based upon standard protocols or electric field based electrofusion using a Cyto Pulse large chamber cell fusion electroporator (Cyto Pulse Sciences, Inc., Glen Burnie, MD). The resulting hybridomas were then screened for the production of antigen-specific antibodies. Single cell suspensions of splenocytes from immunized mice were fused to one-fourth the number of SP2/0 nonsecreting mouse myeloma cells (ATCC, CRL 1581) with 50% PEG (Sigma). Cells were plated at approximately $1x10^5$/well in flat bottom microtiter plate, followed by about two week incubation in selective medium containing 10% fetal bovine serum, 10% P388D1 (ATCC, CRL TIB-63) conditioned medium, 3-5% origen (IGEN) in DMEM (Mediatech, CRL 10013, with high glucose, L-glutamine and sodium pyruvate) plus 5 mM HEPES, 0.055 mM 2-mercaptoethanol, 50 mg/ml gentamycin and 1x HAT (Sigma, CRL P-7185). After 1-2 weeks, cells were cultured in medium in which the HAT was replaced with HT. Individual wells were then screened by ELISA (described above) for human anti-PD-1 monoclonal IgG antibodies. Once extensive hybridoma growth occurred, medium was monitored usually after 10-14 days. The antibody-secreting hybridomas were replated, screened again and, if still positive for human IgG, anti-PD-1 monoclonal antibodies were subcloned at least twice by limiting dilution. The stable subclones were then cultured *in vitro* to generate small amounts of antibody in tissue culture medium for further characterization.

Hybridoma clones 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 were selected for further analysis.

Example 2: Structural Characterization of Human Monoclonal Antibodies 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4

The cDNA sequences encoding the heavy and light chain variable regions of the 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 monoclonal antibodies were obtained from the 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 hybridomas, respectively, using standard PCR techniques and were sequenced using standard DNA sequencing techniques.

The nucleotide and amino acid sequences of the heavy chain variable region of 17D8 are shown in Figure 1A and in SEQ ID NO: 57 and 1, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 17D8 are shown in Figure 1B and in SEQ ID NO: 64 and 8, respectively.

Comparison of the 17D8 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 17D8 heavy chain utilizes a VH segment from human germline VH 3-33, an undetermined D segment, and a JH segment from human germline JH 4b. The alignment of the 17D8 VH sequence to the germline VH 3-33 sequence is shown in Figure 8. Further analysis of the 17D8 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 1A and 8, and in SEQ ID NOs: 15, 22 and 29, respectively.

Comparison of the 17D8 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 17D8 light chain utilizes a VL segment from human germline VK L6 and a JK segment from human germline JK 4. The alignment of the 17D8 VL sequence to the germline VK L6 sequence is shown in Figure 9. Further analysis of the 17D8 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 1B and 9, and in SEQ ID NOs: 36, 43 and 50, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 2D3 are shown in Figure 2A and in SEQ ID NO: 58 and 2, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 2D3 are shown in Figure 2B and in SEQ ID NO: 65 and 9, respectively.

Comparison of the 2D3 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 2D3 heavy chain utilizes a VH segment from human germline VH 3-33, a D segment from human germline 7-27, and a JH segment from human germline JH 4b. The alignment of the 2D3 VH sequence to the germline VH 3-33 sequence is shown in Figure 8. Further analysis of the 2D3 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 2A and 8, and in SEQ ID NOs: 16, 23 and 30, respectively.

Comparison of the 2D3 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 2D3 light chain utilizes a VL segment from human germline VK L6 and a JK segment from human germline JK 4. The alignment of the 2D3 VL sequence to the germline VK L6 sequence is shown in Figure 9. Further analysis of the 2D3 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 2B and 9, and in SEQ ID NOs: 37, 44 and 51, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 4H1 are shown in Figure 3A and in SEQ ID NO: 59 and 3, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 4H1 are shown in Figure 3B and in SEQ ID NO: 66 and 10, respectively.

Comparison of the 4H1 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 4H1 heavy chain utilizes a VH segment from human germline VH 3-33, an undetermined D segment, and a JH segment from human germline JH 4b. The alignment of the 4H1 VH sequence to the germline VH 3-33 sequence is shown in Figure 8. Further analysis of the 4H1 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 3A and 8, and in SEQ ID NOs: 17, 24 and 31, respectively.

Comparison of the 4H1 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 4H1 light chain utilizes a VL segment from human germline VK L6 and a JK segment from human germline JK 1. The alignment of the 4H1 VL sequence to the germline VK L6 sequence is shown in Figure 10. Further analysis of the 4H1 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 3B and 10, and in SEQ ID NOs: 38, 45 and 52, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 5C4 are shown in Figure 4A and in SEQ ID NO: 60 and 4, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 5C4 are shown in Figure 4B and in SEQ ID NO: 67 and 11, respectively.

Comparison of the 5C4 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 5C4 heavy chain utilizes a VH segment from human germline VH 3-33, an undetermined D segment, and a JH segment from human germline JH 4b. The alignment of the 5C4 VH sequence to the germline VH 3-33 sequence is shown in Figure 8. Further analysis of the 5C4 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 4A and 8, and in SEQ ID NOs: 18, 25 and 32, respectively.

Comparison of the 5C4 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 5C4 light chain utilizes a VL segment from human germline VK L6 and a JK segment from human germline JK 1. The alignment of the 5C4 VL sequence to the germline VK L6 sequence is shown in Figure 10. Further analysis of the 5C4 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 4B and 10, and in SEQ ID NOs: 39, 46 and 53, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 4A11 are shown in Figure 5A and in SEQ ID NO: 61 and 5, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 4A11 are shown in Figure 5B and in SEQ ID NO: 68 and 12, respectively.

Comparison of the 4A11 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 4A11 heavy chain utilizes a VH segment from human germline VH 4-39, a D segment from human germline 3-9, and a JH segment from human germline JH 4b. The alignment of the 4A11 VH sequence to the germline VH 4-39 sequence is shown in Figure 11. Further analysis of the 4A11 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 5A and 11, and in SEQ ID NOs: 19, 26 and 33, respectively.

Comparison of the 4A11 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 4A11 light chain utilizes a VL segment from human germline VK L15 and a JK segment from human germline JK 1. The alignment of the 4A11 VL sequence to the germline VK L6 sequence is shown in Figure 12. Further analysis of the 4A11 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 5B and 12, and in SEQ ID NOs: 40, 47 and 54, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 7D3 are shown in Figure 7A and in SEQ ID NO: 62 and 6, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 7D3 are shown in Figure 7B and in SEQ ID NO: 69 and 13, respectively.

Comparison of the 7D3 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 7D3 heavy chain utilizes a VH segment from human germline VH 3-33, a human germline 7-27 D segment, and a JH segment from human germline JH 4b. The alignment of the 7D3 VH sequence to the germline VH 3-33 sequence is shown in Figure 8. Further analysis of the 7D3 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 6A and 8, and in SEQ ID NOs: 20, 27 and 34, respectively.

Comparison of the 7D3 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 7D3 light chain utilizes a VL segment from human germline VK L6 and a JK segment from human germline JK 4. The alignment of the 7D3 VL sequence to the germline VK L6 sequence is shown in Figure 9. Further analysis of the 7D3 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 6B and 9, and in SEQ ID NOs: 41, 48 and 55, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 5F4 are shown in Figure 7A and in SEQ ID NO: 63 and 7, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 5F4 are shown in Figure 7B and in SEQ ID NO: 70 and 14, respectively.

Comparison of the 5F4 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 5F4 heavy chain utilizes a VH segment from human germline VH 4-39, a D segment from human germline 3-9, and a JH segment from human germline JH 4b. The alignment of the 5F4 VH sequence to the germline VH 4-39 sequence is shown in Figure 11. Further analysis of the 5F4 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 7A and 11, and in SEQ ID NOs: 21, 28 and 35, respectively.

Comparison of the 5F4 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 5F4 light chain utilizes a VL segment from human germline VK L15 and a JK segment from human germline JK 1. The alignment of the 5F4 VL sequence to the germline VK L6 sequence is shown in Figure 12. Further analysis of the 5F4 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 7B and 12, and in SEQ ID NOs: 42, 49 and 56, respectively.

Example 3: Characterization of Binding Specificity and Binding Kinetics of Anti-PD-1 Human Monoclonal Antibodies

In this example, binding affinity and binding kinetics of anti-PD-1 antibodies were examined by Biacore analysis. Binding specificity, and cross-competition were examined by flow cytometry.

Binding affinity and kinetics

Anti-PD-1 antibodies were characterized for affinities and binding kinetics by Biacore analysis (Biacore AB, Uppsala, Sweden). Purified recombinant human PD-1 fusion protein was covalently linked to a CM5 chip (carboxy methyl dextran coated chip) via primary amines, using standard amine coupling chemistry and kit provided by Biacore. Binding was measured by flowing the antibodies in HBS EP buffer (provided by Biacore AB) at a concentration of 267 nM at a flow rate of 50 µl/min. The antigen-antibody association kinetics was followed for 3 minutes and the dissociation kinetics was followed for 7 minutes. The association and dissociation curves were fit to a 1:1 Langmuir binding model using BIAevaluation software (Biacore AB). To minimize the effects of avidity in the estimation of the binding constants, only the initial segment of data corresponding to association and dissociation phases were used for fitting. The K_D, k_{on} and k_{off} values that were determined are shown in Table 2.

Table 2. Biacore binding data for PD-1 human monoclonal antibodies.

Sample #	Sample ID	Affinity K_D x 10^{-9} (M)	On rate k_{on} x 10^5 (1/Ms)	Off rate k_{off} x 10^{-4} 1/s
1	17D8	0.16	2.56	0.45
2	2D3	1.20	3.77	4.52
3	4H1	5.46	3.15	1.72
4	5C4	0.73	4.32	3.15
5	4A11	0.13	0.76	0.099
6	7D3	2.49	18.2	4.54
7	5F4	2.91	8.74	2.54

Binding specificity by flow cytometry

Chinese hamster ovary (CHO) cell lines that express recombinant human PD-1 at the cell surface were developed and used to determine the specificity of PD-1 human monoclonal antibodies by flow cytometry. CHO cells were transfected with expression plasmids containing full length cDNA encoding transmembrane forms of PD-1. Binding of the 5C4 and 4H1 anti-PD-1 human monoclonal antibodies was assessed by incubating the transfected cells with the anti-PD-1 human monoclonal antibodies at a concentration of 20 μg/ml. The cells were washed and binding was detected with a FITC-labeled anti-human IgG Ab. Flow cytometric analyses were performed using a FACScan flow cytometry (Becton Dickinson, San Jose, CA). The results are depicted in Figures 13A (5C4) and 13B (4H1). The anti-PD-1 human monoclonal antibodies bound to the CHO cells transfected with PD-1 but not to CHO cells that were not transfected with human PD-1. These data demonstrate the specificity of anti-PD-1 human monoclonal antibodies for PD-1.

Binding specificity by ELISA against other CD28 family members

A comparison of the binding of anti-PD-1 antibodies to CD28 family members was performed by standard ELISA using four different CD28 family members to examine the specificity of binding for PD-1.

Fusion proteins of CD28 family members, ICOS, CTLA-4 and CD28 (R&D Biosystems) were tested for binding against the anti-PD-1 human monoclonal antibodies 17D8, 2D3, 4H1, 5C4, and 4A11. Standard ELISA procedures were performed. The anti-PD-1 human monoclonal antibodies were added at a concentration of 20 μg/ml. Goat-anti-human IgG (kappa chain-specific) polyclonal antibody conjugated with horseradish peroxidase (HRP) was used as secondary antibody. The results are shown in Figure 14. Each of the anti-PD-1 human monoclonal antibodies 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 bound with high specificity to PD-1, but not to the other CD28 family members.

Example 4: Characterization of anti-PD-1 antibody binding to PD-1 expressed on the surface of human and monkey cells

Anti-PD-1 antibodies were tested for binding to cells expressing PD-1 on their cell surface by flow cytometry.

Activated human T-cells, monkey peripheral blood mononuclear cells (PBMC), and CHO cells transfected with PD-1 were each tested for antibody binding. Human T cells and cynomolgous PBMC were activated by anti-CD3 antibody to induce PD-1 expression on T cells prior to binding with a human anti-PD-1 monoclonal antibody. Binding of the 5C4 and 4H1 anti-PD-1 human monoclonal antibodies was assessed by incubating the transfected cells with either IgG1 or IgG4 forms of the anti-PD-1 human monoclonal antibodies at different concentrations. The cells were washed and binding was detected with a FITC-labeled anti-human IgG Ab. Flow cytometric analyses were performed using a FACScan flow cytometry (Becton Dickinson, San Jose, CA). The results are shown in Figures 15A (activated human T cells), 15B (cynomolgous monkey PBMC) and 15C (PD-1-transfected CHO cells). The anti-PD-1 monoclonal antibodies 5C4 and 4H1 bound to activated human T cells, activated monkey PBMCs, and CHO cells transfected with human PD-1, as measured by the mean fluorescent intensity (MFI) of staining. These data demonstrate that the anti-PD-1 HuMAbs bind to both human and cynomolgous monkey cell surface PD-1.

Example 5: Effect of human anti-PD-1 antibodies on cell proliferation and cytokine production in a Mixed Lymphocyte Reaction

A mixed lymphocyte reaction was employed to demonstrate the effect of blocking the PD-1 pathway to lymphocyte effector cells. T cells in the assay were tested for proliferation, IFN-gamma secretion and IL-2 secretion in the presence or absence of an anti-PD-1 HuMAb antibody.

Human T-cells were purified from PBMC using a human CD4+ T cell enrichment column (R&D systems). Each culture contained 10^5 purified T-cells and 10^4 allogeneic dendritic cells in a total volume of 200 µl. Anti-PD-1 monoclonal antibody 5C4, 4H1, 17D8, 2D3 or a Fab fragment portion of 5C4 was added to each culture at different antibody concentrations. Either no antibody or an isotype control antibody was used as a negative control. The cells were cultured for 5 days at 37°C. After day 5, 100 µl of medium was taken from each culture for cytokine measurement. The levels of IFN-gamma and other cytokines were measured using OptEIA ELISA kits (BD Biosciences). The cells were labeled with ^{3}H-thymidine, cultured for another 18 hours, and analyzed for cell proliferation. The results are shown in Figures 16A (T cell proliferation), 16B (IFN-γ secretion) and 16C (IL-2 secretion). The anti-PD-1 human monoclonal antibodies promoted T-cell proliferation, IFN-gamma secretion and IL-2 secretion in a concentration dependent manner. The 5C4-Fab fragment also promoted T-cell proliferation, IFN-gamma secretion and IL-2 secretion in a concentration dependent manner. In contrast, cultures containing the isotype control antibody did not show an increase in T cell proliferation, IFN-gamma or IL-2 secretion.

Example 6: Blocking of ligand binding to PD-1 by human anti-PD-1 antibodies

Anti-PD-1 HuMAbs were tested for the ability to block binding of the ligands PD-L1 and PD-L2 to PD-1 expressed on transfected CHO cells by using a flow cytometry assay.

PD-1 expressing CHO cells were suspended in FACS buffer (PBS with 4% fetal calf serum). Various concentrations of the anti-PD-1 HuMAbs 5C4 and 4H1 were added to the cell suspension and incubated at 4°C for 30 minutes. Unbound antibody was washed off and either FITC-labeled PD-L1 fusion protein or FITC-labeled PD-L2 fusion protein was added into the tubes and incubated at 4°C for 30 minutes. Flow cytometric analyses were performed using a FACScan flowcytometer (Becton Dickinson, San Jose, CA). The results are depicted in Figures 17A (blocking of PD-L1) and 17B (blocking of PD-L2). The anti-PD-1 monoclonal antibodies 5C4 and 4H1 blocked binding of PD-L1 and PD-L2 to CHO cells transfected with human PD-1, as measured by the mean fluorescent intensity (MFI) of staining. These data demonstrate that the anti-PD-1 HuMAbs block binding of ligand (both PD-L1 and PD-L2) to cell surface PD-1.

Example 7: Effect of human anti-PD-1 antibodies on the release of cytokines in human blood

The anti-PD-1 HuMAbs were mixed with fresh human whole blood in order to determine whether the anti-PD-1 HuMAbs alone stimulated the release of certain cytokines from human blood cells.

500 µl of heparinized-fresh human whole blood, was added into each well. Either 10 µg or 100 µg of an anti-PD-1 HuMAb (4H1 or 5C4,the latter either as an IgG1 or IgG4 isotype) was added to each well. Some wells were incubated with anti-CD3 antibody as a positive control, or a human IgG1 or human IgG4 antibody as isotype-matched negative controls. The cells were incubated at 37 °C for either 6 or 24 hours. The cells were spun down and the plasma was collected for measurement of the cytokines IFN-gamma, TNF-alpha, IL-2, IL-4, IL-6, IL-10 and IL-12 using a cytokine cytometric bead array assay (BD Biosciences). The concentration of each cytokine (pg/ml) is shown in Tables 3a, with a 6 hour incubation, and 3b, with a 24 hour incubation, below. The results show that treatment with the human anti-PD-1 antibodies 5C4 and 4H1 alone did not stimulate human blood cells to release any of the cytokines IFN-gamma, TNF-alpha, IL-2, IL-4, IL-6, IL-10 and IL-12.

Table 3a. Cytokine production following 6 hour incubation

Ab	IFN-gamma (pg/ml)	TNF-alpha (pg/ml)	IL-10 (pg/ml)	IL-6 (pg/ml)	IL-4 (pg/ml)	IL-2 (pg/ml)
No Ab	12.3	2	3	5	3.6	1.9
10 mg/ml anti-CD3	5000	530	82.6	510.4	37.2	467.9
100 mg/ml anti-CD3	5000	571	91.3	530	43.9	551.5
10 mg/ml hIgG1	7	1.8	2.8	4.4	2.6	1.5
100 mg/ml hIgG1	0	2.2	2.7	6	2.6	1.4
10 mg/ml hIgG4	5.4	1.4	2.5	4.5	2.1	1.3
100 mg/ml hIgG4	6.4	2.3	3	32.6	2.9	1.4
10 mg/ml 4H1	6.2	1.8	2.4	4.1	2.8	1.6
100 mg/ml 4H1	11.8	2	2.6	3.5	2.6	1.7
10 mg/ml 5C4 IgG1	4.2	1.6	2.3	3.9	2.5	1.3
100 mg/ml 5C4 IgG1	0	1.4	2.2	3.6	2.1	1.2
10 mg/ml 5C4 IgG4	8.3	2.5	1.9	4.8	1.6	1.5
100 mg/ml 5C4 IgG4	3.6	1.7	2.4	3.9	2.3	1.5

Table 3b. Cytokine production following 24 hour incubation

Ab	IFN-gamma (pg/ml)	TNF-alpha (pg/ml)	IL-10 (pg/ml)	IL-6 (pg/ml)	IL-4 (pg/ml)	IL-2 (pg/ml)
No Ab	11.2	2	6.1	5.9	2.6	1.7
10 mg/ml anti-CD3	5000	565.9	432	5000	64.5	1265.3
100 mg/ml anti-CD3	5000	535	461	5000	73.8	1334.9
10 mg/ml hIgG1	0	0	0	0	0	0
100 mg/ml hIgG1	11.5	1.7	7.9	60.8	2.9	1.5
10 mg/ml hIgG4	24.6	3.1	8.3	63.4	3.1	2.3
100 mg/ml hIgG4	11.2	1.8	8	27.7	3.1	2.4
10 mg/ml 4H1	27.3	2.9	8	13.9	5.3	2.6
100 mg/ml 4H1	17.5	2.5	4.4	7	4	2.1
10 mg/ml 5C4 IgG1	9.1	2	7.6	68.5	3.5	1.8
100 mg/ml 5C4 IgG1	12.9	1.9	6.1	25.3	2.9	1.7
10 mg/ml 5C4 IgG4	14	1.9	4.4	3.3	2.6	1.9
100 mg/ml 5C4 IgG4	0	0	0	0	0	0

Example 8: Effect of anti-PD-1 antibodies on the apoptosis of T-cells

The effect of anti-PD-1 antibodies on the induction of apoptosis of T-cells was measured using an annexin V staining test.

T cells were cultured in a mixed lymphocyte reaction, as described above in Example 5. The anti-PD-1 antibody 5C4 was added to the tube at a concentration of 25 μg/ml. A non-specific antibody was used as a control. Annexin V and propidium iodide were added according to standard protocol (BD Biosciences). The mixture was incubated for 15 minutes in the dark at room temperature and then analyzed using a FACScan flowcytometer (Becton Dickinson, San Jose, CA). The results are shown in Figure 18. The anti-PD-1 antibody 5C4 did not have an effect on T-cell apoptosis.

Example 9: Effect of anti-PD-1 antibodies on cytokine secretion by viral-stimulated PBMC cells from a virus positive donor

In this example, peripheral blood mononuclear cells (PBMC) from a donor positive for CMV were isolated and exposed to a CMV lysate in the presence or absence of anti-PD-1 antibodies to examine the effect of the antibodies on cytokine secretion simulated by antigen.

$2x10^5$ human PMBCs from a CMV positive donor were cultured in a total volume of 200 µl and added into each well along with a lysate of CMV-infected cells. The anti-PD-1 HuMAb 5C4 was added to each well in various concentrations for 4 days. After day 4, 100 µl of medium was taken from each culture for cytokine measurement. The level of IFN-gamma was measured using OptEIA ELISA kits (BD Biosciences). The cells were labeled with ^{3}H-thymidine, cultured for another 18 hours, and analyzed for cell proliferation. The cell proliferation was analyzed using the Cell Titer-Glo reagent (Promega). The results are shown in Figure 19. The anti-PD-1 HuMab 5C4 increased IFN gamma secretion in a concentration dependent manner. These results shows that anti-PD-1 HuMAbs can stimulate IFN-gamma release in a memory T cell response from PBMC cells previously stimulated against an antigen.

Example 10: Effect of anti-PD-1 antibody on secondary antibody response to antigen

Mice were immunized and rechallenged with a TI -antigen (DNP-Ficoll) and also treated with a rat anti-mouse-PD-1 antibody, or a control antibody to examine the effect of the anti-PD-1 antibody on antibody titers.

Female C57BL6 mice were divided into two groups, with 6 mice/group. One group was treated with a control rat IgG and the other with a rat anti-mouse PD-1 antibody. The mice were immunized with 5µg of DNP-Ficoll (a T1-antigen) in 50µl CFA by i.p. at day 0. Either the control rat IgG antibody or the rat-mPD-1 antibody (200µg/mouse) was given by i.p. at days -1, 0 and 2. Four weeks later, mice were rechallenged with 5µg of DNP-Ficoll in 50µl IFA by i.p. at day 0. Rat anti-mPD-1 antibody or control antibody (200µg/mouse) was given by i.p. at days 0 and 1. Antibody titers were measured by standard ELISA assay at day 7 following the boost. The results are shown in Table 4 below. In the mice treated with the anti-mPD-1 antibody, both IgM and IgG3 isotypes showed the greatest increase in titer following challenge with the T1-antigen, as compared to mice treated with a control antibody. These results demonstrate that anti-PD-1 treatment can increase antibody titers in response to T1-antigen.

Table 4. Murine secondary response following treatment with anti-PD-1 antibody

Antibody Isotype	Control group	Rat anti-mouse PD-1 antibody	P value
IgM	606	1200	0.026
IgG	9	15.55	0.18
IgG1	1.2	1.1	0.83
IgG2b	5.05	9.26	0.18
IgG3	21.9	81.2	0.03

* Results shown are average concentration of antibody isotype (μg/ml)

Example 11: Treatment of *in vivo* tumor model using anti-PD-1 antibodies

Mice implanted with a cancerous tumor were treated *in vivo* with anti-PD-1 antibodies to examine the *in vivo* effect of the antibodies on tumor growth. As a positive control, an anti-CTLA-4 antibody was used, since such antibodies have been shown to inhibit tumor growth *in vivo*.

In this experiment, the anti-PD-1 antibody used was a chimeric rat anti-mouse-PD-1 antibody generated using well known laboratory techniques. To generate the rat anti-mouse PD-1 antibody, rats were immunized with mouse cells transfected to express a recombinant mouse PD-1 fusion protein (R&D Systems Catalog No. 1021-PD) and monoclonal antibodies were screened for binding to mouse PD-1 antigen by ELISA assay. The rat anti-PD-1 antibody V regions were then recombinantly linked to a murine IgG1 constant region using standard molecular biology techniques and rescreened for binding to mouse PD-1 by ELISA and FACS. The chimeric rat anti-mouse-PD-1 antibody used herein is referred to as 4H2.

For the tumor studies, female AJ mice between 6-8 weeks of age (Harlan Laboratories) were randomized by weight into 6 groups. The mice were implanted subcutaneously in the right flank with 2 x 10^6 SA1/N fibrosarcoma cells dissolved in 200 μl of DMEM media on day 0. The mice were treated with PBS vehicle, or antibodies at 10 mg/kg. The animals were dosed by intraperitoneal injection with approximately 200 μl of PBS containing antibody or vehicle on days 1, 4, 8 and 11. Each group contained 10 animals and the groups consisted of: (i) a vehicle group, (ii) control mouse IgG, (iii) control hamster IgG, (iv) hamster anti-mouse CTLA-4 antibody and (v) the chimeric anti-PD-1 antibody 4H2. The mice were monitored twice weekly for tumor growth for approximately 6 weeks. Using an electronic caliper, the tumors were measured three dimensionally (height x width X length) and tumor volume was calculated. Mice were euthanized when the tumors reached tumor end point (1500 mm^3) or show greater than 15% weight loss. The results are shown in Figure 20. The anti-PD-1 antibody extended the mean time to reaching the tumor end point volume (1500 mm^3) from ~25 days in the control groups to ~40 days. Thus, treatment with an anti-PD-1 antibody has a direct *in vivo* inhibitory effect on tumor growth.

Example 12: Generation of Chimeric (Rat–Mouse) anti-PD-1 Antibody 4H2

Rat monoclonal antibody against mouse PD-1 antibodies (rat anti-mPD-1) were generated from rats immunized with mPD-1-hFc fusion protein using standard hybridoma production methods (see Kohler and Milstein (1975) *Nature* 256:495; and Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor New York). Eight hybridomas were subcloned, and antibodies were isolated and screened for their ability to block mouse PD-L2 (mPD-L2) binding to mPD-1. Several anti-mPD-1 antibodies capable of blocking mPD-L2 binding to mPD-1 were identified (*see, e.g.*, activity of 4H2, Figure 41) and the binding affinity of several of these antibodies to mPD-1-Fc fusion protein was determined by ELISA (Figure 42).

Antibody 4H2.B3 was further characterized, which is referred to interchangeably herein as "4H2." CHO cells expressing mouse PD-1 were constructed and incubated with 4H2 anti-mPD-1 antibody at a concentration ranging from 200 μg/ml to 0.012 μg/ml to determine the binding affinity of 4H2 to PD-1. Binding of anti-mPD-1 antibody to the PD-1 expressing CHO cells was detected by incubating with donkey-anti-rat IgG, FITC conjugated and measured by FACS. The anti-mPD-1 antibody had an EC_{50} (50% effective concentration) of about 0.38 μg (Figure 43) and a K_D of 4.7×10^{-9} M. To examine the inhibition of PD-L1 binding to PD-1, the same assay was performed except that the cells were also incubated with 0.16 μg mPD–L1-hFc fusion protein, then binding of PD-L1 to the PD-1 expressing CHO cells was detected by incubating with goat-anti-human IgG (Fc specific), FITC conjugated and measuring binding signal by FACS (MFI, mean fluorescence intensity). The anti-mPD-1 antibody had an EC_{50} of about 0.72 μg (Figure 44).

For use in the mouse tumor models, the 4H2 rat anti-mPD-1 needed to be modified so the mouse immune system would not neutralize the immunotherapeutic antibody (*i.e.*, so the antibody would have better pharmacokinetics) and to avoid antibody-dependent cellular cytotoxicity (ADCC) by reducing Fc receptor interactions (*i.e.*, so blockade by anti-PD-1 could be evaluated with being compromised by ADCC effects). The original rat anti-mPD-1 antibody, 4H2, was determined to be a rat IgG2a isotype. Hence, the Fc-portion of the 4H2 antibody was replaced with an Fc-portion from a mouse IgG1 isotype. Using the assay described above,the binding affinity of the rat–mouse chimeric 4H2 to mPD-1 was found to be comparable to the rat 4H2.B3 anti-mPD-1 antibody (Figure 45). Similarly, inhibition of PD-L1 binding to PD-1 was comparable for both antibodies (Figure 46). Thus, the rat-mouse chimeric 4H2 anti-mPD-1 antibody was used to examine the therapeutic efficacy of anti-PD-1 in combination with anti-CTLA-4.

Example 13: *In Vivo* Efficacy of Combination Therapy (anti-CTLA-4 and anti-PD-1 Antibodies) on Tumor Establishment and Growth

MC38 colorectal cancer cells (PD-L1$^-$) (available from Dr. N. Restifo, National Cancer Institute, Bethesda, MD; or Jeffrey Schlom, National Institutes of Health, Bethesda, MD) were

implanted in C57BL/6 mice (2 x 10^6 cells/mouse). On day 0 (*i.e.*, the day the MC38 cells were implanted in the mice), each of four groups of 10 mice each was injected intraperitoneally (IP) with one of the following: (1) mouse IgG (control), (2) anti-CTLA-4 monoclonal antibody 9D9 (mouse anti-mouse CTLA-4, obtained from J. Allison, Memorial Sloan–Kettering Cancer Center, New York, NY), (3) anti-PD-1 monoclonal antibody 4H2 (chimeric antibody in which a rat anti-mouse PD-1 was modified with a mouse Fc region, as described in Example 6), or (4) anti-CTLA-4 antibody 9D9 and anti-PD-1 antibody 4H2. Antibody injections were then further administered on days 3, 6 and 10. The single antibody treatments were dosed at 10 mg/kg, and the combination of anti-CTLA-4 antibody and anti-PD-1 antibody was dosed at 5 mg/kg of each antibody (*i.e.*, 10 mg/kg of total antibody). Using an electronic caliper, the tumors were measured three dimensionally (height x width x length) and tumor volume was calculated. Mice were euthanized when the tumors reached a designated tumor end-point. The results are shown in Table 5 and Figure 21.

Table 5. Percentage of Tumor-Free Mice Following Anti–PD-1 and/or Anti–CTLA–4 Treatment

Treatment	Total mice studied	Tumor-free mice (%)
mIgG1	10	0
anti-CTLA-4	10	1 (10)
anti-PD-1	10	3 (30)
anti-CTLA-4 + anti-PD-1	10	6 (60)

Eight mice in the IgG group reached the tumor end-point by about day 30 and two mice (86066 and 87260) in the IgG group had ulcerated tumors (Figure 21A). In the anti-CTLA-4 antibody alone group, seven mice reached the tumor end-point by about day 60, one mouse had an ulcerated tumor (84952), one mouse had a tumor with a volume of less than 1500 mm^3 (85246), and one mouse was tumor-free (86057) (Figure 21B). In the anti-PD-1 antibody alone group, six mice reached the tumor end-point by about day 60, one mouse had an ulcerated tumor (86055), and three mice were tumor-free (84955, 85239 and 86750) (Figure 21C). In the anti-CTLA-4 antibody and anti-PD-1 antibody combination group, four mice reached the tumor end-point by about Day 40, and six mice were tumor-free (84596, 85240, 86056, 86071, 86082 and 86761) (Figure 21D).

Figure 22 shows that the mean tumor volume measured at day 21 was about 2955 mm^3 for the IgG control group; about 655 mm^3 for the CTLA-4 antibody alone group, about 510 mm^3 for the PD-1 antibody alone group, and about 280 mm^3 for the anti-CTLA-4 antibody and anti-PD-1 antibody combination group. Figure 23 shows that the median tumor volume measured at day 21 was about 2715 mm^3 for the IgG group; about 625 mm^3 for the CTLA-4 antibody alone group; about 525 mm^3 for the PD-1 antibody alone group; and about 10 mm^3

for the CTLA-4 antibody and PD-1 antibody combination group (and down to 0 mm^3 by day 32).

This study indicates that, in a murine tumor model, CTLA-4 antibody treatment alone and PD-1 antibody treatment alone have a modest effect on tumor growth, and that the combination treatment of CTLA-4 antibody and PD-1 antibody has a significantly greater effect on tumor growth. It is interesting to note that the combination treatment with CTLA-4 antibody and PD-1 antibody had a more significant effect on tumor growth at a dose of 5 mg/kg of each antibody as compared to the effect of either antibody alone when each is administered at a higher dose of 10 mg/kg.

Example 14: *In Vivo* Efficacy of Combination Therapy (anti-CTLA-4 and anti-PD-1 Antibodies) on Established Tumor Growth

MC38 colorectal cancer cells (PD-L1$^-$) were implanted in C57BL/6 mice (2×10^6 cells/mouse) for a time sufficient (about 6 to 7 days) to permit the formation of tumors. On day 6 post-implantation (day -1), tumor measurements were taken and mice were randomized based on mean tumor volume (about 250 mm^3) into 11 groups for subsequent antibody therapy. At day 0 (*i.e.*, one week after the MC38 cells were implanted), mice were injected IP with (1) mouse IgG (control), (2) anti-CTLA-4 monoclonal antibody 9D9, (3) anti-PD-1 monoclonal antibody 4H2, or (4) anti-CTLA-4 monoclonal antibody 9D9 and anti-PD-1 antibody monoclonal antibody 4H2, at a concentration of 10 mg/kg per mouse. Antibody injections were also administered on days 3, 6 and 10. The monoclonal antibody compositions used had low levels of endotoxin and did not significantly aggregate. Using an electronic caliper, the tumors were measured three dimensionally (height x width x length) and tumor volume was calculated. Tumor measurements were taken on day 0 (tumors at the beginning of treatment had a volume of about 125 mm^3), and on days 3, 6, 10, 13, 17 and 20 post-antibody injection. Mice were euthanized when the tumors reached a designated tumor end-point (a particular tumor volume such as 1500 mm^3 and/or when the mice showed greater than about 15% weight loss).

All eleven mice in the IgG group reached the tumor end-point by about day 17 (Figure 24A). In the anti-CTLA-4 antibody alone group, seven of eleven mice reached the tumor end-point by about day 12 (Figure 24B). In the anti-PD-1 antibody alone group, four mice reached the tumor end-point by about day 13 and two mice were tumor-free (Figure 24C). In the anti-CTLA-4 antibody and anti-PD-1 antibody combination group, one mouse reached the tumor end-point by about day 17, one mouse reached the tumor end-point by about day 45 and nine mice were tumor-free on day 45 (Figure 24D).

Figure 25 shows that the mean tumor volume measured at day 10 was about 1485 mm^3 for the IgG control group; about 1010 mm^3 for the CTLA-4 antibody alone group; about 695 mm^3 for the PD-1 antibody alone group; and about 80 mm^3 for the anti-CTLA-4 antibody and anti-PD-1 antibody combination group. Figure 26 shows that the median tumor volume

measured at day 10 was about 1365 mm^3 for the IgG group; about 1060 mm^3 for the anti-CTLA-4 antibody alone group; about 480 mm^3 for the anti-PD-1 antibody alone group; and about 15 mm^3 for the anti-CTLA-4 antibody and anti-PD-1 antibody combination group (which was down to 0 mm^3 by day 17).

This study indicates that, in a murine tumor model, treatment with the combination of CTLA-4 antibody and PD-1 antibody has a significantly greater effect on tumor growth than either antibody alone, even when a tumor is already well established.

Example 15: Dose Titration of Combination Therapy (anti-CTLA-4 and anti-PD-1 Antibodies) on Established Tumor Growth

MC38 colorectal cancer cells (PD-L1$^-$) were implanted in C57BL/6 mice (2 x 10^6 cells/mouse) for a time sufficient (about 6 to 7 days) to permit the formation of tumors as described in Example 3. Groups of 10 mice were injected IP at days 0, 3, 6 and 10 as follows: Group (A) mouse IgG (control, 20 mg/kg), Group (B) anti-PD-1 monoclonal antibody 4H2 (10 mg/kg) and mouse IgG (10 mg/kg), Group (C) anti-CTLA-4 monoclonal antibody 9D9 (10 mg/kg) and mouse IgG (10 mg/kg), Group (D) anti-CTLA-4 monoclonal antibody 9D9 (10 mg/kg) and anti-PD-1 antibody monoclonal antibody 4H2 (10 mg/kg), Group (E) anti-CTLA-4 monoclonal antibody 9D9 (3 mg/kg) and anti-PD-1 antibody monoclonal antibody 4H2 (3 mg/kg), or Group (F) anti-CTLA-4 monoclonal antibody 9D9 (1 mg/kg) and anti-PD-1 antibody monoclonal antibody 4H2 (1 mg/kg). Using an electronic caliper, the tumors were measured three dimensionally (height x width x length) and tumor volume was calculated. Tumor measurements were taken at the beginning of treatment (*i.e.*, on day 0 tumors had an average volume of about 90 mm^3), and on days 3, 6, 10, 13, 17 and 20 post-antibody treatment. Mice were euthanized when the tumors reached a designated tumor end-point (a particular tumor volume such as 1500 mm^3 and/or when the mice showed greater than about 15% weight loss).

Figure 27A shows that all 10 control mice had reached a tumor end-point. Figure 27B shows that the group treated with 10 mg/kg anti-PD-1 antibody (Group B) had 6 mice that reached the tumor end–point and 4 mice with tumors having a volume of about 750 mm^3 or less. Figure 27C shows that the group treated with 10 mg/kg anti-CTLA-4 antibody (Group C) had 3 mice that reached the tumor end-point and 7 mice with tumors having a volume of about 1000 mm^3 or less. Figure 27D shows that the group treated with a combination of 10 mg/kg anti-PD-1 antibody with 10 mg/kg anti-CTLA-4 antibody (Group D) had 2 mice with tumors having a volume of about 1000 mm^3 or less, and 8 mice that were tumor free. Figure 27E shows that the group treated with a combination of 3 mg/kg anti-PD-1 antibody with 3 mg/kg anti-CTLA-4 antibody (Group E) had one mouse that had reached the tumor end-point, 7 mice with tumors having a volume of about 500 mm^3 or less, and 2 mice that were tumor free. Figure 27F shows that the group treated with a combination of 1 mg/kg anti-PD-1 antibody with 1 mg/kg anti-CTLA-4 antibody (Group F) had 4 mice that had reached the

tumor end-point, 5 mice with tumors having a volume of about 1100 mm^3 or less, and one mouse that was tumor free.

Figures 27G and 27H show the tumor volumes in mice treated sequentially with anti–PD-1 antibody first and anti-CTLA-4 antibody second, and vice versa. The mice of Figure 27G first received 10 mg/kg anti-CTLA-4 on each of days 0 and 3, and then received 10 mg/kg anti-PD-1 antibody on each of days 6 and 10. The mice of Figure 27H first received 10 mg/kg anti-PD-1 antibody on each of days 0 and 3, and then received 10 mg/kg anti-CTLA-4 antibody on each of days 6 and 10. For group G at day 27, 8 mice reached the tumor end-point, one mouse had a very small tumor (which, after a significant delay, eventually grew out) and one mouse was tumor free. For group H at day 27, 8 mice reached the tumor end-point and 2 were tumor free.

Figure 28 shows that the mean tumor volume measured at day 10 was about 1250 mm^3 for the IgG control group; about 470 mm^3 for the PD-1 antibody with the IgG control; about 290 mm^3 for the CTLA-4 antibody with the IgG control (measured at day 6); about 40 mm^3 for the anti-CTLA-4 antibody (10 mg/kg) and anti-PD-1 antibody (10 mg/kg) combination group; about 165 mm^3 for the anti-CTLA-4 antibody (3 mg/kg) and anti-PD-1 antibody (3 mg/kg) combination group; and about 400 mm^3 for the anti-CTLA-4 antibody (1 mg/kg) and anti-PD-1 antibody (1 mg/kg) combination group. Figure 29 shows that the median tumor volume measured at day 13 was about 1680 mm^3 for the IgG control group; about 400 mm^3 for the PD-1 antibody with the IgG control; about 660 mm^3 for the CTLA-4 antibody with the IgG control; 0 mm^3 for the anti-CTLA-4 antibody (10 mg/kg) and anti-PD-1 antibody (10 mg/kg) combination group; about 90 mm^3 for the anti-CTLA-4 antibody (3 mg/kg) and anti-PD-1 antibody (3 mg/kg) combination group; and about 650 mm^3 for the anti-CTLA-4 antibody (1 mg/kg) and anti-PD-1 antibody (1 mg/kg) combination group. For the combination treatment of the anti-PD-1 antibody with the anti-CTLA-4 antibody, the number of mice per group that were tumor free at day 27 of the study was 8/10 (10 mg/kg), 2/10 (3 mg/kg) and 1/10 (1 mg/kg) (data not shown).

This study indicates that, in a murine tumor model, treatment with the combination of CTLA-4 antibody and PD-1 antibody functions in a dose dependent manner and has a significantly greater effect on tumor growth than both antibodies alone, even at a lower dose and even when a tumor is already well established. Moreover, the antibodies may be administered sequentially (anti-CTLA-4 antibody first and anti-PD-1 antibody second, or vice versa) and the combination is still superior to the antibody monotherapies.

Example 16: *In Vivo* Efficacy of Combination Therapy (anti-CTLA-4 and anti-PD-1 Antibodies) on Fibrosarcoma Establishment and Growth

SA1/N fibrosarcoma cells (PD-L1$^-$) (Leach *et al.* (1996) *Science* 271:1734-1736) were implanted subcutaneously in A/J mice (2 x 10^6 cells/mouse) on day 0. On days 1, 4, 7 and 11 post-implantation, mice were injected IP as follows: Group (A) PBS alone (referred to as the

"vehicle"); Group (B) mouse IgG (control, 10 mg/kg per mouse), Group (C) anti-PD-1 monoclonal antibody 4H2 (10 mg/kg per mouse), Group (D) anti-CTLA-4 monoclonal antibody 9D9 (10 mg/kg or 0.2 mg/kg per mouse), and Group (E) anti-PD-1 monoclonal antibody 4H2 (10 mg/kg per mouse) in combination with anti-CTLA-4 monoclonal antibody 9D9 (0.2 mg/kg per mouse). The study lasted 41 days and tumor measurements were taken on various days throughout the course of the study (*see* Figure 29). Tumor volume was calculated by measuring tumors in three dimensions (height x width x length) using an electronic caliper. Mice were euthanized when the tumors reached a designated tumor end-point-a volume of 1500 mm^3 and/or an ulcerated tumor.

Figures 30A and 30B show that 19 out of the 20 control (9/10 in Group A and 10/10 in Group B) mice had either reached a tumor end-point or had developed ulcerated tumors. Figure 30C shows that the group treated with 10 mg/kg anti-PD-1 antibody (Group C) had 6 mice that reached a tumor end-point (2 with a volume greater than 1500 mm^3 and 4 with an ulcerated tumor) and 4 mice that were tumor free. Figure 30D shows that the group treated with 10 mg/kg anti-CTLA-4 antibody (Group D) had 5 mice that reached a tumor end-point (2 with a volume greater than 1500 mm^3 and 3 with an ulcerated tumor), one mouse with a small tumor (volume of about 70 mm^3) and 4 mice that were tumor free. Figure 30E shows that the group treated with 0.2 mg/kg anti-CTLA-4 antibody (Group E) had 10 mice that reached a tumor end-point (6 with a volume greater than 1500 mm^3 and 4 with an ulcerated tumor). Figure 30F shows that the group treated with a combination of 10 mg/kg anti-PD-1 antibody with 0.2 mg/kg anti-CTLA-4 antibody (Group F) had 2 mice that reached a tumor end–point (one with a volume greater than 1500 mm^3 and one with an ulcerated tumor) and 8 mice that were tumor free.

Figures 31 and 32 show the mean and median tumor volume, respectively, that developed in treated and untreated mice over the course of this study. The tumor growth inhibition in mice treated with these antibodies, as compared to mice treated with the control antibody mouse IgG, is summarized in Table 6.

Table 6. Tumor Growth Inhibition and Tumor Free Mice Following Anti-PD-1 and/or Anti-CTLA-4 Treatment

Group†	Median Tumor Volume – mm^3 (Day 15)	TGI* (%) (Day 15)	Median Tumor Volume – mm^3 (Day 19)	TGI (%) (Day 19)	No. of Tumor Free Mice (Day 41)
A	985	–	1140	–	0/10
B	635	–	1060	–	0/10
C	465	27	310	71	4/10
D	235	63	90	91	4/10
E	600	6	805	24	0/10
F	330	48	90	92	8/10

* TGI = tumor growth inhibition; the median could be calculated only when fewer than 50% of the mice reached the tumor end point.

† Groups are as defined in Figure 30. A = vehicle (PBS); B = mouse IgG; C = anti-PD-1, 10 mg/kg; D = anti-CTLA-4, 10 mg/kg; E = anti-CTLA-4, 0.2 mg/kg; and F = anti-PD-1, 10 mg/kg with anti-CTLA-4, 0.2 mg/kg.

These data further indicate that the combination therapy comprising anti-PD-1 and anti-CTLA-4 antibodies is substantially more effective than treatment with either antibody alone. Indeed, the combination is still more effective than single antibody treatments even when the combination therapy contains a subtherapeutic dose of anti-CTLA-4 antibody. These data also indicate that surprisingly the presence or absence of PD-L1 on the tumor may have no effect on the efficacy of treatment with this antibody combination, although the presence of PD-L1 may influence the effect of the antibody monotherapies in that expression of PD-L1 on the tumor may also lead to inhibition of anti-tumor T cell responses (*see* Figure 40).

Example 17: *In Vivo* Efficacy and Dose Titration of Combination Therapy (anti-CTLA-4 and anti-PD-1 Antibodies) on PD-L1⁻ Fibrosarcoma Growth

SA1/N fibrosarcoma cells (PD-L1⁻) were implanted subcutaneously in A/J mice (2 x 10^6 cells/mouse) on day 0 for a time sufficient (about 7 days) to permit the establishment of a tumor. On days 7, 10, 13 and 16 post-implantation, ten groups of 8 mice having an average tumor volume of 110 mm^3 were injected IP as follows: Group (A) PBS alone (referred to as the "vehicle"); Group (B) mouse IgG (control, 10 mg/kg per mouse); Group (C) anti-CTLA-4 monoclonal antibody 9D9 (0.25 mg/kg); Group (D) anti-CTLA-4 monoclonal antibody 9D9 (0.5 mg/kg per mouse); Group (E) anti-CTLA-4 monoclonal antibody 9D9 (5 mg/kg); Group (F) anti-PD-1 monoclonal antibody 4H2 (3 mg/kg per mouse); Group (G) anti-PD-1 monoclonal antibody 4H2 (10 mg/kg per mouse); Group (H) anti-PD-1 monoclonal antibody 4H2 (10 mg/kg per mouse) in combination with anti-CTLA-4 monoclonal antibody 9D9 (0.25 mg/kg per mouse); Group (I) anti-PD-1 monoclonal antibody 4H2 (10 mg/kg per mouse) in combination with anti-CTLA-4 monoclonal antibody 9D9 (0.5 mg/kg per mouse); and Group

(J) anti-PD-1 monoclonal antibody 4H2 (3 mg/kg per mouse) in combination with anti-CTLA-4 monoclonal antibody 9D9 (0.5 mg/kg per mouse).

On days 10, 13, 16 and 19 post-implantation, two groups of 6 mice having an average tumor volume of 255 mm^3 were injected IP as follows: Group (K) mouse IgG (control, 10 mg/kg per mouse); and Group (L) anti-PD-1 monoclonal antibody 4H2 (10 mg/kg per mouse) in combination with anti-CTLA-4 monoclonal antibody 9D9 (1 mg/kg per mouse). The study lasted 51 days and tumor measurements were taken on various days throughout the course of the study (*see* Figures 33-38). Tumor volume was calculated by measuring tumors in three dimensions (height x width x length) using an electronic caliper. Mice were euthanized when the tumors reached a designated tumor end-point-a volume of 1500 mm^3 and/or an ulcerated tumor.

Figure 33 shows the response to immunostimulatory antibody treatment in mice with tumors having an initial volume of about 110 mm^3 (*i.e.*, at the time of the first antibody treatment. Figures 33A and 33B show that all 16 control mice (Groups A and B) reached a tumor end-point (15 with a tumor volume greater than 1500 mm^3 and 1 with an ulcerated tumor). Figures 33C-33E show that tumor bearing mice respond to treatment with anti-CTLA-4 antibody in a dose-dependent manner (*e.g.*, Group C receiving 0.25 mg/kg had 7/8 mice reach the tumor end-point and one mouse with a tumor volume less than 200 mm^3, whereas Group E receiving 5 mg/kg had 6/8 mice reach the tumor end-point and two mice were tumor free). Figures 33F and 33G show that mice responded about the same regardless of the anti-PD-1 antibody dose (Group F received 3 mg/kg and Group G received 10 mg/kg). In contrast, the mice receiving a combination treatment of 10 or 3 mg/kg anti-PD-1 antibody with 0.25 or 0.5 mg/kg anti-CTLA-4 antibody (Groups H, I and J) showed a significant reduction in tumor growth. For example, Figure 33J shows that the group treated with a combination of 3 mg/kg anti-PD-1 antibody with 0.5 mg/kg anti-CTLA-4 antibody (Group J) had 2 mice that had ulcerated tumors, 2 mice with a tumor volume less than 500 mm^3, and 4 mice that were tumor free. The unexpected synergistic effect of an anti-PD-1 antibody combined with an anti-CTLA-4 antibody, along with the surprising effectiveness of subtherapeutic levels of anti-CTLA-4 antibody in the combination, are shown in Figures 34 (mean tumor volume) and 35 (median tumor volume).

Figure 36 shows the response to immunostimulatory antibody treatment in mice with larger tumors, those having an initial volume of about 250 mm^3 (*i.e.*, at the time of the first antibody treatment). Figure 36A shows that all 6 control mice (Group K) reached a tumor end-point (4 with a tumor volume greater than 1500 mm^3 and 2 with an ulcerated tumor). Figure 36B shows that the group treated with a combination of 10 mg/kg anti-PD-1 antibody with 1 mg/kg anti-CTLA-4 antibody (Group L) had one mouse with an ulcerated tumor, 4 mice with a tumor volume greater than 1500 mm^3, and one mouse that was tumor free. The mean and median tumor volumes are shown in Figures 37 and 38.

The tumor growth inhibition in mice treated with these antibodies, as compared to mice treated with the control antibody mouse IgG, is summarized in Table 7 and Figure 39.

Table 7. Tumor Growth Inhibition Following Anti-PD-1 and/or Anti-CTLA-4 Treatment

Group	Mean Tumor Volume – mm^3 (Day 23)	TGI* (Mean)	Median Tumor Volume – mm^3 (Day 23)	TGI (Median)	Tumor Free Mice (Day 51)	No. Mice at Tumor End Point
A	700	–	1,380	–	–	–
B	1,710	–	1,360	–	–	–
C	1,050	39%	925	32	–	–
D	770	55%	505	63	–	–
E	155	91%	100	93	2/8	6/8
F	1,050	39%	675	50	–	7/8
G	1,070	37%	1,145	16	–	6/8
H	85	95%	25	98	4/8	3/8
I	75	96%	60	95	4/8	1/8
J	80	95%	5	99	4/8	0/8
K	1,900	–	2,125	–	–	–
L	1,115	41	1,090	49	1/6	–

* TGI = tumor growth inhibition; the median could only be calculated when fewer than 50% of the mice reached the tumor end point.

† Groups are as defined in Figures 33 and 36. For smaller initial tumor: A = vehicle (PBS); B = mouse IgG, 10 mg/kg; C = anti-CTLA-4, 0.25 mg/kg; D = anti-CTLA-4, 0.5 mg/kg; E = anti-CTLA-4, 5 mg/kg; F = anti-PD-1, 3 mg/kg; G = anti-PD-1, 10 mg/kg; H = anti-PD-1, 10 mg/kg with anti-CTLA-4, 0.25 mg/kg; I = anti-PD-1, 10 mg/kg with anti-CTLA-4, 0.5 mg/kg; and J = anti-PD-1, 3 mg/kg with anti-CTLA-4, 0.5 mg/kg. For larger initial tumor: K = mouse IgG, 10 mg/kg; and L = anti-PD-1, 10 mg/kg with anti-CTLA-4, 0.25 mg/kg.

Together these data indicate that the combination therapy comprising anti-PD-1 and anti-CTLA-4 antibodies is substantially more effective than treatment with either antibody alone. In addition, surprisingly the dose of each antibody can be reduced without affecting the synergistic efficacy of this combination of immunostimulatory therapeutic antibodies. The combination therapy still seems to be effective even when the tumor mass is more mature (*i.e.*, larger).

Example 18: Tumor Immunity in Mice Following anti-PD-1 Antibody Treatment and Re-Challenge with PD-L1$^-$ Fibrosarcoma Cells

Mice that survived tumor-free from a challenge with tumor cells and treatment with anti-PD-1 antibody (*i.e.*, treatment similar to the efficacy studies described in Examples 5 and 6) were then re-challenged with tumor cells to investigate immunity to tumor formation after such a treatment. Briefly, in the initial challenge, SA1/N fibrosarcoma cells (PD-L1$^-$) were implanted subcutaneously in A/J mice (1 x 10^6 cells/mouse) on day 0. On days 1, 4, 7, 10, 14, 17 and 20 post-implantation, groups of mice were injected IP with either mouse IgG (control, 10 mg/kg per mouse) or with one of various doses of anti-PD-1 monoclonal antibody 4H2 (30,

10, 3, 1 and 0.3 mg/kg per mouse). Tumor formation and volume was monitored with a precision electronic caliper twice a week until the study was complete. A group of 8 mice were tumor-free after the anti-PD1 antibody treatment (4 that were treated with 30 mg/kg, 2 with 3 mg/kg, one with 1 mg/kg, and one with 0.3 mg/kg).

The eight treated, tumor-free A/J mice were re-challenged by subcutaneously implanting 1 x 10^6 SA1/N fibrosarcoma cells/mouse. As a control, nine naïve mice were subcutaneously implanted with 1 x 10^6 SA1/N fibrosarcoma cells/mouse. Tumor formation and volume was monitored with a precision electronic caliper twice a week until day 62 post-implantation. All nine naïve (control) mice reached the tumor end-point by day 22 post-implantation of the fibrosarcoma cells. In contrast, the eight tumor-free mice re-challenged with fibrosarcoma cells did not develop tumors up to 62 days post-implantation. Figure 47 shows the mean tumor volume for the naïve and re-challenged mice. These results demonstrate that treatment with an immunostimulatory antibody, such as anti-PD-1, provides the treated subject with immunity to further tumor formation, even in the presence of cells capable of forming a tumor.

Example 19: Tumor Immunity in Mice Following Single Antibody Therapy (anti-PD-1) or Combination Antibody Therapy (anti-CTLA-4 and anti-PD-1 Re-Challenged with PD-L1$^-$ Colorectal Cancer Cells

Mice that survived tumor-free from a challenge with tumor cells and treatment with either anti-PD-1 antibody alone or anti-PD-1 antibody combined with anti-CTLA-4 antibody (*i.e.*, treatment similar to the efficacy studies described in Examples 2-4) were then re-challenged with tumor cells to investigate immunity to tumor formation after such treatments. Briefly, in the initial challenge, MC38 colorectal cancer cells (PD-L1$^-$) were implanted in C57BL/6 mice (2 x 10^6 cells/mouse) on day 0. On days 0, 3, 6 and 10 post-implantation, groups of mice were injected IP with one of the following treatments: (1) mouse IgG (control, 10 mg/kg per mouse), anti-PD-1 monoclonal antibody 4H2, or (3) anti-PD-1 monoclonal antibody 4H2 in combination with anti-CTLA-4 monoclonal antibody 9D9. Tumor growth was monitored with a precision electronic caliper as described in Example 15. A group of 11 mice were tumor-free after the anti-PD1 antibody treatment (2 total) or the combination anti-PD-1/anti-CTLA-4 antibody treatment (9 total).

The 11 treated, tumor-free C57BL/6 mice were re-challenged by implantation of 2 x 10^7 MC38 colorectal cancer cells/mouse (*i.e.*, a dose of cells 10 x greater than the initial challenge). As a control, seven naïve mice were implanted with 2 x 10^7 MC38 colorectal cancer cells/mouse. Tumor formation and volume was monitored with a precision electronic caliper for the duration of the re-challenge experiment (at least 20 days). Figure 48 shows that all seven naïve (control) mice developed a tumor and reached the tumor end-point by day 18 post-implantation of the colorectal cancer cells. In contrast, all 11 tumor-free mice re-challenged with colorectal cancer cells did not develop tumors up to 18 days post-implantation.

Figure 49 shows the mean tumor volume for the naïve and re-challenged mice. These data indicate that, similar to the antibody monotherapy, the combination antibody therapy resulting in PD-1 and CTLA-4 blockade produces a persistent immunity to tumor relapse.

Example 20: *In Vivo* Efficacy of Combination Therapy (anti-CTLA-4 and anti-PD-1 Antibodies) on Established Tumor Growth

CT26 colorectal cancer cells were implanted in BALB/Cmice (2×10^6 cells/mouse) for a time sufficient (about 10 days) to permit the formation of tumors. On day 10 post-implantation, tumor measurements were taken and mice were randomized based on mean tumor volume (about 250 mm^3) into 5 groups for subsequent antibody therapy. At day 0 (*i.e.*, 10 days after the CT26 cells were implanted), mice were injected IP with (1) mouse IgG (control), (2) anti-CTLA-4 monoclonal antibody 9D9, (3) anti-PD-1 monoclonal antibody 4H2, or (4) anti-CTLA-4 monoclonal antibody 9D9 and anti-PD-1 antibody monoclonal antibody 4H2, at a concentration of 10 mg/kg per mouse. Antibody injections were also administered on days 3, 6 and 10. The monoclonal antibody compositions used had low levels of endotoxin and did not significantly aggregate. Using an electronic caliper, the tumors were measured three dimensionally (height x width x length) and tumor volume was calculated. Tumor measurements were taken on day 0 (tumors at the beginning of treatment had a volume of about 125 mm^3), and on days 3, 6, 10, 13, 17 and 20 post-antibody injection. Mice were euthanized when the tumors reached a designated tumor end-point (a particular tumor volume such as 1500 mm^3 and/or when the mice showed greater than about 15% weight loss). The results are shown in Figure 50. This study indicates that, in a murine tumor model, treatment with the combination of CTLA-4 antibody and PD-1 antibody has a significantly greater effect on tumor growth than either antibody alone, even when a tumor is already well established.

Example 21: Effect of human anti-PD-1 antibody on function of T regulatory cells

T regulatory cells are lymphocytes that suppress the immune response. In this example, T regulatory cells were tested for its inhibitory function on proliferation and IFN-gamma secretion of CD4+CD25- T cells in the presence or absence of an anti-PD-1 human monoclonal antibody.

T regulatory cells were purified from PBMC using a CD4+CD25+ regulatory T cell isolation kit (Miltenyi Biotec). T regulatory cells were added into a mixed lymphocyte reaction (see above) containing purified CD4+CD25- T cells and allogeneic dendritic cells in a 2:1 ratio of CD4+CD25- to T regulatory cells. Anti-PD-1 monoclonal antibody 5C4 was added at a concentration of 10 μg/ml. Either no antibody or an isotype control antibody was used as a negative control. Culture supernatants were harvested on Day 5 for cytokine measurement using a Beadlyte cytokine detection system (Upstate). The cells were labeled with ^{3}H-thymidine, cultured for another 18 hours, and analyzed for cell proliferation. The results are shown in Figures 51A (T cell proliferation) and 51B (IFN-gamma secretion). The

addition of anti-PD-1 human monoclonal antibody 5C4 partially released inhibition imposed by Treg cells on proliferation and IFN-gamma secretion of CD4+CD25- T cells, indicating that anti-PD-1 antibodies have an effect on T regulatory cells.

Example 22: Effect of human anti-PD-1 antibody on T cell activation

In this example, effect of blockade of PD-1 pathway by anti-PD-1 antibody 5C4 on T cell activation was examined. Purified human CD4+ T cells (Dynal CD4 T cell purification kit) were activated with 1μg/ml soluble anti-CD3 antibody (BD) in the presence of autologous monocytes or monocyte-derived dendritic cells (DCs). Monocytes were purified using Miltenyi CD14 monocyte purification kit, and DCs was generated in vitro after culture of monocytes with GM-CSF and IL-4 (PeproTech) for 7 days. After three days of activation in the presence or absence of titrated anti-PD-1 antibody or irrelevant isotype control mAb, culture supernatants were harvested for ELISA analysis of IFNγ secretion while tritiated thymidine was added during the final 18 hours of the assay in order to measure T cell proliferation. The results shown in Figures 52A and 52B demonstrate that PD-1 blockade by anti-PD-1 antibody resulted in enhanced T cell proliferation and IFN-γ secretion. Synergic effect by anti-PD-1 antibody and anti-CTLA-4 antibody on T cell activation (specifically on IFN-γ secretion) in the presence of monocytes was also observed.

Example 23: Assessment of ADCC activity of anti-PD-1 antibody

In this example, an antibody-dependent cellular cytotoxicity (ADCC) assay was performed to evaluate whether anti-PD-1 antibody could induce ADCC to target cells. Two versions of 5C4, one with an Fc region of human IgG1 (5C4-IgG1) and the other with an Fc region of human IgG4 (5C4-IgG4), were tested in the assay. The Delfia Cell Cytotoxicity Kit from Perkin Elmer was used for the assay. Briefly, purified human CD4 T cells (Dynal CD4 T cell purification kit) were activated by plate-bound anti-CD3 antibody (BD) to induce PD-1 expression. Target activated CD4 T cells were then labeled with BATDA reagent. Labeled CD4 T cells were added to a V-bottom 96-well plate, followed by the addition of human PBMC (an effector to target (E/T) cell ratio of 50:1) and designed antibody. After incubation for 1 hour at 37°C, the plate was spun down. Supernatant was transferred into a flat bottom 96-well plate and the plate was read using a RubyStar plate reader. Results showed that 5C4-IgG4 did not mediate ADCC on activated CD4 T cells, while 5C4-IgG1 did mediate ADCC on activated CD4 T cells (Figure 53), indicating that ADCC activity is related to its Fc region of the anti-PD-1 antibody.

Example 24: Assessment of Complement-dependent Cytotoxicity of anti-PD-1 antibody

In this example, complement dependant cytotoxicity (CDC) of anti-PD-1 antibody was examined. Two versions of 5C4, one with Fc region of human IgG1 (5C4-IgG1) and the other with Fc region of human IgG4 (5C4-IgG4), were tested in the assay. Briefly, purified human

CD4 T cells (Dynal CD4 T cell purification kit) were activated by plate-bound anti-CD3 antibody (BD) to induce PD-1 expression. Serial dilutions of anti-PD-1 antibody (5C4) and control antibodies from 50 μg/mL to 640 pg/mL were tested for CDC in the presence of human complement (Quidel-A113). Alamar blue (Biosource International) was used to measure cytotoxicity. The plate was read on a fluorescent plate reader (EX530 EM590). Viable cell counts are proportional to fluorescence units. Results showed that neither 5C4-IgG1 or 5C4-IgG4 mediated CDC on activated CD4 T cells, while the positive control antibody (anti-HLA-ABC antibody) did (Figure 54).

Example 25: Assessment of PD-1 expression on human T cells

In this example, human PBMCs from different donors were examined for PD-1 expression on various cell subsets by FACS. Biotinylated anti-PD-1 antibody, which has displayed a much higher sensitivity than commercially available anti-PD-1 antibody on detection of PD-1 molecules on cell surface, was used in the assay. Bound antibody was detected using an PE-conjugated streptavidin. Flow cytometric analyses were performed using a FACScan flow cytometry (Becton Dickinson) and Flowjo software (Tree Star). PD-1 expression was detected on some peripheral human T cells, but not on B cells or monocytes. Further examination of T cell subsets indicates that PD-1 is expressed on CD4 and CD8 memory and effector T cells, but absent on naïve CD4 or CD8 T cells.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention, in addition to those described herein, will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims. The invention is, therefore, to be limited only by the terms of the appended claims along with the full scope of equivalents to which the claims are entitled.

Claims

1. An isolated human monoclonal antibody, or an antigen-binding portion thereof, wherein the antibody binds to PD-1 and wherein the antibody exhibits at least one of the following properties:
   a) binds to human PD-1 with a K_D of 1 x 10^{-7} M or less;
   b) does not substantially bind to human CD28, CTLA-4 or ICOS;
   c) increases T-cell proliferation in an Mixed Lymphocyte Reaction (MLR) assay;
   d) increases interferon-gamma production in an MLR assay; or
   e) increases interleukin-2 (IL-2) secretion in an MLR assay.

2. The antibody of claim 1 which is full-length antibody of an IgG1 or IgG4 isotype.

3. The antibody of claim 1 which is an antibody fragment or a single chain antibody.

4. The antibody of claim 1, wherein the antibody binds to human PD-1 with a K_D of 5 x 10^{-8} M or less.

5. The antibody of claim 1, wherein the antibody binds to human PD-1 with a K_D of 1 x 10^{-8} M or less.

6. The antibody of claim 1, wherein the antibody binds to human PD-1 with a K_D of 5 x 10^{-9} M or less.

7. The antibody of claim 1, wherein the antibody binds to human PD-1 with a K_D of between 1 x 10^{-8} M and 1 x 10^{-10} M.

8. An isolated human monoclonal antibody, or an antigen-binding portion thereof, wherein the antibody cross-competes for binding to PD-1 with a reference antibody comprising:
   a) a human heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6 and 7; and
   b) a human light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13 and 14.

9. The antibody of claim 8, wherein the human heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 1 and the human light chain variable region comprises the amino acid sequence of SEQ ID NO: 8.

10. The antibody of claim 8, wherein the human heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 2 and the human light chain variable region comprises the amino acid sequence of SEQ ID NO: 9

11. The antibody of claim 8, wherein the human heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 3 and the human light chain variable region comprises the amino acid sequence of SEQ ID NO: 10.

12. The antibody of claim 8, wherein the human heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 4 and the human light chain variable region comprises the amino acid sequence of SEQ ID NO: 11.

13. The antibody of claim 8, wherein the human heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 5 and the human light chain variable region comprises the amino acid sequence of SEQ ID NO: 12.

14. The antibody of claim 8, wherein the human heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 6 and the human light chain variable region comprises the amino acid sequence of SEQ ID NO: 13.

15. The antibody of claim 8, wherein the human heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 7 and the human light chain variable region comprises the amino acid sequence of SEQ ID NO: 14.

16. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 3-33 gene, wherein the antibody specifically binds to PD-1.

17. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 4-39 gene, wherein the antibody specifically binds to PD-1.

18. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L6 gene, wherein the antibody specifically binds to PD-1.

19. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L15 gene, wherein the antibody specifically binds to PD-1.

20. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising:
a) a heavy chain variable region of a human V_H 3-33 gene; and
b) a light chain variable region of a human Vk L6 gene;
wherein the antibody specifically binds to PD-1.

21. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising:
a) a heavy chain variable region of a human V_H 4-39 gene; and
b) a light chain variable region of a human Vk L15 gene;
wherein the antibody specifically binds to PD-1.

22. An isolated monoclonal antibody, or antigen-binding portion thereof, comprising a heavy chain variable region that comprises CDR1, CDR2, and CDR3 sequences; and a light chain variable region that comprises CDR1, CDR2, and CDR3 sequences, wherein:
a) the heavy chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35, and conservative modifications thereof;
b) the light chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56, and conservative modifications thereof; and
c) the antibody specifically binds to PD-1.

23. The antibody of claim 22, wherein the heavy chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28, and conservative modifications thereof; and the light chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49, and conservative modifications thereof.

24. The antibody of claim 23, wherein the heavy chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21, and conservative modifications thereof; and the light chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42, and conservative modifications thereof.

25. An isolated monoclonal antibody, or antigen-binding portion thereof, comprising a heavy chain variable region and a light chain variable region, wherein:

a) the heavy chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6 and 7;
b) the light chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13 and 14;
c) the antibody binds to human PD-1 with a K_D of 1×10^{-7} M or less; and
d) the antibody does not substantially bind to human CD28, CTLA-4 or ICOS.

26. The antibody of claim 25, wherein the antibody further comprises at least one of the following properties:
a) the antibody increases T-cell proliferation in an Mixed Lymphocyte Reaction (MLR) assay;
b) the antibody increases interferon-gamma production in an MLR assay; or
c) the antibody increases interleukin-2 (IL-2) secretion in an MLR assay.

27. An isolated monoclonal antibody, or antigen-binding portion thereof, comprising:
a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21;
b) a heavy chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28;
c) a heavy chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35;
d) a light chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42;
e) a light chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49; and
f) a light chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56;
wherein the antibody specifically binds PD-1.

28. The antibody of claim 27, which comprises:
a) a heavy chain variable region CDR1 comprising SEQ ID NO: 15;
b) a heavy chain variable region CDR2 comprising SEQ ID NO: 22;
c) a heavy chain variable region CDR3 comprising SEQ ID NO: 29;
d) a light chain variable region CDR1 comprising SEQ ID NO: 36;
e) a light chain variable region CDR2 comprising SEQ ID NO: 43; and
f) a light chain variable region CDR3 comprising SEQ ID NO: 50.

29. The antibody of claim 27, which comprises:
    a) a heavy chain variable region CDR1 comprising SEQ ID NO: 16;
    b) a heavy chain variable region CDR2 comprising SEQ ID NO: 23;
    c) a heavy chain variable region CDR3 comprising SEQ ID NO:30;
    d) a light chain variable region CDR1 comprising SEQ ID NO: 37;
    e) a light chain variable region CDR2 comprising SEQ ID NO: 44; and
    f) a light chain variable region CDR3 comprising SEQ ID NO: 51.

30. The antibody of claim 27, which comprises:
    a) a heavy chain variable region CDR1 comprising SEQ ID NO: 17;
    b) a heavy chain variable region CDR2 comprising SEQ ID NO: 24;
    c) a heavy chain variable region CDR3 comprising SEQ ID NO: 31;
    d) a light chain variable region CDR1 comprising SEQ ID NO: 38;
    e) a light chain variable region CDR2 comprising SEQ ID NO: 45; and
    f) a light chain variable region CDR3 comprising SEQ ID NO: 52.

31. The antibody of claim 27, which comprises:
    a) a heavy chain variable region CDR1 comprising SEQ ID NO: 18;
    b) a heavy chain variable region CDR2 comprising SEQ ID NO: 25;
    c) a heavy chain variable region CDR3 comprising SEQ ID NO: 32;
    d) a light chain variable region CDR1 comprising SEQ ID NO: 39;
    e) a light chain variable region CDR2 comprising SEQ ID NO: 46; and
    f) a light chain variable region CDR3 comprising SEQ ID NO: 53.

32. The antibody of claim 27, which comprises:
    a) a heavy chain variable region CDR1 comprising SEQ ID NO: 19;
    b) a heavy chain variable region CDR2 comprising SEQ ID NO: 26;
    c) a heavy chain variable region CDR3 comprising SEQ ID NO: 33;
    d) a light chain variable region CDR1 comprising SEQ ID NO: 40;
    e) a light chain variable region CDR2 comprising SEQ ID NO: 47; and
    f) a light chain variable region CDR3 comprising SEQ ID NO: 54.

33. The antibody of claim 27, which comprises:
    a) a heavy chain variable region CDR1 comprising SEQ ID NO: 20;
    b) a heavy chain variable region CDR2 comprising SEQ ID NO: 27;
    c) a heavy chain variable region CDR3 comprising SEQ ID NO: 34;
    d) a light chain variable region CDR1 comprising SEQ ID NO: 41;
    e) a light chain variable region CDR2 comprising SEQ ID NO: 48; and
    f) a light chain variable region CDR3 comprising SEQ ID NO: 55.

34. The antibody of claim 27, which comprises:
    a) a heavy chain variable region CDR1 comprising SEQ ID NO: 21;
    b) a heavy chain variable region CDR2 comprising SEQ ID NO: 28;
    c) a heavy chain variable region CDR3 comprising SEQ ID NO: 35;
    d) a light chain variable region CDR1 comprising SEQ ID NO: 42;
    e) a light chain variable region CDR2 comprising SEQ ID NO: 49; and
    f) a light chain variable region CDR3 comprising SEQ ID NO: 56.

35. An isolated monoclonal antibody, or antigen-binding portion thereof comprising:
    a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6 and 7; and
    b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13 and 14;
    wherein the antibody specifically binds PD-1.

36. The antibody of claim 35, which comprises:
    a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 1; and
    b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8.

37. The antibody of claim 35, which comprises:
    a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 2; and
    b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 9.

38. The antibody of claim 35, which comprises:
    a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 3; and
    b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 10.

39. The antibody of claim 35, which comprises:
    a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4; and
    b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 11.

40. The antibody of claim 35, which comprises:
    a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 5; and

b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 12.

41. The antibody of claim 35, which comprises:
a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6; and
b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 13.

42. The antibody of claim 35, which comprises:
a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7; and
b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14.

43. A composition comprising the antibody, or antigen-binding portion thereof, of any one of claims 1-36, and a pharmaceutically acceptable carrier.

44. An immunoconjugate comprising the antibody, or antigen-binding portion thereof, of any one of claims 1-36, linked to a therapeutic agent.

45. A composition comprising the immunoconjugate of claim 44 and a pharmaceutically acceptable carrier.

46. The immunoconjugate of claim 44, wherein the therapeutic agent is a cytotoxin.

47. A composition comprising the immunoconjugate of claim 46 and a pharmaceutically acceptable carrier.

48. The immunoconjugate of claim 44, wherein the therapeutic agent is a radioactive isotope.

49. A composition comprising the immunoconjugate of claim 48 and a pharmaceutically acceptable carrier.

50. A bispecific molecule comprising the antibody, or antigen-binding portion thereof, of any one of claims 1-36, linked to a second functional moiety having a different binding specificity than said antibody, or antigen-binding portion thereof.

51. A composition comprising the bispecific molecule of claim 50, and a pharmaceutically acceptable carrier.

52. An isolated nucleic acid molecule encoding the antibody, or antigen-binding portion thereof, of any one of claims 1-36.

53. An expression vector comprising the nucleic acid molecule of claim 52.

54. A host cell comprising the expression vector of claim 53.

55. A transgenic mouse comprising human immunoglobulin heavy and light chain transgenes, wherein the mouse expresses the antibody of any one of claims 1-36.

56. A hybridoma prepared from the mouse of claim 55, wherein the hybridoma produces said antibody.

57. A method of modulating an immune response in a subject comprising administering to the subject the antibody, or antigen-binding portion thereof, of any one of claims 1-36 such that the immune response in the subject is modulated.

58. A method of inhibiting growth of tumor cells in a subject, comprising administering to the subject a therapeutically effective amount of an anti-PD-1 antibody, or antigen-binding portion thereof.

59. The method of claim 58, wherein the antibody is a chimeric antibody.

60. The method of claim 58, wherein the antibody is a humanized antibody.

61. The method of claim 58, wherein the antibody is a fully human antibody.

62. The method of claim 58, wherein the tumor cells are of a cancer selected from the group consisting of melanoma, renal cancer, prostate cancer, breast cancer, colon cancer and lung cancer.

63. The method of claim 58, wherein the tumor cells are of a cancer selected from the list consisting of bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of

the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers.

64. A method of inhibiting growth of tumor cells in a subject, comprising administering to a subject the antibody, or antigen-binding portion thereof, of any one of claims 1-36 in an amount effective to inhibit growth of the tumor cells.

65. A method of treating an infectious disease in a subject comprising administering to the subject the antibody, or antigen-binding portion thereof, of any one of claims 1-36 such that the subject is treated for the infectious disease.

66. The method of claim 65, wherein the infectious disease is selected from the list consisting of: HIV, Influenza, Herpes, Giardia, Malaria, Leishmania, the pathogenic infection by the virus Hepatitis (A, B, & C), herpes virus (e.g., VZV, HSV-1, HAV-6, HSV-II, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus, pathogenic infection by the bacteria chlamydia, rickettsial bacteria, mycobacteria, staphylococci, streptococci, pneumonococci, meningococci and conococci, klebsiella, proteus, serratia, pseudomonas, legionella, diphtheria, salmonella, bacilli, cholera, tetanus, botulism, anthrax, plague, leptospirosis, and Lymes disease bacteria, pathogenic infection by the fungi Candida (albicans, krusei, glabrata, tropicalis, etc.), Cryptococcus neoformans, Aspergillus (fumigatus, niger, etc.), Genus Mucorales (mucor, absidia, rhizophus), Sporothrix schenkii, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Coccidioides immitis and Histoplasma capsulatum, and pathogenic infection by the parasites Entamoeba histolytica, Balantidium coli, Naegleriafowleri, Acanthamoeba sp., Giardia lambia, Cryptosporidium sp., Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondi, and Nippostrongylus brasiliensis.

67. A method of enhancing an immune response to an antigen in a subject, comprising administering to the subject: (i) the antigen; and (ii) the antibody, or antigen-binding portion thereof, of any one of claims 1-36 such that an immune response to the antigen in the subject is enhanced.

68. The method of claim 67, wherein the antigen is a tumor antigen, a viral antigen, a bacterial antigen or an antigen from a pathogen.

69. A method for treating a hyperproliferative disease, which method comprises:
(a) administering a anti-PD-1 antibody to a subject, and
(b) administering a anti-CTLA-4 antibody to the subject.

70. The method of claim 69, wherein the anti-PD-1 antibody is administered at a subtherapeutic dose.

71. The method of claim 69, wherein the anti-CTLA-4 antibody is administered at a subtherapeutic dose.

72. The method of claim 69, wherein the anti-PD-1 antibody and the anti-CTLA-4 antibody are each administered at a subtherapeutic dose.

73. The method according to claim 69 wherein the anti-PD-1 antibody and the anti-CTLA-4 antibody are administered sequentially.

74. The method according to claim 73 wherein the anti-PD-1 antibody is administered before the anti-CTLA-4 antibody.

75. The method according to claim 73 wherein the anti-CTLA-4 antibody is administered before the anti-PD-1 antibody.

76. The method according to claim 73 wherein the anti-PD-1 antibody and the anti-CTLA-4 antibody are administered concurrently.

77. The method according to claim 73 wherein the anti-PD-1 antibody and the anti-CTLA-4 antibody are admixed as a single composition and administered concurrently.

78. The method according to claim 73 comprising administering to a subject (a) a composition comprising the anti-PD-1 antibody and a pharmaceutically acceptable

carrier and (b) a composition comprising the anti-CTLA-4 antibody and a pharmaceutically acceptable carrier.

79. The method of any one of claims 69 to 78 wherein the hyperproliferative disease is cancer.

80. The method of claim 79 wherein the cancer is colon cancer.

81. The method of any one of claims 68 to 79 wherein the subject is a human.

82. The method of any one of claims 68 to 79 wherein the anti-PD-1 antibody is a human sequence antibody.

83. The method of claim 82 wherein the anti-PD-1 human sequence antibody is a monoclonal antibody.

84. The method of claim 83 wherein the anti-PD-1 human sequence monoclonal antibody is 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4.

85. The method of any one of claims 68 to 79 wherein the anti-CTLA-4 antibody is a human sequence antibody.

86. The method of claim 85 wherein the anti-CTLA-4 human sequence antibody is a monoclonal antibody.

87. The method of claim 86 wherein the anti-CTLA-4 human sequence monoclonal antibody is 10D1 (MDX-010).

88. A method for altering an adverse event associated with treatment of a hyperproliferative disease with an immunostimulatory agent, which method comprises:
(a) administering an anti-PD-1 antibody to a subject, and
(b) administering a subtherapeutic dose of anti-CTLA-4 antibody to the subject.

89. The method of claim 86 wherein the anti-PD-1 antibody is administered in a subtherapeutic dose.

90. The method according to claim 88 wherein the anti-PD-1 antibody and the anti-CTLA-4 antibody are administered sequentially.

91. The method according to claim 90 wherein the anti-PD-1 antibody is administered before the anti-CTLA-4 antibody.

92. The method according to claim 90 wherein the anti-CTLA-4 antibody is administered before the anti-PD-1 antibody.

93. The method according to claim 88 wherein the anti-PD-1 antibody and the anti-CTLA-4 antibody are administered concurrently.

94. The method according to claim 88 wherein the anti-PD-1 antibody and the anti-CTLA-4 antibody are admixed as a single composition and administered concurrently.

95. The method according to claim 88 comprising administering to a subject (a) a composition comprising the anti-PD-1 antibody and a pharmaceutically acceptable carrier and (b) a composition comprising the anti-CTLA-4 antibody and a pharmaceutically acceptable carrier.

96. The method of any one of claims 88 to 95 wherein the hyperproliferative disease is cancer.

97. The method of claim 96 wherein the cancer is colon cancer.

98. The method of any one of claims 88 to 95 wherein the subject is a human.

99. The method of any one of claims 88 to 95 wherein the anti-PD-1 antibody is a human sequence antibody.

100. The method of claim 99 wherein the anti-PD-1 human sequence antibody is a monoclonal antibody.

101. The method of claim 100 wherein the anti-PD-1 human sequence monoclonal antibody is 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4.

102. The method of any one of claims 88 to 95 wherein the anti-CTLA-4 antibody is a human sequence antibody.

103. The method of claim 102 wherein the anti-CTLA-4 human sequence antibody is a monoclonal antibody.

104. The method of claim 103 wherein the anti-CTLA-4 human sequence monoclonal antibody is 10D1 (MDX-010).

105. A method for preparing an anti-PD-1 antibody comprising:
(a) providing: (i) a heavy chain variable region antibody sequence comprising a CDR1 sequence that is selected from the group consisting of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21, a CDR2 sequence that is selected from the group consisting of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28; and a CDR3 sequence that is selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35; or (ii) a light chain variable region antibody sequence comprising a CDR1 sequence that is selected from the group consisting of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42, a CDR2 sequence that is selected from the group consisting of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49, and a CDR3 sequence that is selected from the group consisting of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56;

(b) altering at least one amino acid residue within at least one variable region antibody sequence, said sequence being selected from the heavy chain variable region antibody sequence and the light chain variable region antibody sequence, to create at least one altered antibody sequence; and

(c) expressing the altered antibody sequence as a protein.

Anti-PD1 17D8 VH

V segment: 3-33
D segment: unknown
J segment: JH4b

```
     Q   V   Q   L   V   E   S   G   G   D   V   V   Q   P   G   G   S   L
1    CAG GTG CAG CTG GTG GAG TCT GGG GGA GAC GTG GTC CAG CCT GGG GGG TCC CTG

                                                        CDR1
                                                        ~~~~~~~~~~~~~~~~~~~
     R   L   S   C   A   A   S   G   V   A   F   S   N   Y   G   M   H   W
55   AGA CTC TCC TGT GCA GCG TCT GGA GTC GCC TTC AGT AAC TAT GGC ATG CAC TGG

                                                            CDR2
                                                            ~~~~~~~~~~~~~~~~~~~
     V   R   Q   A   P   G   K   G   L   E   W   V   A   V   I   W   Y   D
109  GTC CGC CAG GCT CCC GGC AAG GGG CTG GAG TGG GTG GCA GTT ATC TGG TAT GAT

                     CDR2
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     G   S   N   K   Y   Y   A   D   S   V   K   G   R   F   T   I   S   R
163  GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG AAG GGC CGG TTC ACC ATC TCC AGA


     D   N   S   K   N   M   L   Y   L   Q   M   N   S   L   R   A   E   D
217  GAC AAT TCC AAG AAC ATG CTC TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC

                                        CDR3
                                        ~~~~~~~~~~~~~~~~
     T   A   M   Y   Y   C   A   R   N   D   D   Y   W   G   Q   G   T   L
271  ACG GCT ATG TAT TAC TGT GCG AGG AAC GAT GAC TAC TGG GGC CAG GGA ACC CTG

                                        ┗━▶ JH4b

     V   T   V   S   S
325  GTC ACC GTC TCC TCA
```

Figure 1A

RECTIFIED SHEET(RULE91)

Anti-PD1 17D8 VK

V segment: L6
J segment: JK4

```
      E   I   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R
1     GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                CDR1
                            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      A   T   L   S   C   R   A   S   Q   S   V   S   S   Y   L   A   W   Y
55    GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC TAC TTA GCC TGG TAC

                                                                CDR2
                                                            ~~~~~~~~~~~~~~~~~~~
      Q   Q   K   P   G   Q   A   P   R   L   I   I   Y   D   A   S   N   R
109   CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC ATC ATC TAT GAT GCA TCC AAC AGG

      CDR2
      ~~~~~~~
      A   T   G   I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
163   GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                                    CDR3
                                                                ~~~~~~~~
      L   T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q
217   CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAG

                CDR3
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      R   S   N   W   P   L   T   F   G   G   G   T   K   V   E   I   K
271   CGT AGC AAC TGG CCT CTC ACT TTC GGC GGA GGG ACC AAG GTG GAG ATC AAA
                                  ↳
```

Figure 1B

RECTIFIED SHEET(RULE91)

Anti-PD-1 2D3 VH

V segment: 3-33
D segment: 7-27
J segment: JH4b

```
     Q   V   Q   L   V   E   S   G   G   D   V   V   Q   P   G   R   S   L
1    CAG GTG CAG CTG GTG GAA TCT GGG GGA GAC GTG GTC CAG CCT GGG AGG TCC CTG

                                                        CDR1
                                                        ~~~~~~~~~~~~~~~~~~~
     R   L   S   C   A   A   S   G   L   T   F   T   N   Y   G   F   H   W
55   AGA CTC TCC TGT GCA GCG TCT GGA TTA ACC TTC ACT AAC TAT GGC TTC CAC TGG

                                                            CDR2
                                                            ~~~~~~~~~~~~~~~~~~~
     V   R   Q   A   P   G   K   G   L   E   W   V   A   V   I   W   Y   D
109  GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCT GTT ATA TGG TAT GAT

                         CDR2
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     G   S   K   K   Y   Y   A   D   S   V   K   G   R   F   T   I   S   R
163  GGA AGT AAG AAA TAT TAT GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA

     D   N   S   K   N   T   L   Y   L   Q   M   N   N   L   R   A   E   D
217  GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AAC CTG AGA GCC GAG GAC

                                        CDR3
                                        ~~~~~~~~~~~~~~~~
     T   A   V   Y   Y   C   A   T   G   D   D   Y   W   G   Q   G   T   L
271  ACG GCT GTG TAT TAC TGT GCG ACT GGG GAT GAC TAC TGG GGC CAG GGA ACC CTG

     V   T   V   S   S
325  GTC ACC GTC TCC TCA
```

Figure 2A

RECTIFIED SHEET(RULE91)

Anti-PD-1 2D3 VK

V segment: L6
J segment: JK4

```
     E   I   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R
1    GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                CDR1
                            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     A   T   L   S   C   R   A   S   Q   S   V   S   S   Y   L   A   W   Y
55   GCC ACC CTG TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC TAC TTA GCC TGG TAC

                                                                CDR2
                                                            ~~~~~~~~~~~~~~~~~~~~
     Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   D   T   S   N   R
109  CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GAT ACA TCC AAC AGG

     CDR2
     ~~~~~~~
     A   T   G   I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
163  GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                                    CDR3
                                                                ~~~~~~~~
     L   T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q
217  CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAG

             CDR3
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     R   S   N   W   P   L   T   F   G   G   G   T   K   V   E   I   K
271  CGT AGC AAC TGG CCG CTC ACT TTC GGC GGA GGG ACC AAG GTG GAG ATC AAA
```

Figure 2B

RECTIFIED SHEET(RULE91)

Anti-PD-1 4H1 VH

V segment: 3-33
D segment: undetermined
J segment: JH4b

```
     Q   V   Y   L   V   E   S   G   G   G   V   V   Q   P   G   R   S   L
1    CAG GTG TAC TTG GTA GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG

                                                        CDR1
                                                    ~~~~~~~~~~~~~~~~~~~~
     R   L   S   C   A   A   S   G   F   T   F   S   N   Y   G   M   H   W
55   AGA CTC TCC TGT GCA GCG TCT GGA TTC ACC TTC AGT AAC TAT GGC ATG CAC TGG

                                                            CDR2
                                                        ~~~~~~~~~~~~~~~~~~~~
     V   R   Q   A   P   G   K   G   L   E   W   V   A   L   I   W   Y   D
109  GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA CTT ATA TGG TAT GAT

                        CDR2
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     G   S   N   K   Y   Y   A   D   S   V   K   G   R   F   T   I   S   R
163  GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA


     D   N   S   K   N   T   L   Y   L   Q   M   T   S   L   R   V   E   D
217  GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG ACC AGT CTG AGA GTC GAG GAC

                                        CDR3
                                    ~~~~~~~~~~~~~~~~
     T   A   V   Y   Y   C   A   S   N   V   D   H   W   G   Q   G   T   L
271  ACG GCT GTG TAT TAT TGT GCG AGC AAC GTT GAC CAT TGG GGC CAG GGA ACC CTG


     V   T   V   S   S
325  GTC ACC GTC TCC TCA
```

Figure 3A

RECTIFIED SHEET(RULE91)

Anti-PD-1 4H1 VK

V segment: L6
J segment: JK1

```
      E   I   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R.
1     GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                  CDR1
                          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      A   T   L   S   C   R   A   S   Q   S   V   S   S   Y   L   A   W   Y
55    GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGT AGT TAC TTA GCC TGG TAC

                                                                CDR2
                                                          ~~~~~~~~~~~~~~~~~~~
      Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   D   A   S   N   R
109   CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GAT GCA TCC AAC AGG

      CDR2
      ~~~~~~~
      A   T   G   I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
163   GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                                    CDR3
                                                                    ~~~~~~~
      L   T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q
217   CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAG

              CDR3
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      S   S   N   W   P   R   T   F   G   Q   G   T   K   V   E   I   K
271   AGT AGC AAC TGG CCT CGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA
```

Figure 3B

RECTIFIED SHEET(RULE91)

Anti-PD1 5C4 VH

V segment: 3-33
D segment: unknown
J segment: JH4b

```
     Q   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R   S   L
1    CAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG

                                                    CDR1
                                                    ~~~~~~~~~~~~~~~~~~~
     R   L   D   C   K   A   S   G   I   T   F   S   N   S   G   M   H   W
55   AGA CTC GAC TGT AAA GCG TCT GGA ATC ACC TTC AGT AAC TCT GGC ATG CAC TGG

                                                        CDR2
                                                        ~~~~~~~~~~~~~~~~~~~
     V   R   Q   A   P   G   K   G   L   E   W   V   A   V   I   W   Y   D
109  GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA GTT ATT TGG TAT GAT

                    CDR2
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     G   S   K   R   Y   Y   A   D   S   V   K   G   R   F   T   I   S   R
163  GGA AGT AAA AGA TAC TAT GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA


     D   N   S   K   N   T   L   F   L   Q   M   N   S   L   R   A   E   D
217  GAC AAT TCC AAG AAC ACG CTG TTT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC

                                        CDR3
                                        ~~~~~~~~~~~~~~~~
     T   A   V   Y   Y   C   A   T   N   D   D   Y   W   G   Q   G   T   L
271  ACG GCT GTG TAT TAC TGT GCG ACA AAC GAC GAC TAC TGG GGC CAG GGA ACC CTG
                                            |_____>
                                                JH4b

     V   T   V   S   S
325  GTC ACC GTC TCC TCA
```

Figure 4A

RECTIFIED SHEET(RULE91)

Anti-PD1 5C4 VK

V segment: L6
J segment: JK1

```
      E   I   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R
1     GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                CDR1
                            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      A   T   L   S   C   R   A   S   Q   S   V   S   S   Y   L   A   W   Y
55    GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGT AGT TAC TTA GCC TGG TAC

                                                            CDR2
                                                        ~~~~~~~~~~~~~~~~~~~~
      Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   D   A   S   N   R
109   CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GAT GCA TCC AAC AGG

      CDR2
      ~~~~~~~
      A   T   G   I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
163   GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                                    CDR3
                                                                    ~~~~~~~
      L   T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q
217   CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAG

                CDR3
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~
      S   S   N   W   P   R   T   F   G   Q   G   T   K   V   E   I   K
271   AGT AGC AAC TGG CCT CGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA
                                  └─►
```

Figure 4B

RECTIFIED SHEET(RULE91)

Anti-PD-1 4A11 VH

V segment: 4-39
D segment: 3-9
J segment: JH4b

```
        Q   L   Q   L   Q   E   S   G   P   G   L   V   K   P   S   E   T   L
1       CAG CTG CAG CTG CAG GAG TCG GGC CCA GGA CTG GTG AAG CCT TCG GAG ACC CTG

                                                            CDR1
                                                            ~~~~~~~~~~~~~~~~~~~~~~~
        S   L   T   C   T   V   S   G   G   S   L   S   R   S   S   F   F   W
55      TCC CTC ACC TGC ACT GTC TCT GGT GGC TCC CTC AGC AGG AGT AGT TTC TTC TGG

        CDR1                                                        CDR2
        ~~~~                                                        ~~~~~~~~~~~
        G   W   I   R   Q   P   P   G   K   G   L   E   W   I   G   S   I   Y
109     GGC TGG ATC CGT CAG CCC CCA GGG AAG GGA CTG GAG TGG ATT GGG AGT ATC TAT

                        CDR2
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
        Y   S   G   S   T   Y   Y   N   P   S   L   K   S   R   V   T   I   S
163     TAT AGT GGG AGC ACC TAC TAC AAC CCG TCC CTC AAG AGT CGA GTC ACC ATA TCC


        V   D   T   S   K   N   Q   F   S   L   K   L   S   S   V   T   A   A
217     GTA GAC ACG TCC AAG AAC CAG TTC TCC CTG AAG CTG AGC TCT GTG ACC GCC GCA

                                                CDR3
                                                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
        D   T   A   V   Y   Y   C   V   R   D   Y   D   I   L   T   G   D   E
271     GAC ACG GCT GTG TAT TAC TGT GTG AGA GAT TAC GAT ATT TTG ACT GGC GAC GAG

        CDR3
        ~~~~~~~
        D   Y   W   G   Q   G   T   L   V   T   V   S   S
325     GAC TAC TGG GGC CAG GGA ACC CTG GTC ACC GTG TCC TCA
```

Figure 5A

RECTIFIED SHEET(RULE91)

Anti-PD-1 4A11 VK

V segment: L15
J segment: JK1

```
        D   I   Q   M   T   Q   S   P   S   S   L   S   A   S   V   G   D   R
1       GAC ATC CAG ATG ACC CAG TCT CCA TCC TCA CTG TCT GCA TCT GTG GGA GAC AGA

                                CDR1
                            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
        V   S   I   T   C   R   A   S   Q   G   I   S   S   W   L   A   W   Y
55      GTC TCC ATC ACT TGT CGG GCG AGT CAG GGT ATT AGC AGC TGG TTA GCC TGG TAT

                                                            CDR2
                                                        ~~~~~~~~~~~~~~~~~~~~
        Q   Q   K   P   E   K   A   P   K   S   L   I   Y   A   A   S   N   L
109     CAG CAG AAA CCA GAG AAA GCC CCT AAG TCC CTG ATC TAT GCT GCA TCC AAT TTA

        CDR2
        ~~~~~~~
        R   S   G   V   P   S   R   F   S   G   S   G   S   G   T   D   F   T
163     CGA AGT GGG GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA GAT TTC ACT

                                                                    CDR3
                                                                ~~~~~~~~
        L   T   I   S   S   L   Q   P   E   D   F   A   T   Y   Y   C   Q   Q
217     CTC ACC ATC AGC AGC CTG CAG CCT GAA GAT TTT GCA ACT TAT TAC TGC CAA CAG

                CDR3
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~
        Y   Y   S   Y   P   R   T   F   G   Q   G   T   K   V   E   I   K
271     TAT TAT AGT TAC CCT AGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA
```

Figure 5B

RECTIFIED SHEET(RULE91)

Anti-PD-1 7D3 VH

V segment: 3-33
D segment: 7-27
J segment: JH4b

```
        Q   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R   S   L
1       CAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG

                                                            CDR1
                                                            ~~~~~~~~~~~~~~~~~~~
        R   L   S   C   T   T   S   G   I   T   F   S   S   Y   G   F   H   W
55      AGA CTC TCC TGT ACA ACG TCT GGA ATC ACC TTC AGT AGC TAT GGC TTT CAC TGG

                                                            CDR2
                                                            ~~~~~~~~~~~~~~~~~~~
        V   R   Q   A   P   G   K   G   L   E   W   V   A   V   I   W   Y   D
109     GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA GTG ATA TGG TAT GAT

                        CDR2
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
        G   S   K   K   Y   Y   A   D   S   V   K   G   R   F   T   L   S   R
163     GGA AGT AAA AAA TAC TAT GCA GAC TCC GTG AAG GGC CGA TTC ACC CTC TCC AGA

        D   D   S   K   N   T   L   Y   L   Q   M   N   S   L   R   A   E   D
217     GAC GAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC

                                        CDR3
                                        ~~~~~~~~~~~~~~~~
        T   A   V   Y   Y   C   V   T   G   D   D   Y   W   G   Q   G   T   L
271     ACG GCT GTG TAT TAC TGT GTT ACT GGG GAT GAC TAC TGG GGC CAG GGA ACC CTG

        V   T   V   S   S
325     GTC ACC GTC TCC TCA
```

Figure 6A

RECTIFIED SHEET(RULE91)

Anti-PD-1 7D3 VK

V segment: L6
J segment: JK4

```
        E   I   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R
1       GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                CDR1
                            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
        A   T   L   S   C   R   A   S   Q   S   V   S   S   Y   L   A   W   Y
55      GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC TAC TTA GCC TGG TAC

                                                            CDR2
                                                        ~~~~~~~~~~~~~~~~~~~
        Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   D   A   S   N   R
109     CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GAT GCA TCC AAC AGG

        CDR2
        ~~~~~~~
        A   T   G   I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
163     GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                                    CDR3
                                                                    ~~~~~~~
        L   T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q
217     CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAG

                CDR3
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~
        R   S   N   W   P   L   T   F   G   G   G   T   K   V   E   I   K
271     CGT AGC AAC TGG CCT CTC ACT TTC GGC GGA GGG ACC AAG GTG GAG ATC AAA
```

Figure 6B

RECTIFIED SHEET(RULE91)

Anti-PD-1 5F4 VH

V segment: 4-39
D segment: 3-9
J segment: JH4b

```
        Q   L   Q   L   Q   E   S   G   P   G   L   V   K   P   S   E   T   L
1       CAG CTG CAG CTG CAG GAG TCG GGC CCA GGA CTG GTG AAG CCT TCG GAG ACC CTG

                                                            CDR1
                                                            ~~~~~~~~~~~~~~~~~~~~~~~
        S   L   T   C   S   V   S   G   G   S   L   S   R   S   S   Y   F   W
55      TCC CTC ACC TGC TCT GTC TCT GGT GGC TCC CTC AGC AGG AGT AGT TAC TTC TGG

        CDR1                                                        CDR2
        ~~~                                                         ~~~~~~~~~~~
        G   W   I   R   Q   P   P   G   K   G   L   E   W   I   A   S   I   F
109     GGC TGG ATC CGC CAG CCC CCA GGG AAG GGG CTG GAG TGG ATT GCG AGT ATC TTT

                        CDR2
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
        Y   S   G   E   T   Y   F   N   P   S   L   K   S   R   V   T   I   S
163     TAT AGT GGG GAA ACC TAC TTC AAT CCG TCC CTC AAG AGT CGA GTC ACC ATA TCC


        V   D   T   S   R   N   Q   F   S   L   K   L   S   S   V   T   A   A
217     GTA GAC ACG TCC AGG AAC CAG TTC TCC CTG AAG CTG AGC TCT GTG ACC GCC GCA

                                                    CDR3
                                                    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
        D   T   A   V   Y   Y   C   A   R   D   Y   D   I   L   T   G   D   E
271     GAC ACG GCT GTG TAT TAC TGT GCG AGA GAT TAC GAT ATT TTG ACT GGC GAC GAG

        CDR3
        ~~~~~~~~
        D   Y   W   G   Q   G   T   L   V   T   V   S   S
325     GAC TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA
```

Figure 7A

RECTIFIED SHEET(RULE91)

Anti-PD-1 5F4 VK

V segment: L15
J segment: JK1

```
          D   I   Q   M   T   Q   S   P   S   S   L   S   A   S   V   G   D   R
1         GAC ATC CAG ATG ACC CAG TCT CCA TCC TCA CTG TCT GCA TCT GTA GGA GAC AGA

                                  CDR1
                              ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          V   T   I   T   C   R   A   S   Q   G   I   S   S   W   L   A   W   Y
55        GTC ACC ATC ACT TGT CGG GCG AGT CAG GGT ATT AGC AGC TGG TTA GCC TGG TAT

                                                                  CDR2
                                                              ~~~~~~~~~~~~~~~~~~~~
          Q   Q   K   P   E   K   A   P   K   S   L   I   Y   A   A   S   S   L
109       CAG CAG AAA CCA GAG AAA GCC CCT AAG TCC CTG ATC TAT GCT GCA TCC AGT TTG

          CDR2
          ~~~~~~~
          Q   S   G   V   P   S   R   F   S   G   S   G   S   G   T   D   F   T
163       CAA AGT GGG GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA GAT TTC ACT

                                                                          CDR3
                                                                          ~~~~~~~
          L   T   I   S   S   L   Q   P   E   D   F   A   T   Y   Y   C   Q   Q
217       CTC ACC ATC AGC AGC CTG CAG CCT GAA GAT TTT GCA ACT TAT TAC TGC CAA CAG

                  CDR3
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          Y   Y   S   Y   P   R   T   F   G   Q   G   T   K   V   E   I   K
271       TAT TAT AGT TAC CCT AGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA
```

Figure 7B

RECTIFIED SHEET(RULE91)

Anti-PD1 17D8, 2D3, 4H1, 5C4 and 7D3 VH region

```
                                                                     ___CDR1__
3-33 Germline:   Q V Q L V E S G G G V V Q P G R S L R L S C A A S G F T F S S Y G M H W V R Q
17D8 VH:         - - - - - - - - - D - - - - - G - - - - - - - - - - V A - - N - - - - - - -
2D3 VH:          - - - - - - - - - D - - - - - - - - - - - - - - - - L - - T N - - F - - - -
4H1 VH:          - - Y - - - - - - - - - - - - - - - - - - - - - - - - - - - N - - - - - - -
5C4 VH:          - - - - - - - - - - - - - - - - - - - - D - K - - - I - - - N S - - - - - -
7D3 VH:          - - - - - - - - - - - - - - - - - - - - - - T T - - I - - - - - - F - - - -

                                     ____________CDR2___________
3-33 Germline:   A P G K G L E W V A V I W Y D G S N K Y Y A D S V K G R F T I S R D N S K N T
17D8 VH:         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
2D3 VH:          - - - - - - - - - - - - - - - - - K - - - - - - - - - - - - - - - - - - - M
4H1 VH:          - - - - - - - - - - L - - - - - - - - - - - - - - - - - - - - - - - - - - -
5C4 VH:          - - - - - - - - - - - - - - - - - K R - - - - - - - - - - - - - - - - - - -
7D3 VH:          - - - - - - - - - - - - - - - - - K - - - - - - - - - - - - L - - - D - - -

                                                         _CDR3__
3-33 Germline:   L Y L Q M N S L R A E D T A V Y Y C A R
JH4b germline:                                               D Y W G Q G T L V T V S S
17D8 VH:         - - - - - - - - - - - - - - M - - - - - - - - - - - - - - - - - - - -  (JH4b)
2D3 VH:          - - - - - - N - - - - - - - - - - - - T G - - - - - - - - - - - - - -  (JH4b)
4H1 VH:          - - - - - T - - - V - - - - - - - - - S - V - H - - - - - - - - - - -  (JH4b)
5C4 VH:          - F - - - - - - - - - - - - - - - - - T N D - - - - - - - - - - - - -  (JH4b)
7D3 VH:          - - - - - - - - - - - - - - - - - - V T G D - - - - - - - - - - - - -  (JH4b)
```

Figure 8

RECTIFIED SHEET (RULE 91)

Anti-PD-1 17D8, 2D3 and 7D3 VK Region

```
                                                                                                CDR1
L6 germline: E I V L T Q S P A T L S L S P G E R A T L S C R A S Q S V S S Y L A
17D8 VK:     - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
2D3 VK:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
7D3 VK:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                CDR2
L6 germline: W Y Q Q K P G Q A P R L L I Y D A S N R A T G I P A R F S G S G S G
17D8 VK:     - - - - - - - - - - - - - I - - - - - - - - - - - - - - - - - - - -
2D3 VK:      - - - - - - - - - - - - - - - - - T - - - - - - - - - - - - - - - -
7D3 VK:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                CDR3
L6 germline: T D F T L T I S S L E P E D F A V Y Y C Q Q R S N W P
JK4 germline:                                                     L T F G G G T
17D8 VK:     - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
2D3 VK:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
7D3 VK:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

JK4 germline: K V E I K
17D8 VK:      - - - - - (JK4)
2C3 VK:       - - - - - (JK4)
7D3 VK:       - - - - - (JK4)
```

Figure 9

RECTIFIED SHEET(RULE91)

Anti-PD-1 4H1 and 5C4 VK Region

```
                                                                      CDR1
L6 germline: E I V L T Q S P A T L S L S P G E R A T L S C R A S Q S V S S Y L A
4H1 VK:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
5C4 VK:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                            CDR2
L6 germline: W Y Q Q K P G Q A P R L L I Y D A S N R A T G I P A R F S G S G S G
4H1 VK:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
5C4 VK:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                          CDR3
L6 germline: T D F T L T I S S L E P E D F A V Y Y C Q Q R S N W P
JK1 germline:                                                      W T F G Q G T
4H1 VK:      - - - - - - - - - - - - - - - - - - - - - - S - - - - R - - - - - -
5C4 VK:      - - - - - - - - - - - - - - - - - - - - - - S - - - - R - - - - - -

JK1 germline:K V E I K
4H1 VK:      - - - - - (JK1)
5C4 VK:      - - - - - (JK1)
```

Figure 10

RECTIFIED SHEET(RULE91)

Anti-PD-1 4A11 and 5F4 VH regions

```
                                                                                                                                CDR1
4-39 germline:   Q L Q L Q E S G P G L V K P S E T L S L T C T V S G G S I S S S S Y Y W
4A11 VH:         - - - - - - - - - - - - - - - - - - - - - - - - - - - L - R - - F F -
5F4 VH:          - - - - - - - - - - - - - - - - - - - - - S - - - - - L - R - - - F -

                                                                CDR2
4-39 germline:   G W I R Q P P G K G L E W I G S I Y Y S G S T Y Y N P S L K S R V T I S
4A11 VH:         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
5F4 VH:          - - - - - - - - - - - - - - - - A - - F - - - E - - F - - - - - - - - -

                                                                                                                CDR3
4-39 germline:   V D T S K N H F S L K L S S V T A A D T A V Y Y C A R
4A11 VH:         - - - - - - Q - - - - - - - - - - - - - - - - - - - V - D Y D I L T G D E
5F4 VH:          - - - - R - - - - - - - - - - - - - - - - - - - - - - D Y D I L T G D E

JH4b germline:   D Y W G Q G T L V T V S S
4A11 VH:         - - - - - - - - - - - - - (JH4b)
5F4 VH:          - - - - - - - - - - - - - (JH4b)
```

Figure 11

RECTIFIED SHEET (RULE 91)

Anti-PD-1 4A11 and 5F4 VK region

```
                                                                                                                CDR1
L15 germline:     D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q G I S S
4A11 VK:          - - - - - - - - - - - - - - - - - - - - S - - - - - - - - - -
5F4 VK1:          - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                  CDR2
L15 germline:     W L A W Y Q Q K P E K A P K S L I Y A A S S L Q S G V P S R F
4A11 VK:          - - - - - - - - - - - - - - - - - - - - N - R - - - - - - - -
5F4 VK1:          - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                                                          CDR3
L15 germline:     S G S G S G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y N S
4A11 VK:          - - - - - - - - - - - - - - - - - - - - - - - - - - - - - Y -
5F4 VK1:          - - - - - - - - - - - - - - - - - - - - - - - - - - - - - Y -

L15 germline:     Y P
JK1 germline:           T F G Q G T K V E I K
4A11 VK:          - - R - - - - - - - - - - (JK1)
5F4 VK1:          - - R - - - - - - - - - - (JK1)
```

Figure 12

RECTIFIED SHEET(RULE91)

19/58

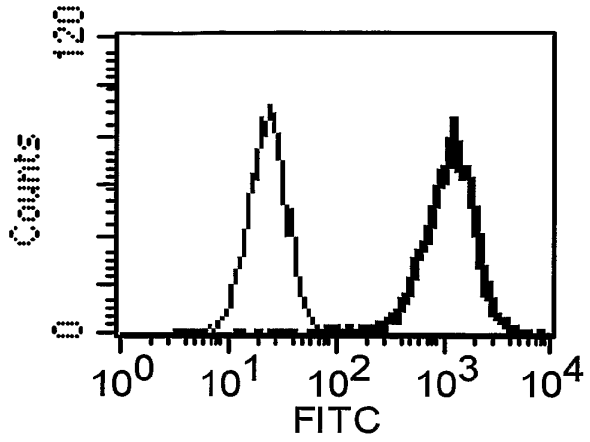


Figure 13A

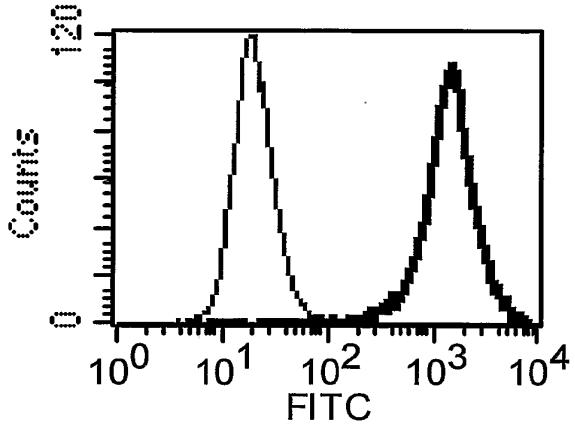


Figure 13B

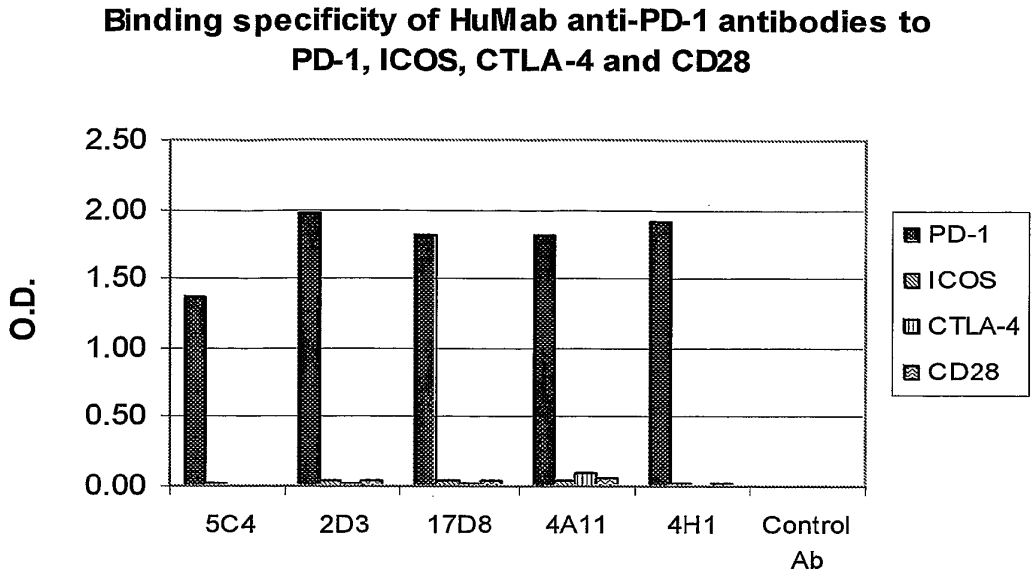

Binding specificity of HuMab anti-PD-1 antibodies to PD-1, ICOS, CTLA-4 and CD28
O.D.
2.50
2.00
1.50
1.00
0.50
0.00
5C4
2D3
17D8
4A11
4H1
Control Ab
PD-1
ICOS
CTLA-4
CD28

Figure 14

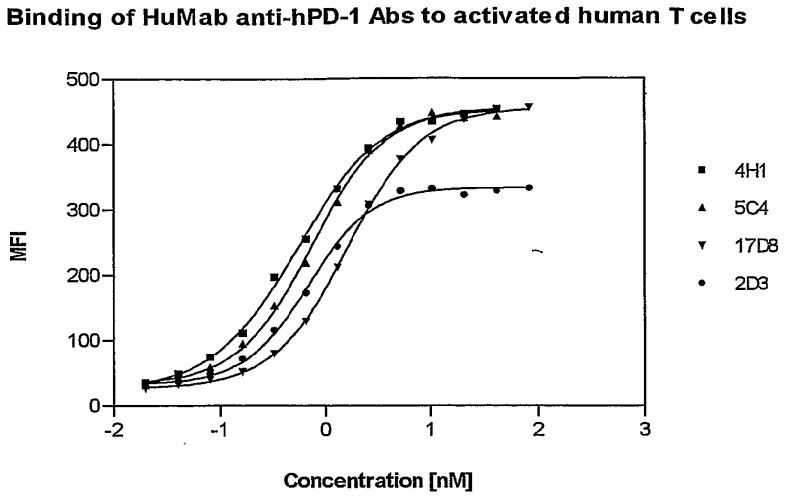


Figure 15A

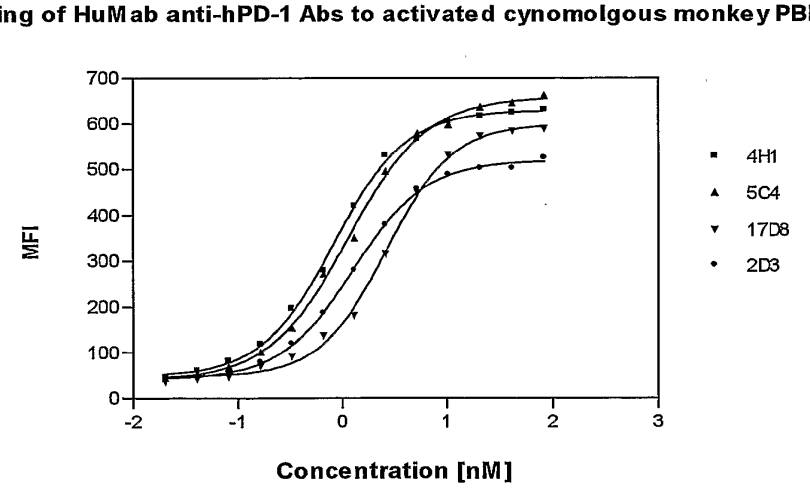


Figure 15B

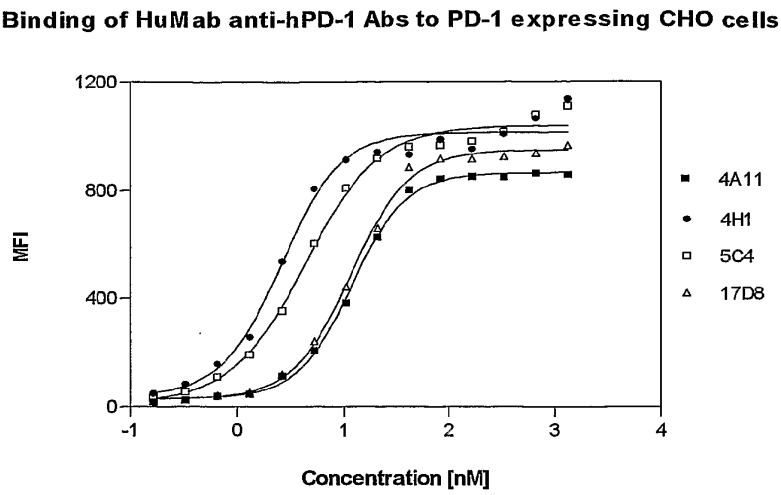

Binding of HuMab anti-hPD-1 Abs to PD-1 expressing CHO cells
MFI
1200
800
400
0
-1
0
1
2
3
4
Concentration [nM]
4A11
4H1
5C4
17D8

Figure 15C

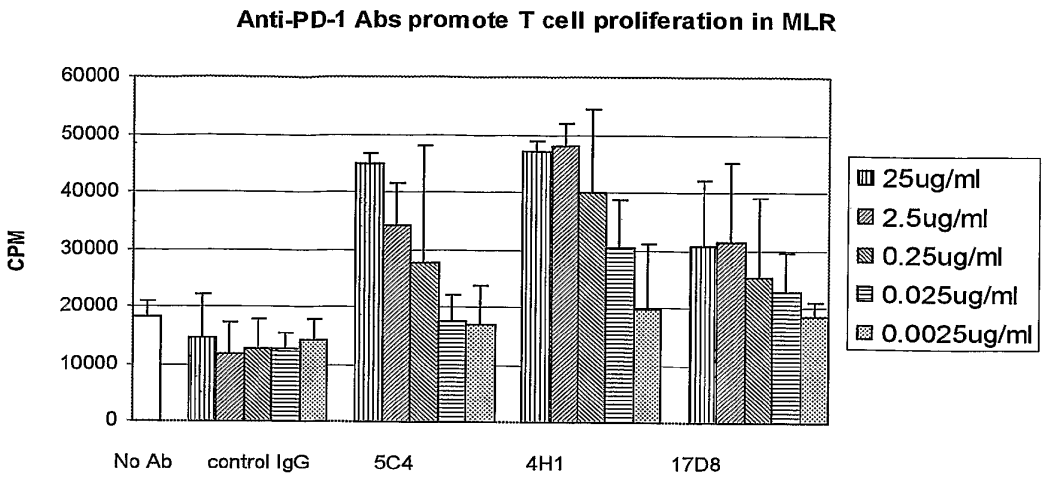


Figure 16A

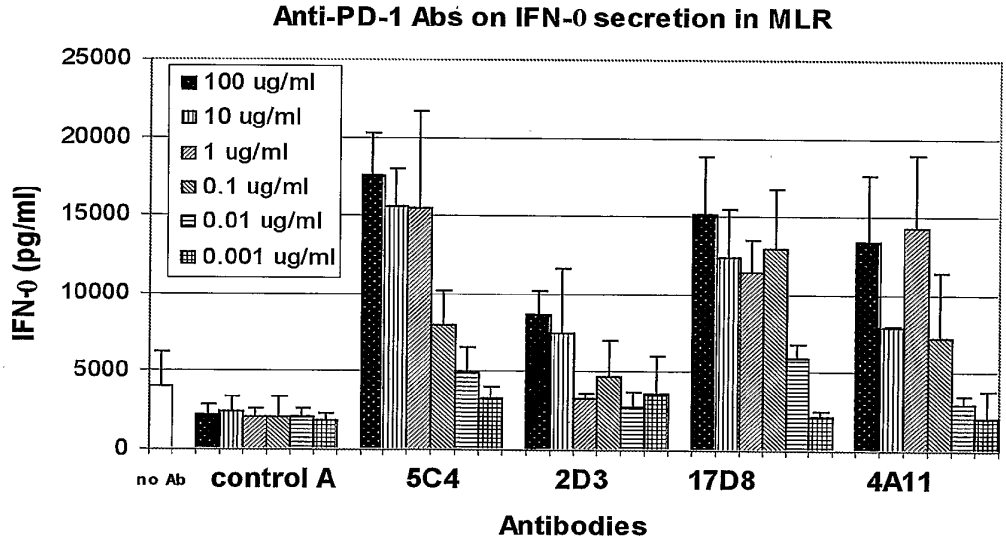


Figure 16B

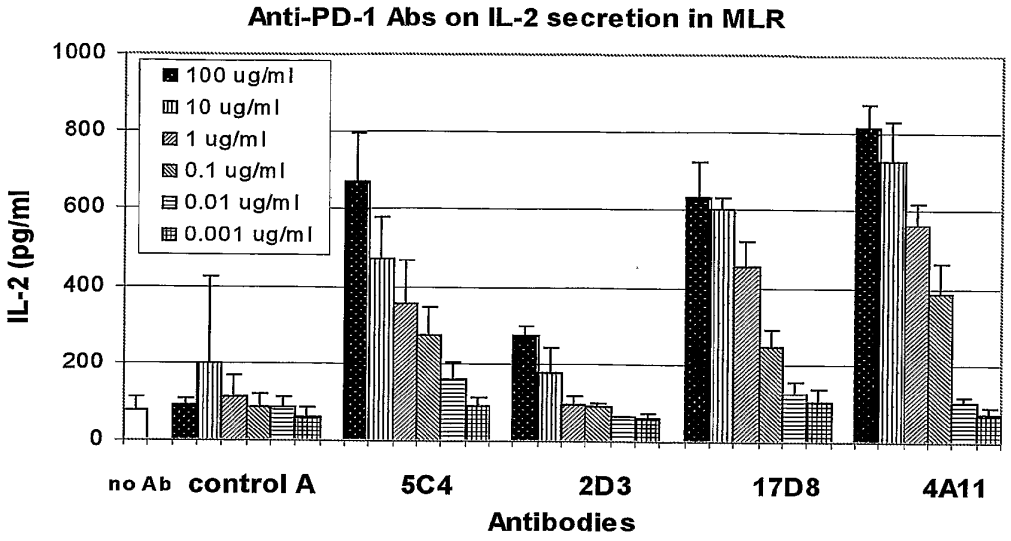

Anti-PD-1 Abs on IL-2 secretion in MLR
IL-2 (pg/ml)
1000
800
600
400
200
0
100 ug/ml
10 ug/ml
1 ug/ml
0.1 ug/ml
0.01 ug/ml
0.001 ug/ml
no Ab
control A
5C4
2D3
17D8
4A11
Antibodies

Figure 16C

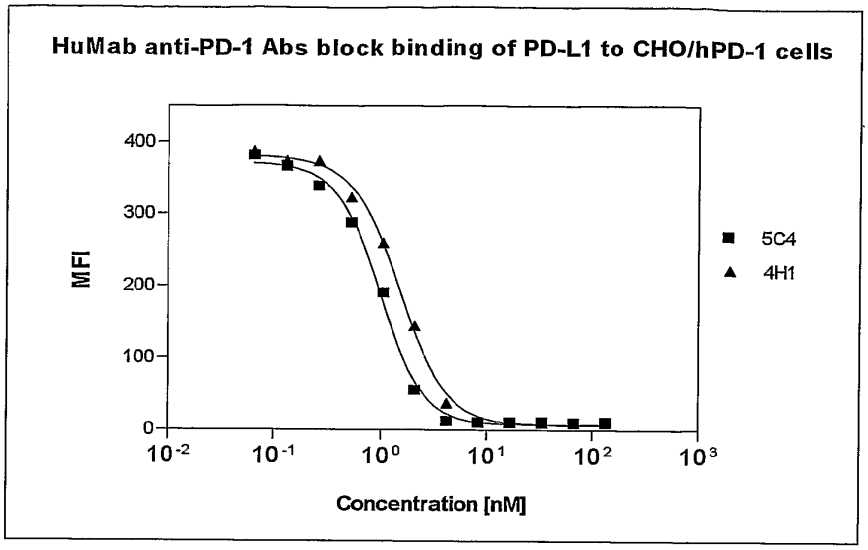


Figure 17A

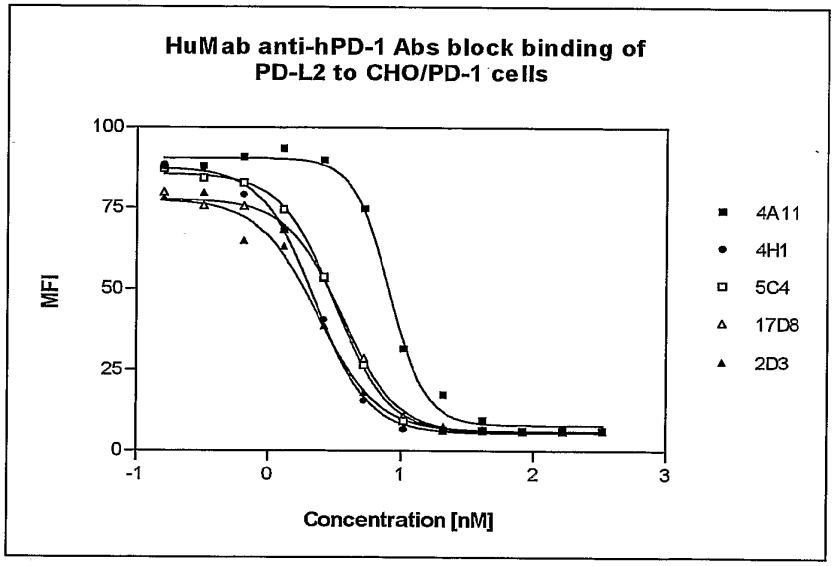


Figure 17B

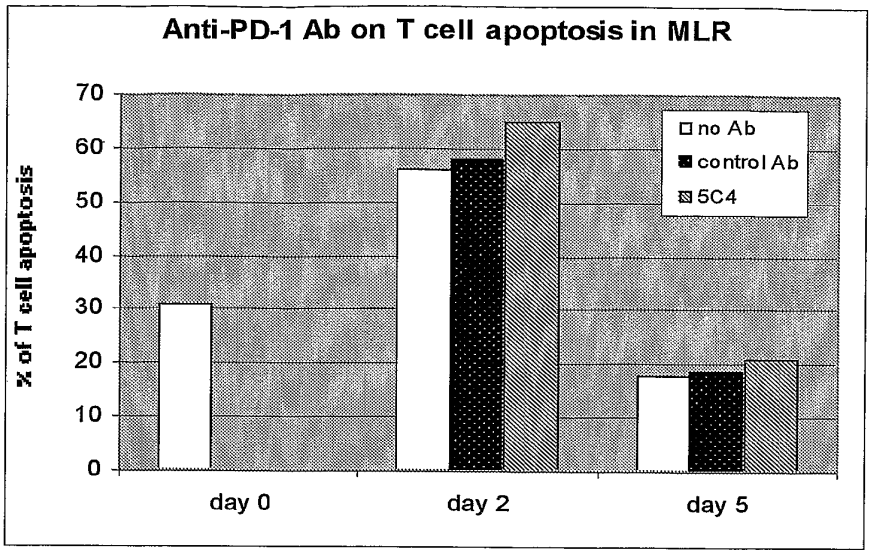


Figure 18

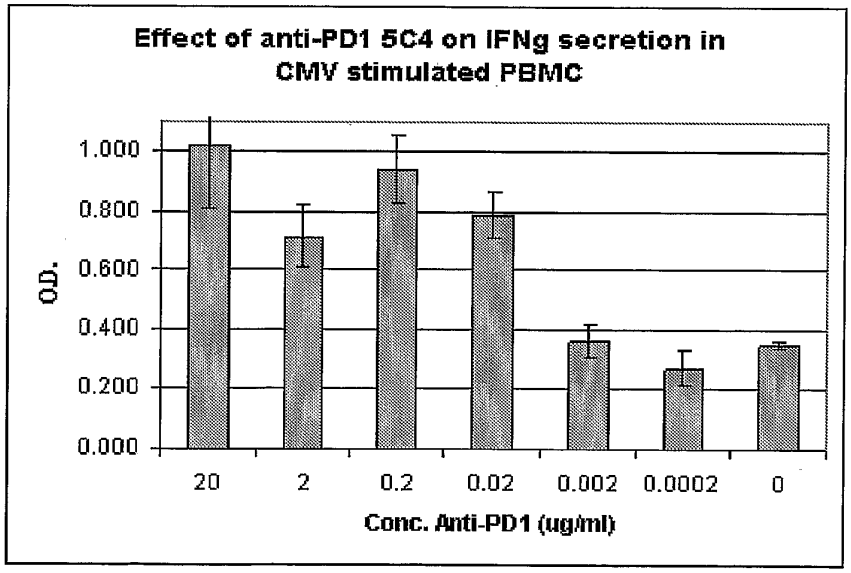


Figure 19

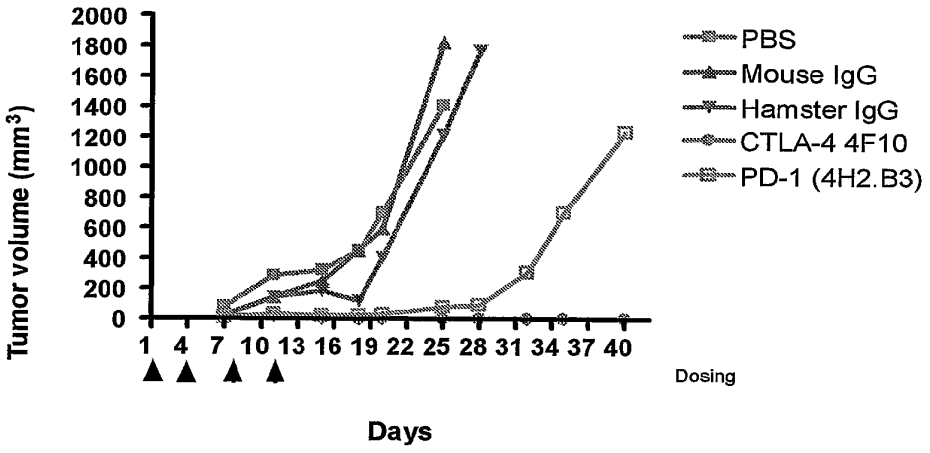

Tumor volume (mm3)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1 4 7 10 13 16 19 22 25 28 31 34 37 40
Dosing
Days
PBS
Mouse IgG
Hamster IgG
CTLA-4 4F10
PD-1 (4H2.B3)

Figure 20

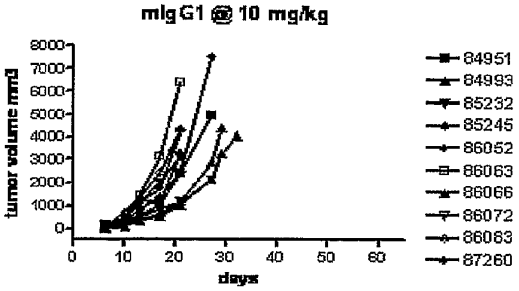


Figure 21A

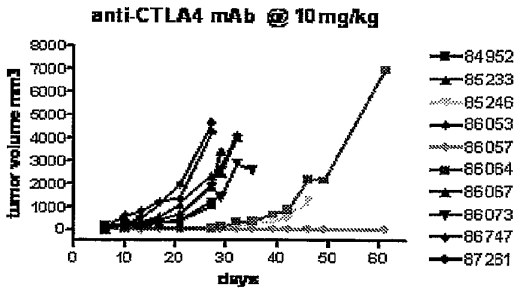


Figure 21B

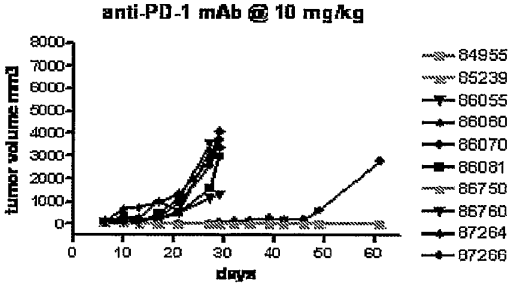


Figure 21C

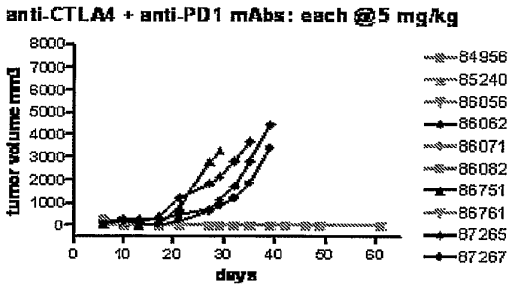


Figure 21D

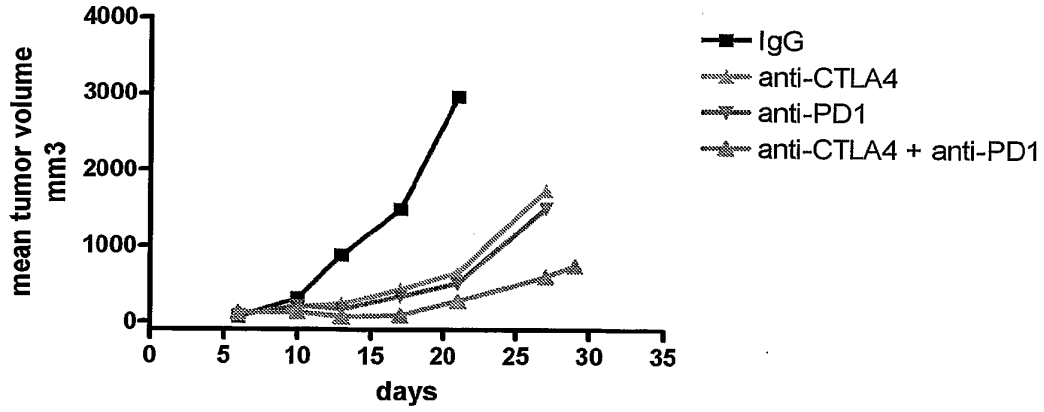


Figure 22

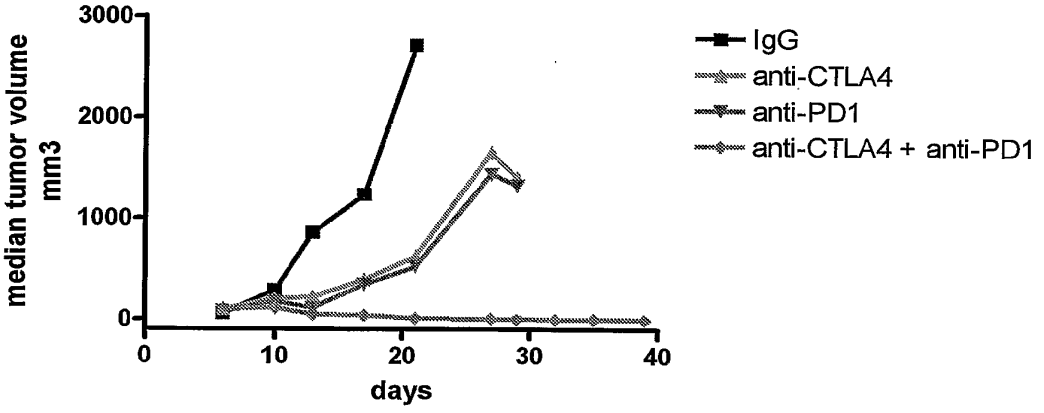


Figure 23

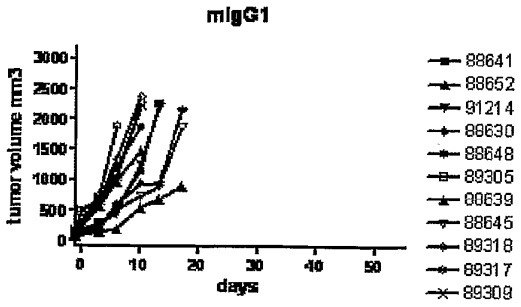


Figure 24A

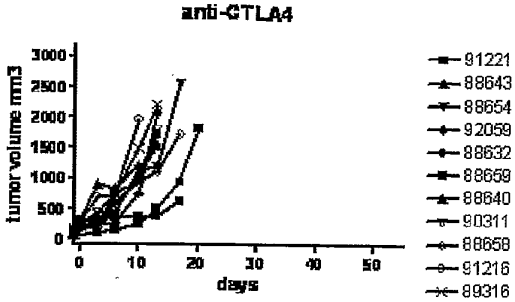


Figure 24B

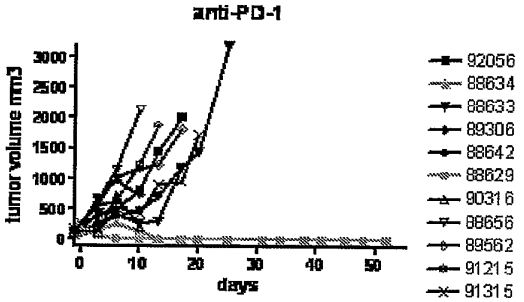


Figure 24C

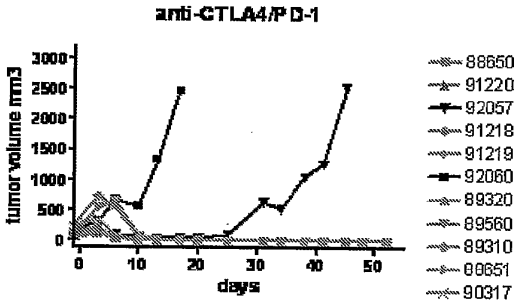


Figure 24D

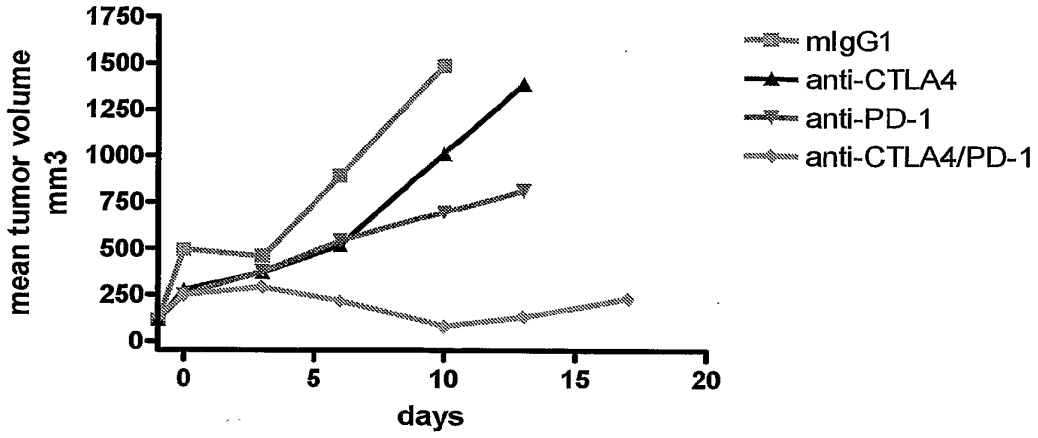


Figure 25

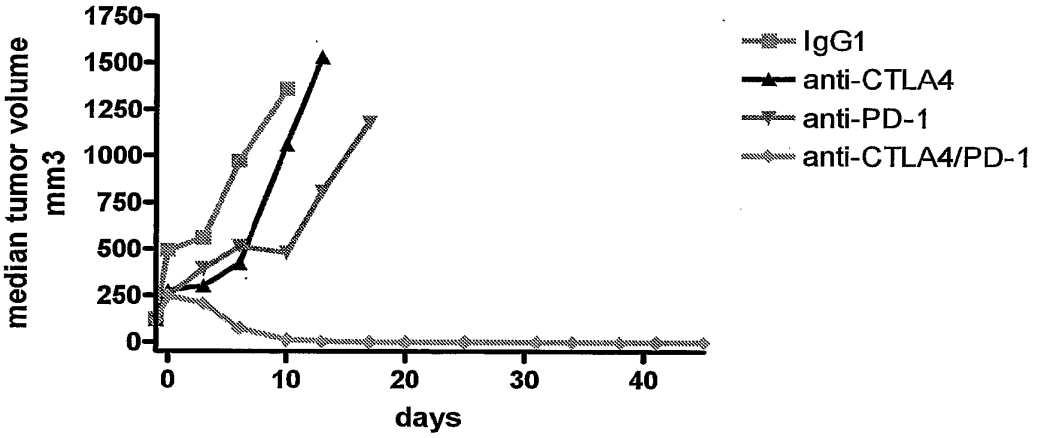


Figure 26

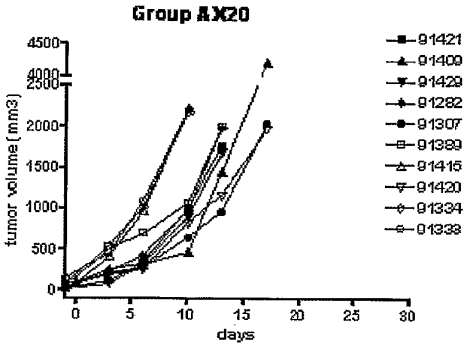


Figure 27A

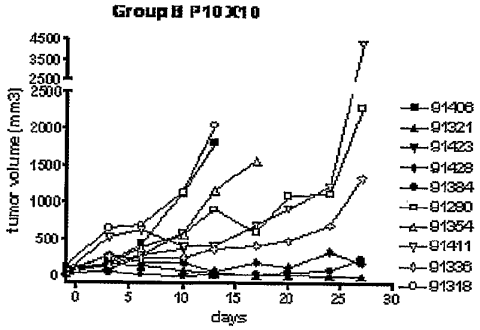


Figure 27B

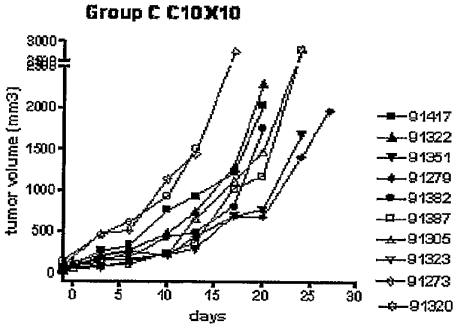


Figure 27C

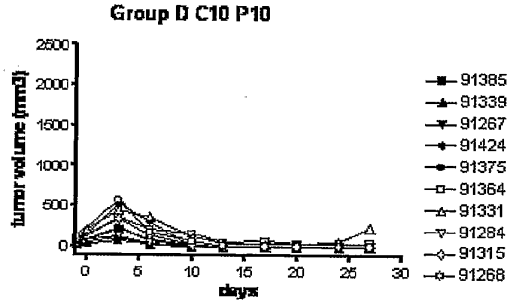


Figure 27D

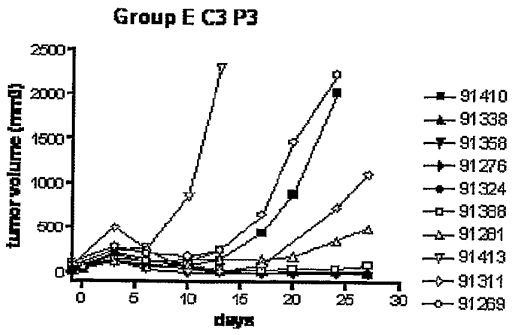


Figure 27E

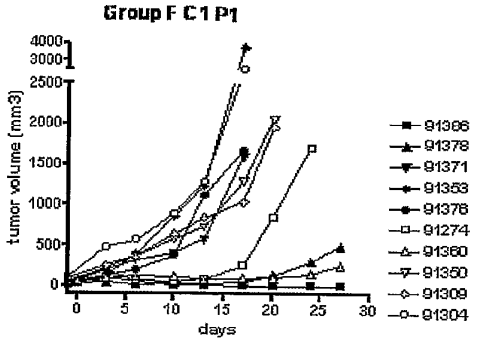


Figure 27F

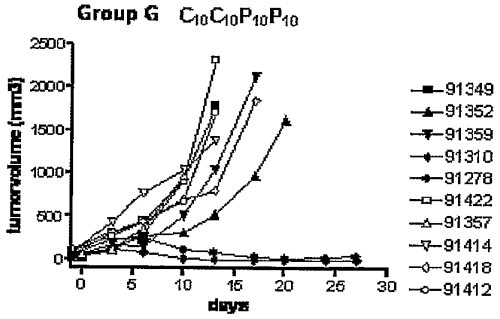


Figure 27G

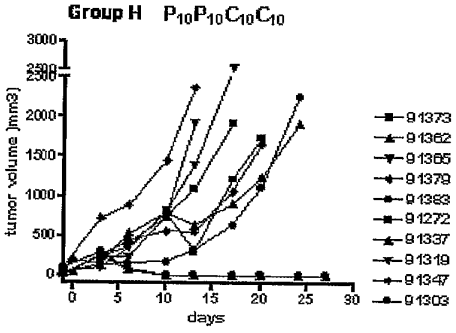


Figure 27H

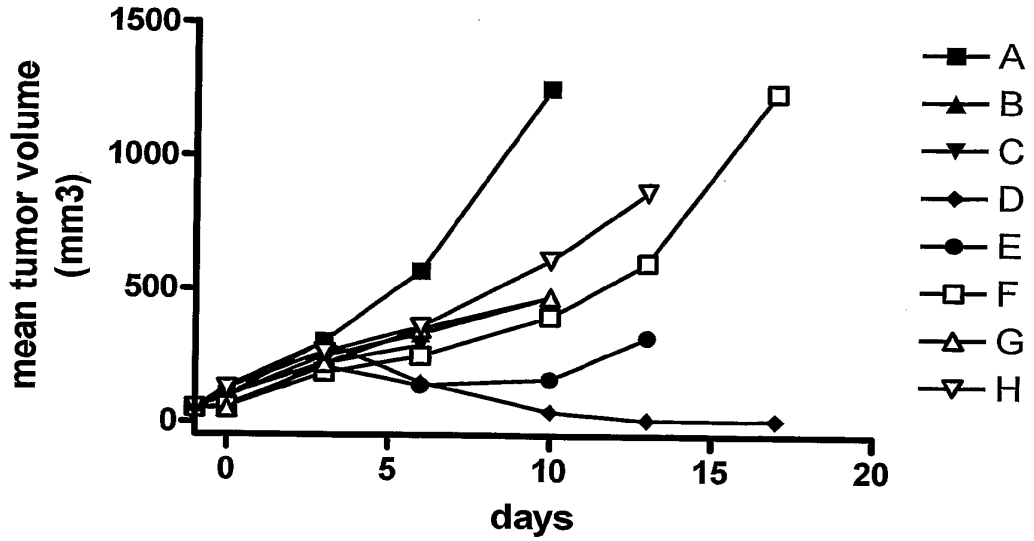


Figure 28

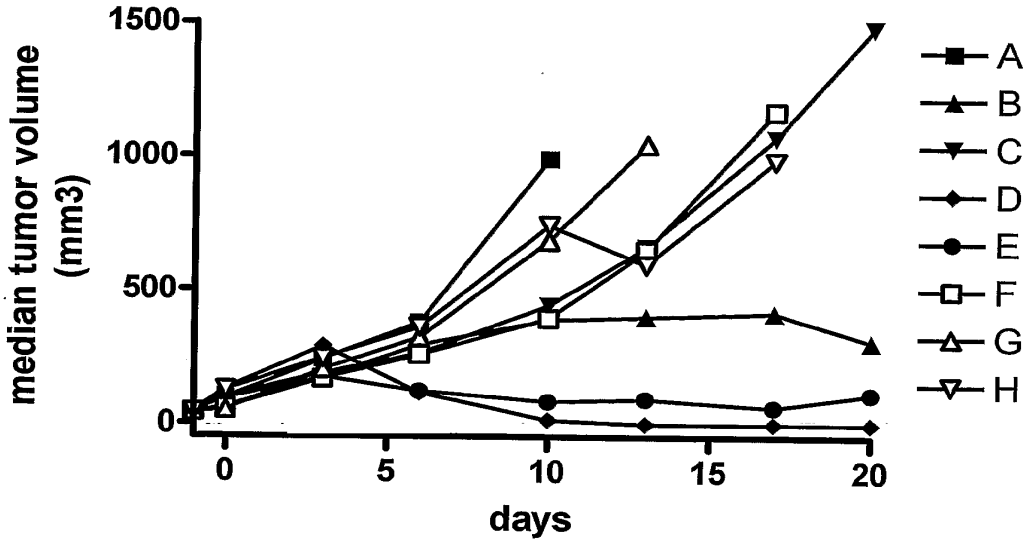


Figure 29

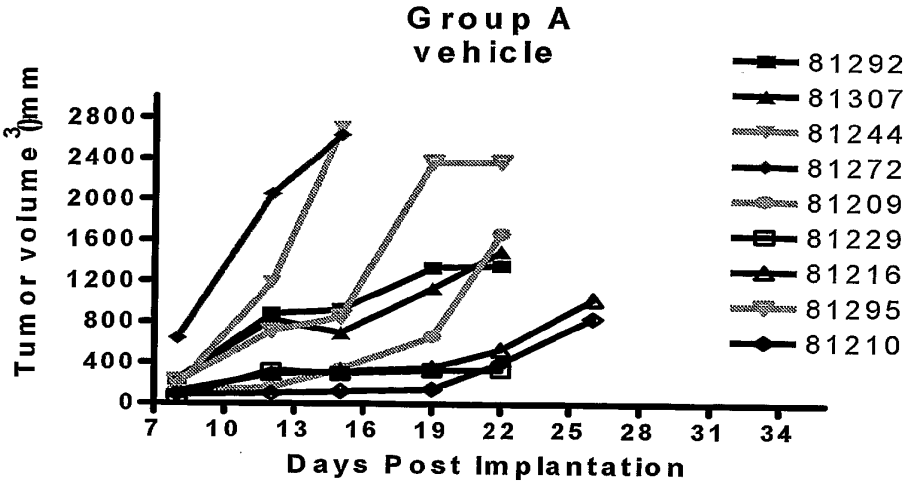


Figure 30A

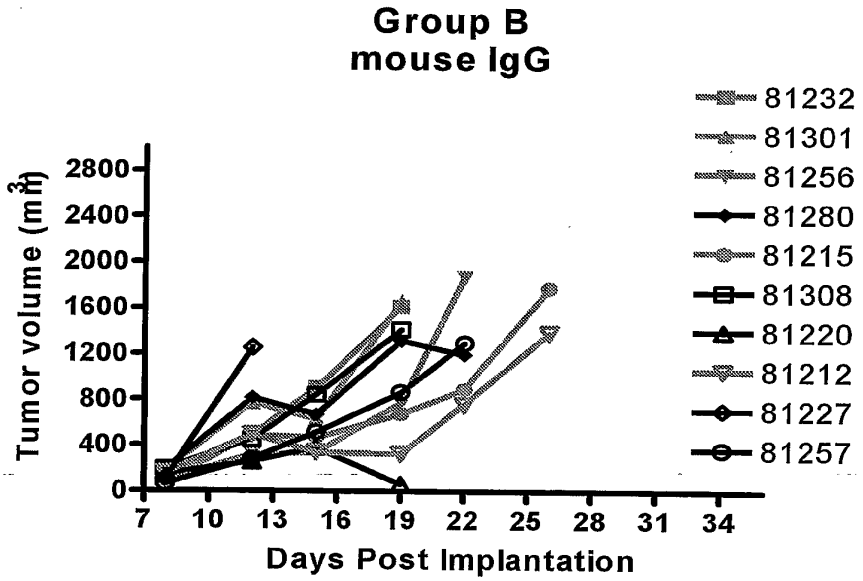


Figure 30B

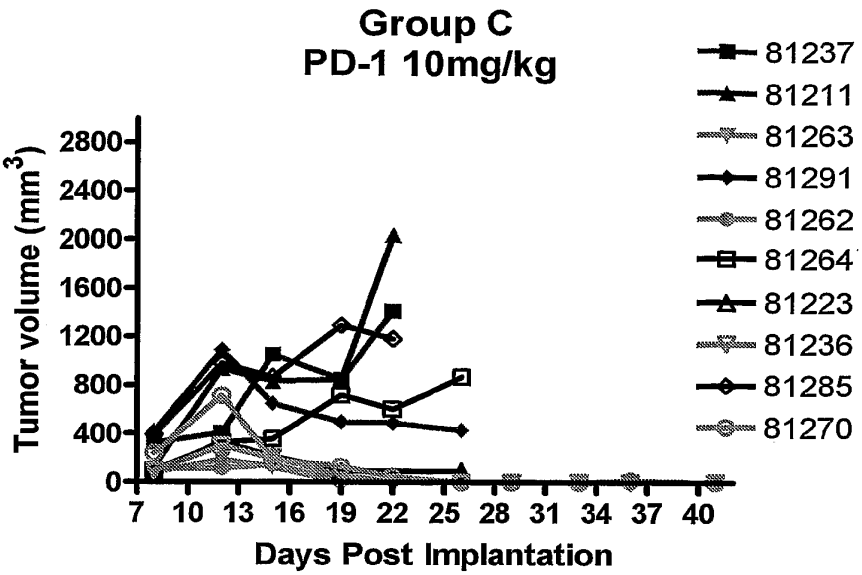


Figure 30C

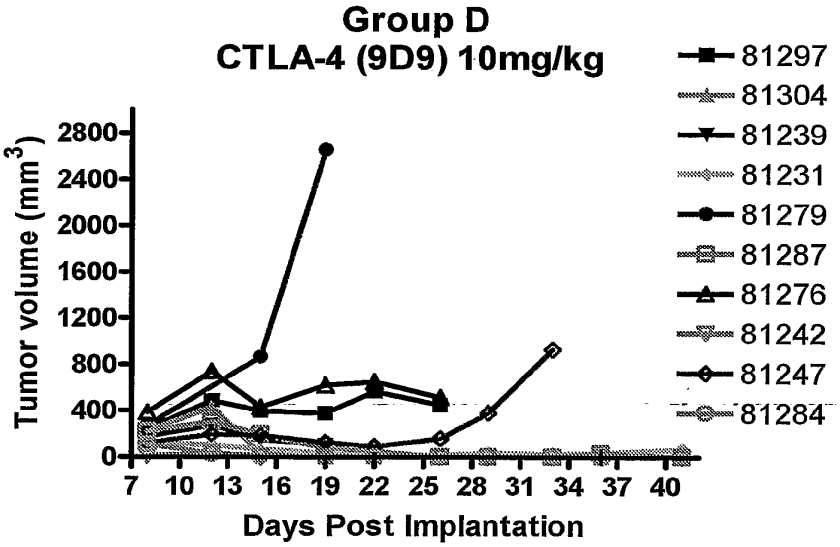


Figure 30D

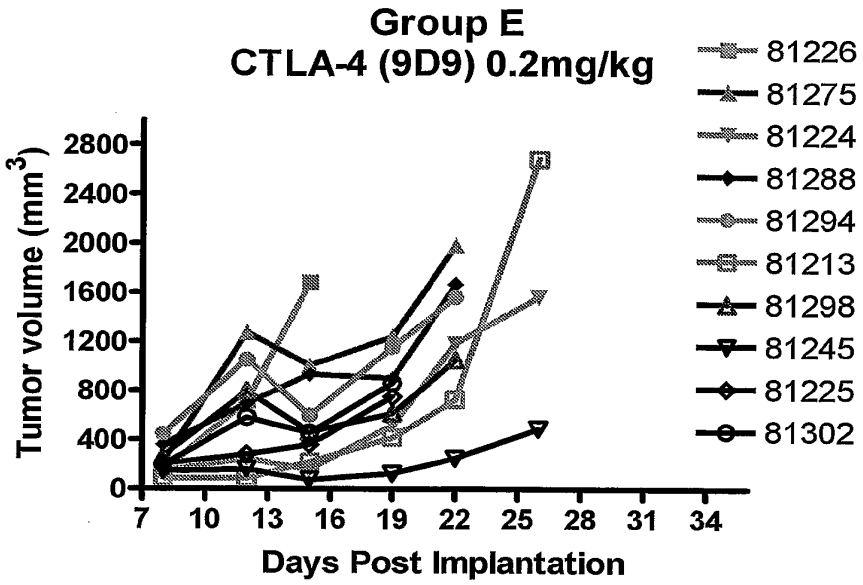


Figure 30E

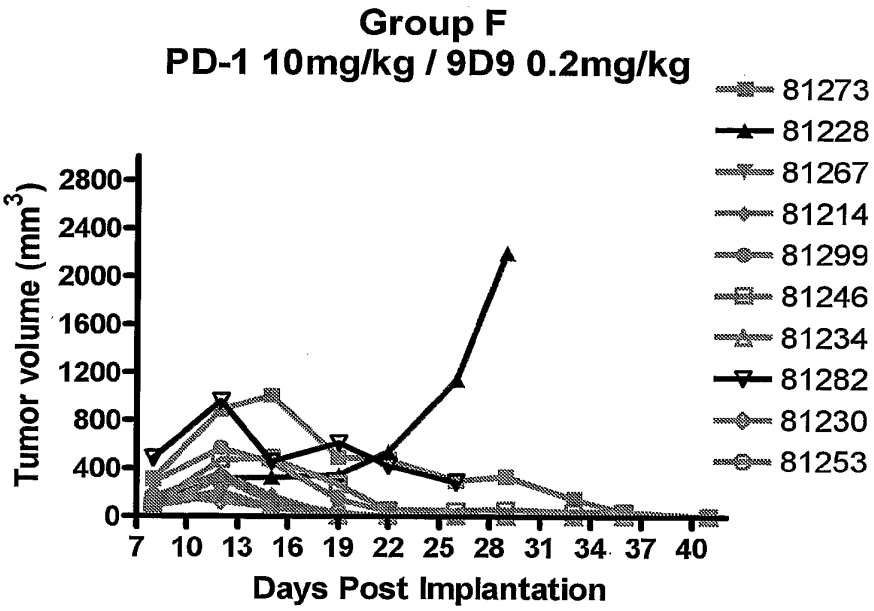


Figure 30F

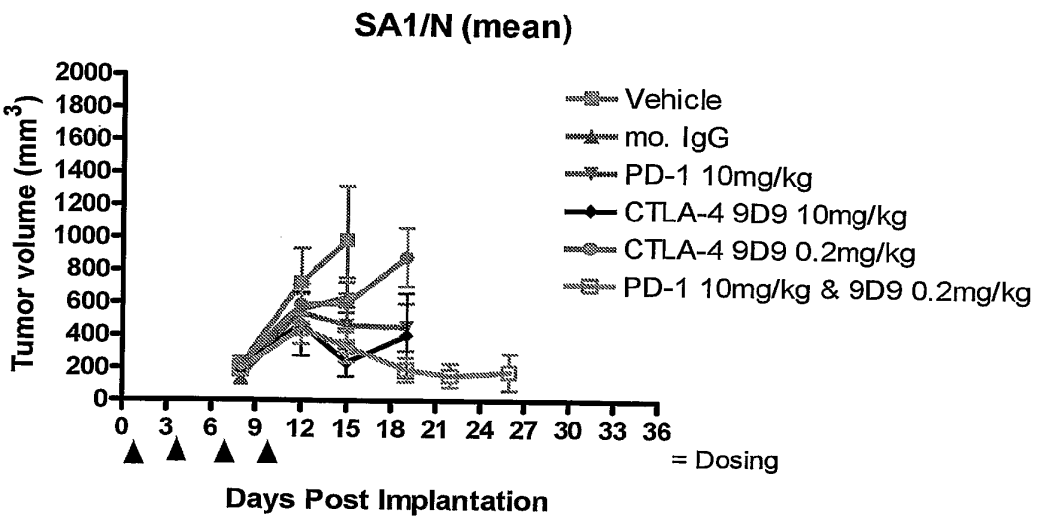


Figure 31

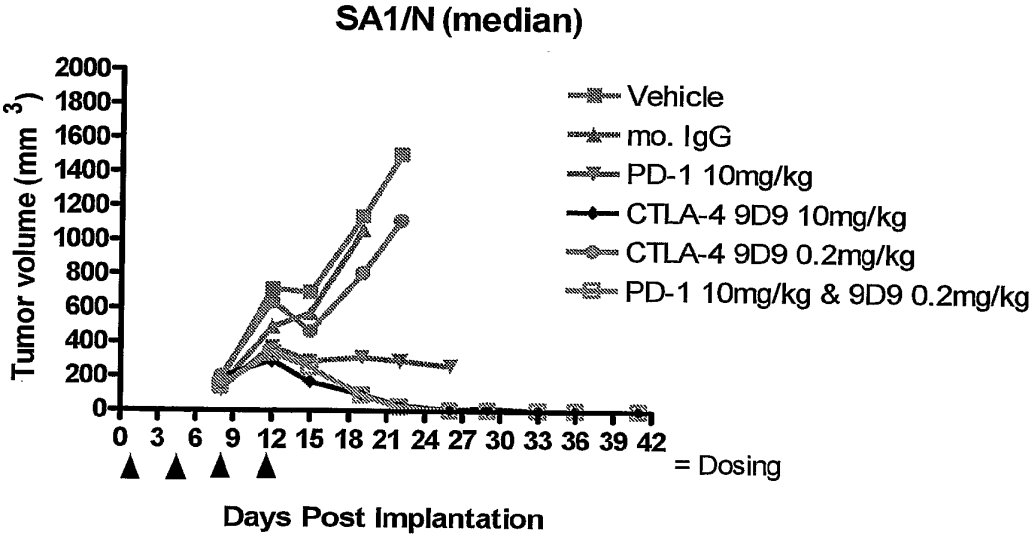


Figure 32

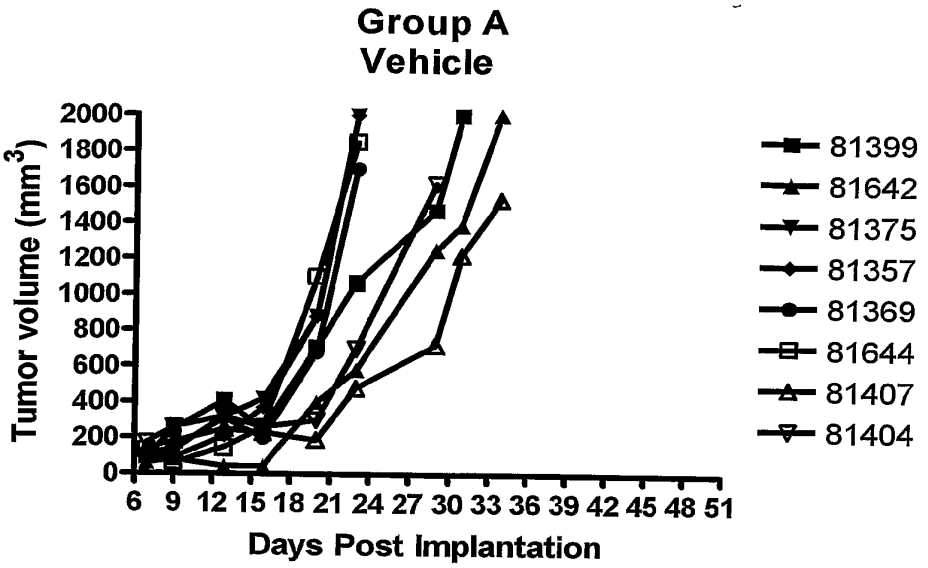


Figure 33A

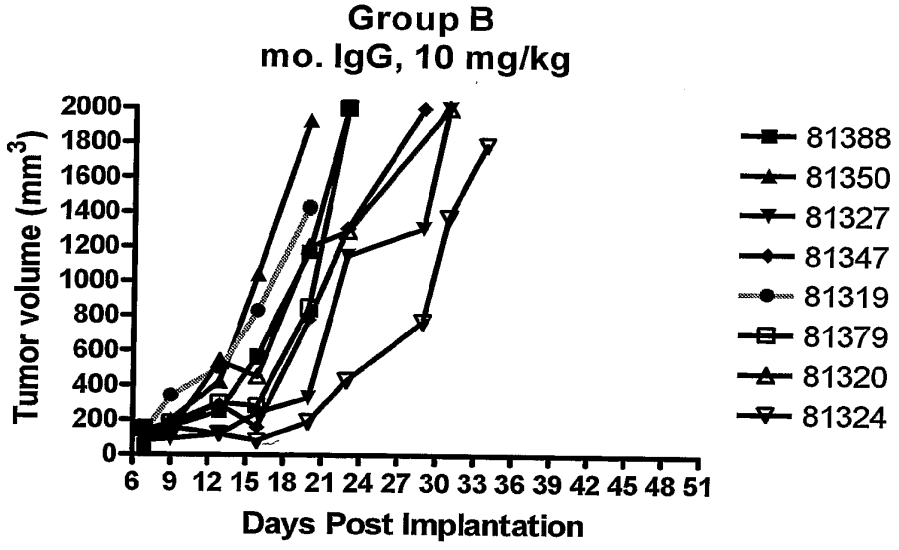


Figure 33B

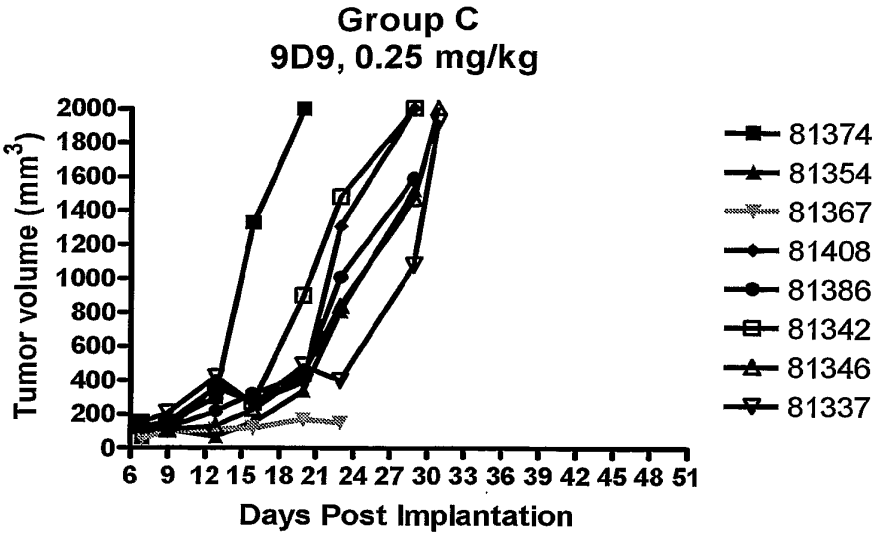


Figure 33C

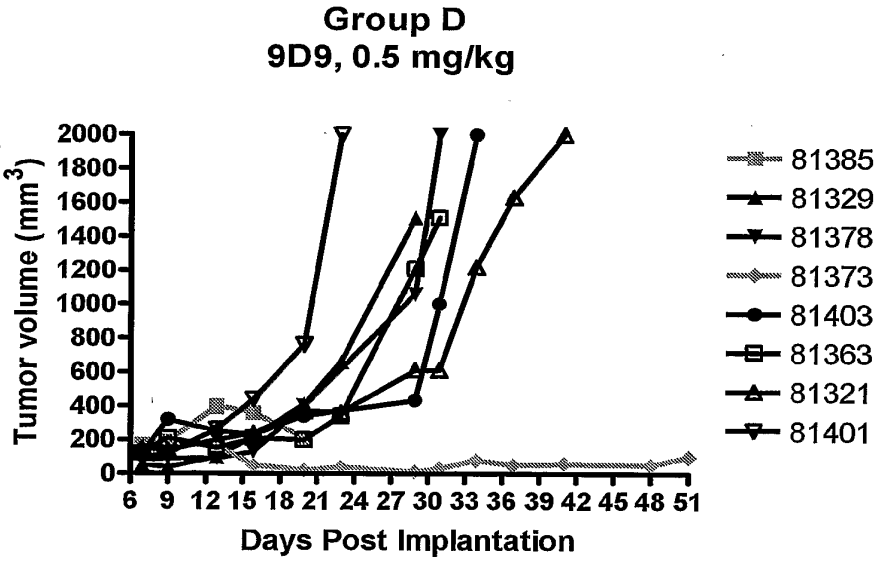


Figure 33D

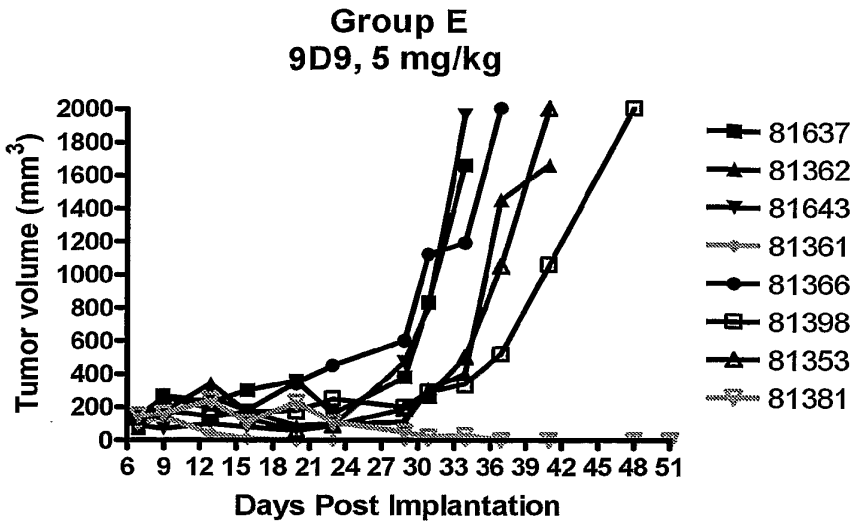


Figure 33E

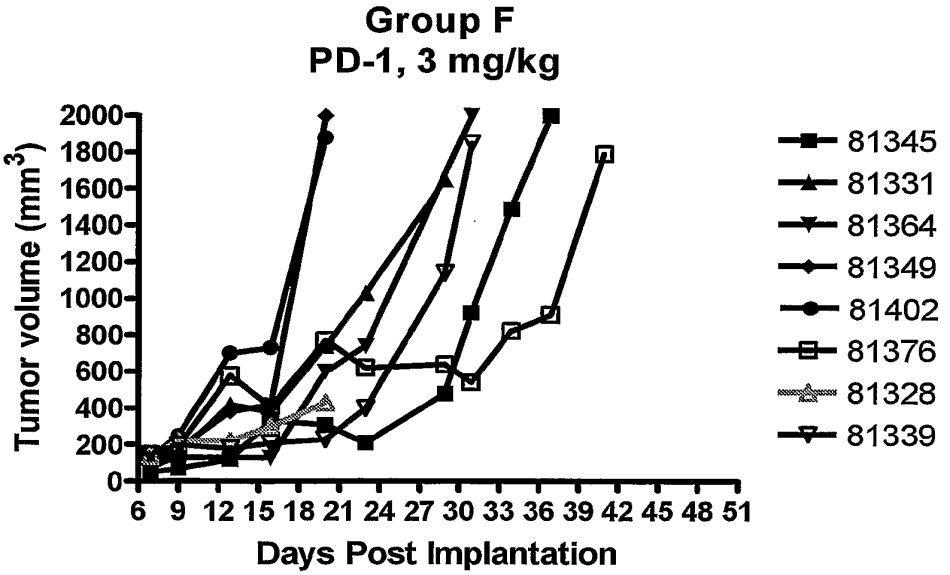


Figure 33F

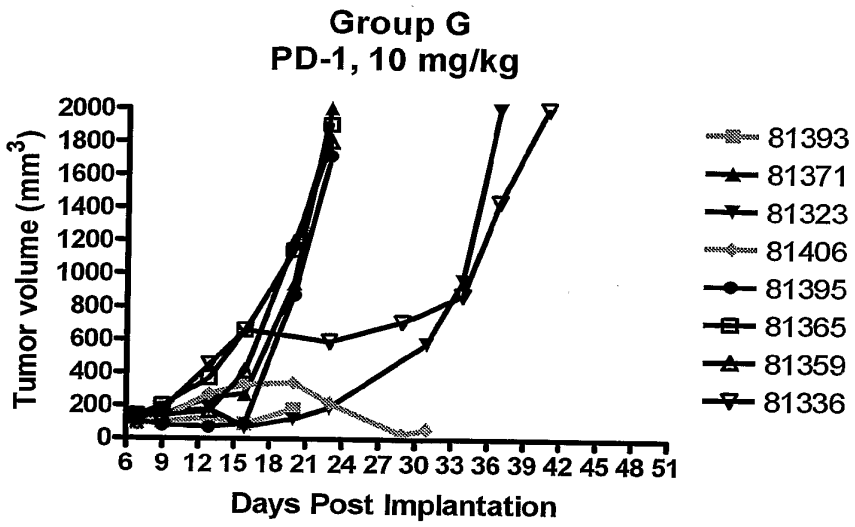


Figure 33G

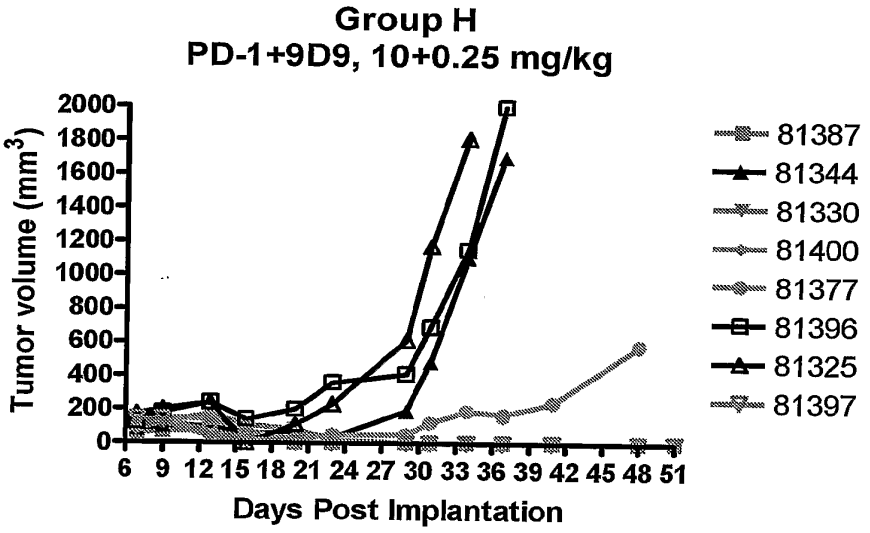


Figure 33H

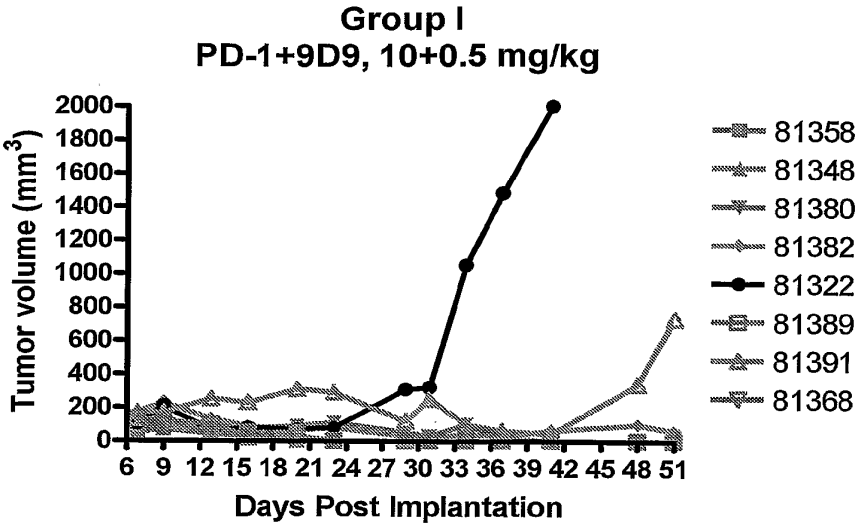


Figure 33I

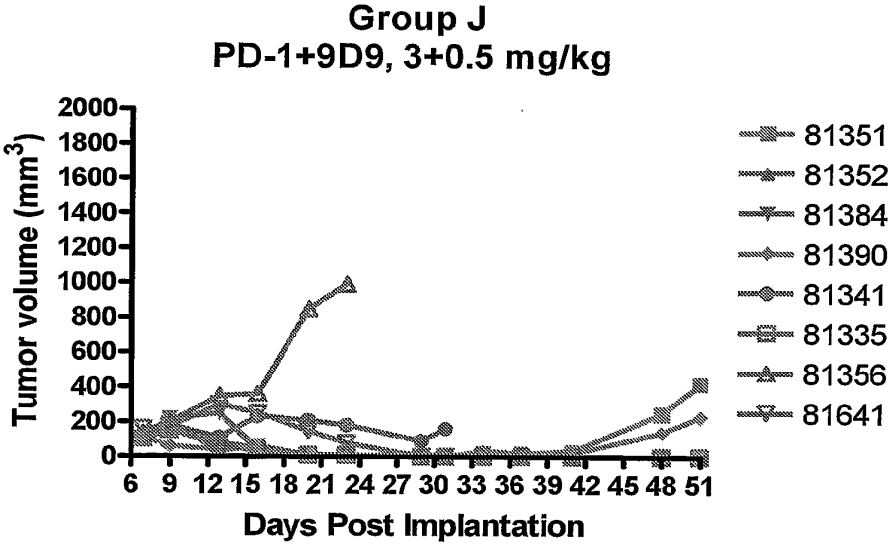


Figure 33J

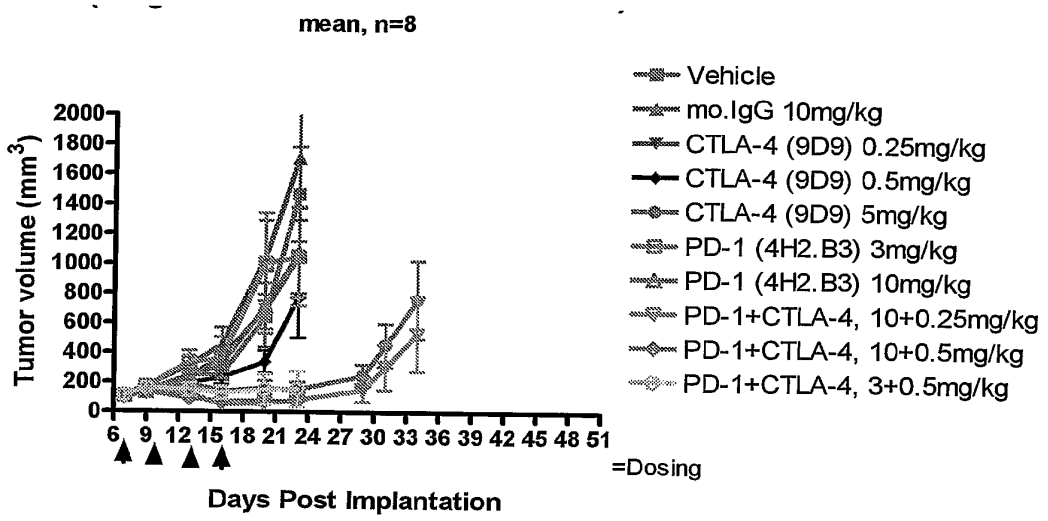


Figure 34

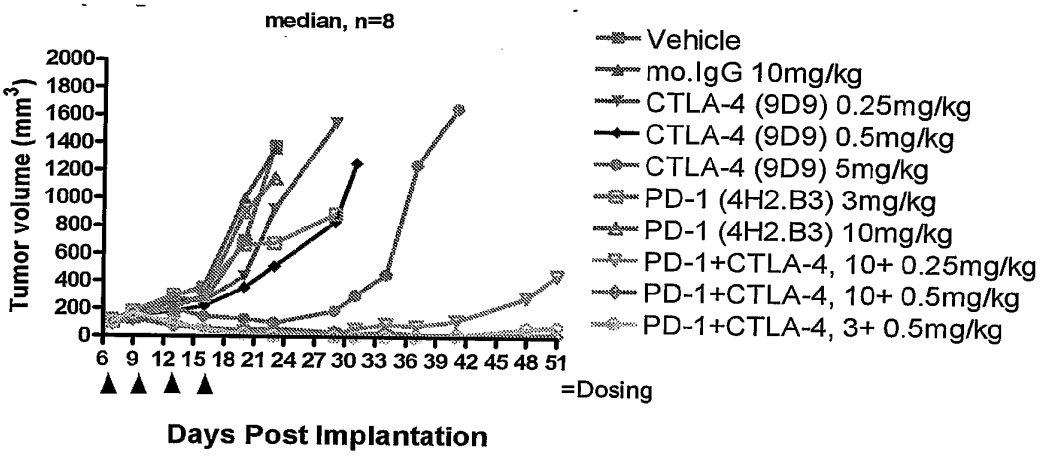


Figure 35

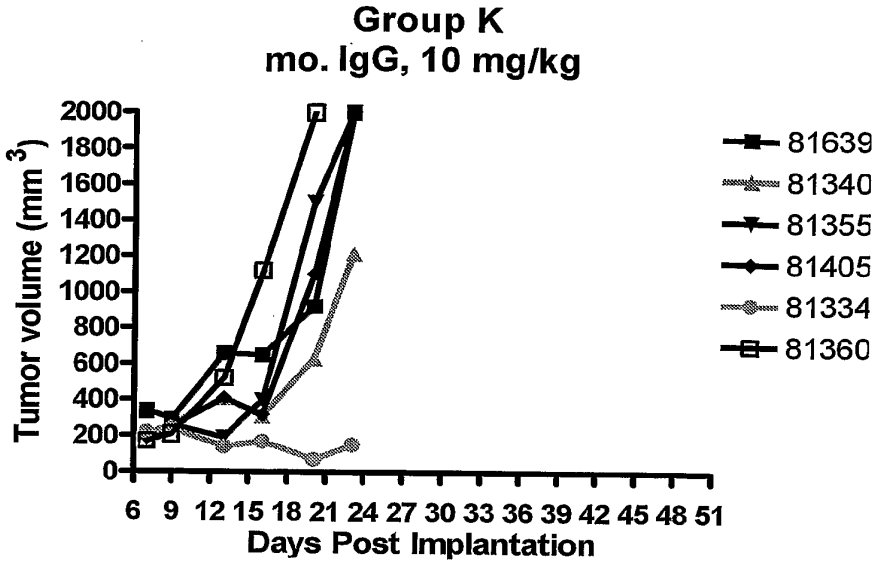


Figure 36A

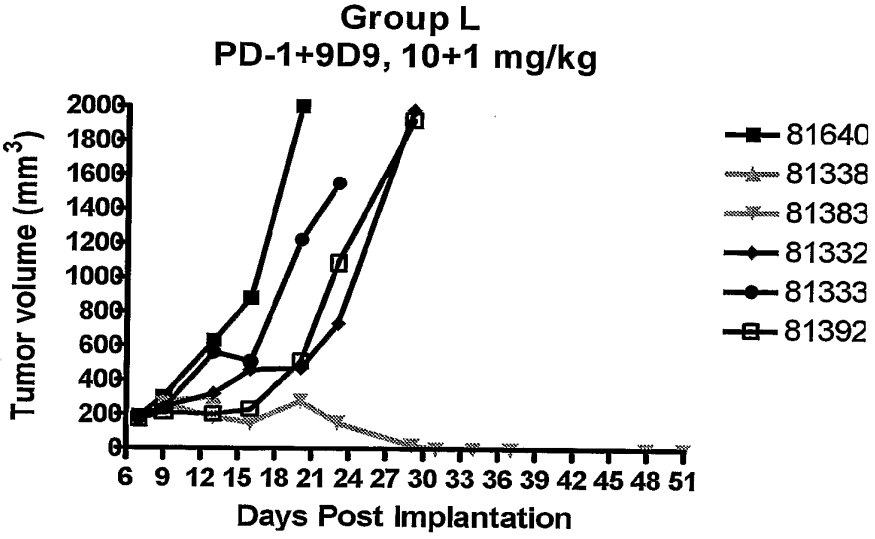


Figure 36B

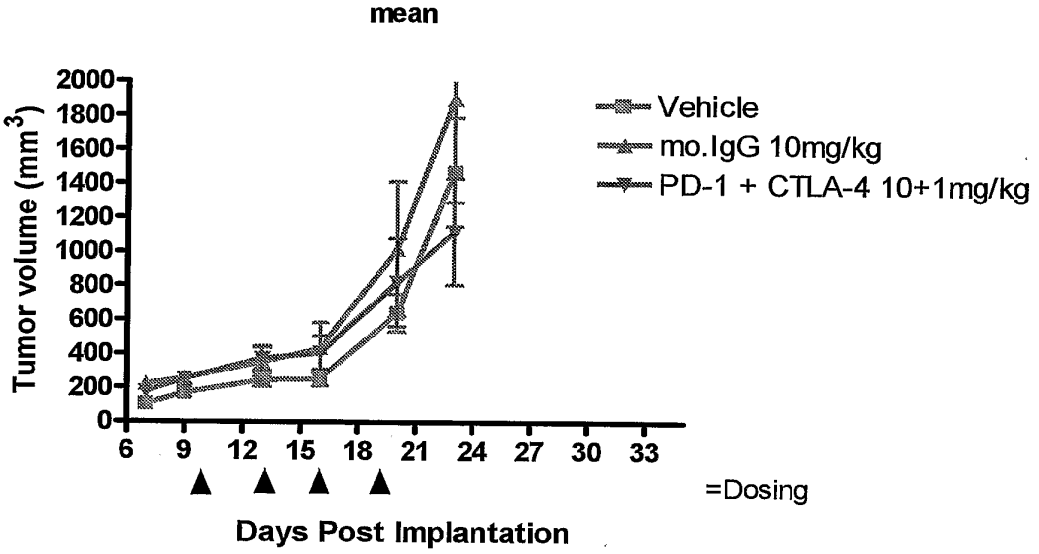


Figure 37

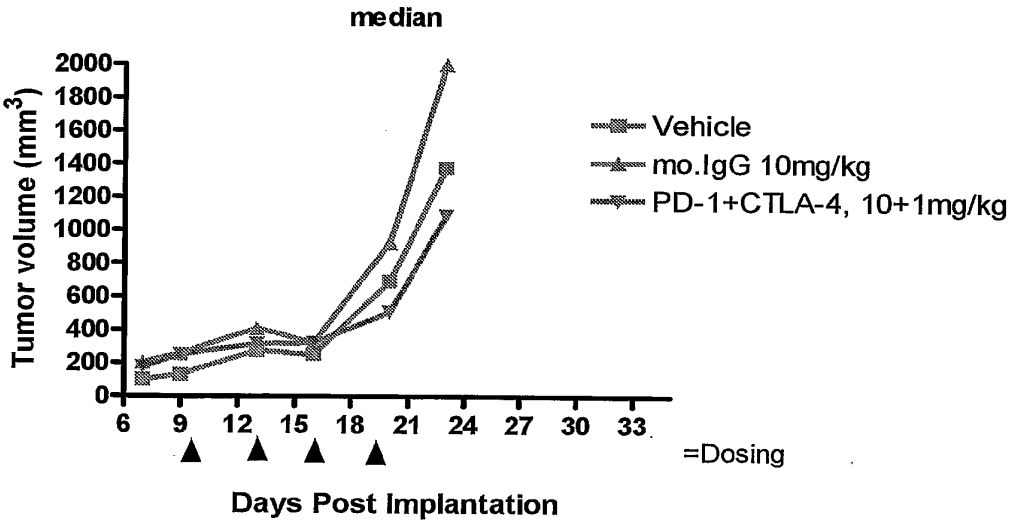


Figure 38

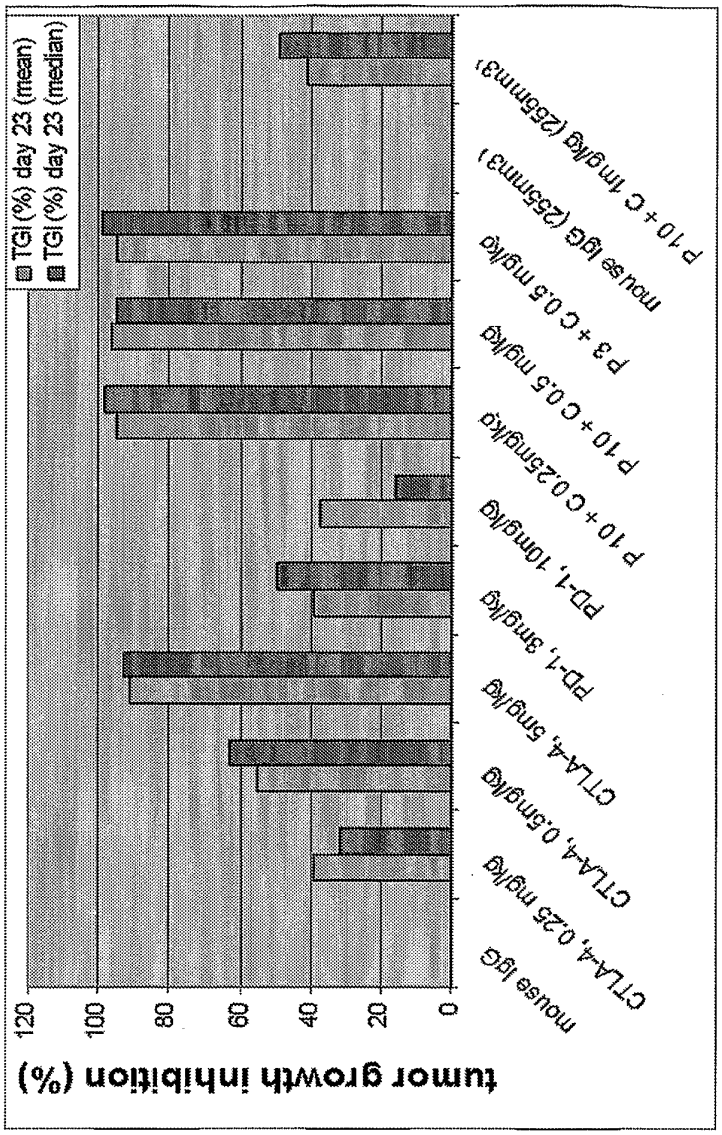

TGI (%) day 23 (mean)
TGI (%) day 23 (median)
tumor growth inhibition (%)
120
100
80
60
40
20
0
mouse IgG
CTLA-4, 0.25 mg/kg
CTLA-4, 0.5mg/kg
CTLA-4, 5mg/kg
PD-1, 3mg/kg
PD-1, 10mg/kg
P10+C 0.25mg/kg
P10+C 0.5 mg/kg
P3+C 0.5 mg/kg
mouse IgG (265mm3)
P10+C 1mg/kg (265mm3)

Figure 39

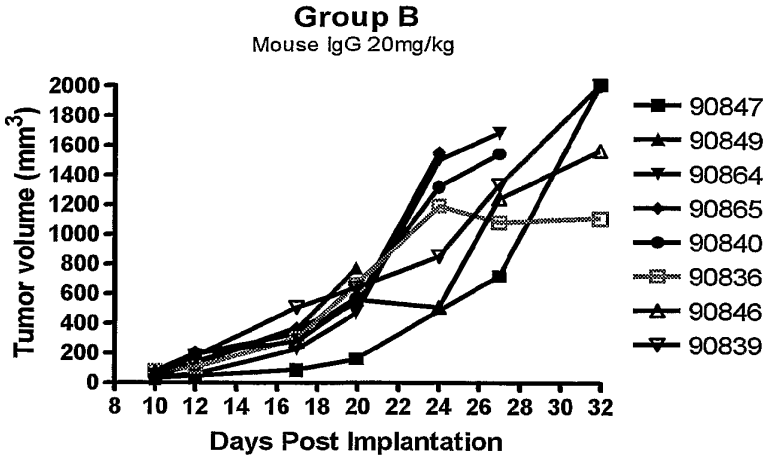


Figure 40A

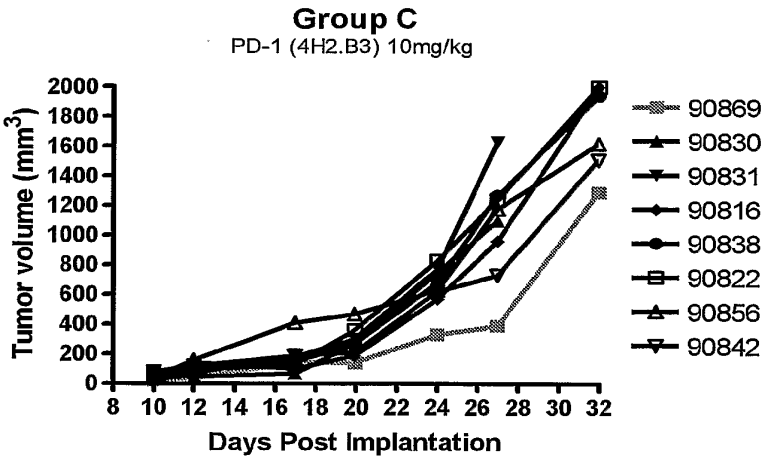


Figure 40B

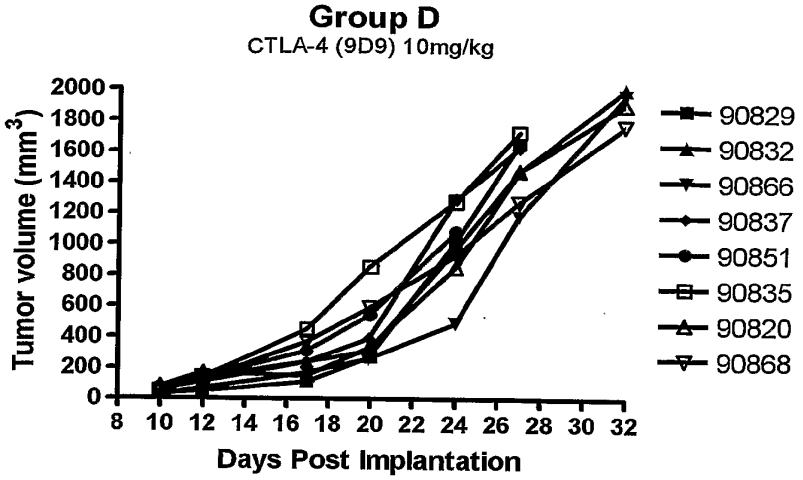


Figure 40C

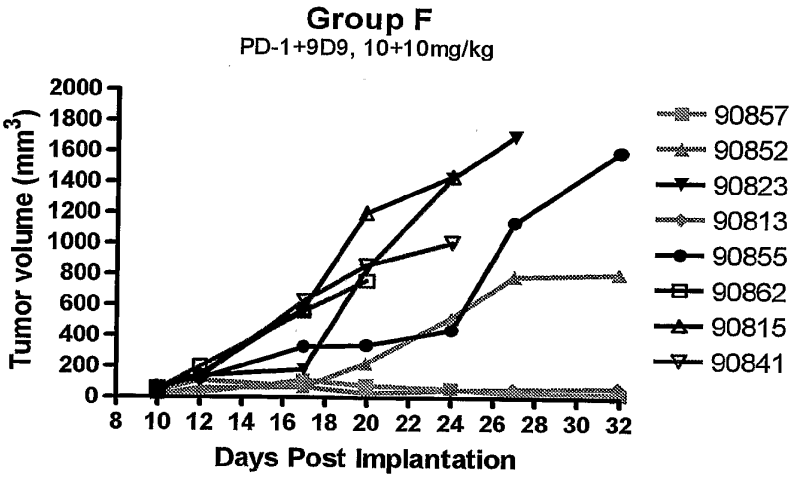


Figure 40D

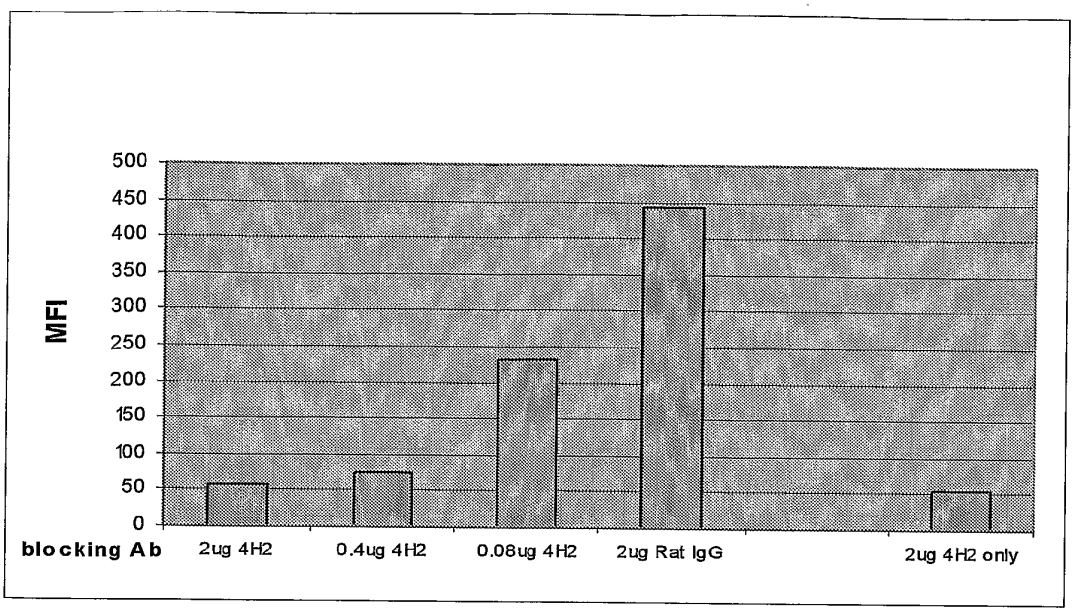


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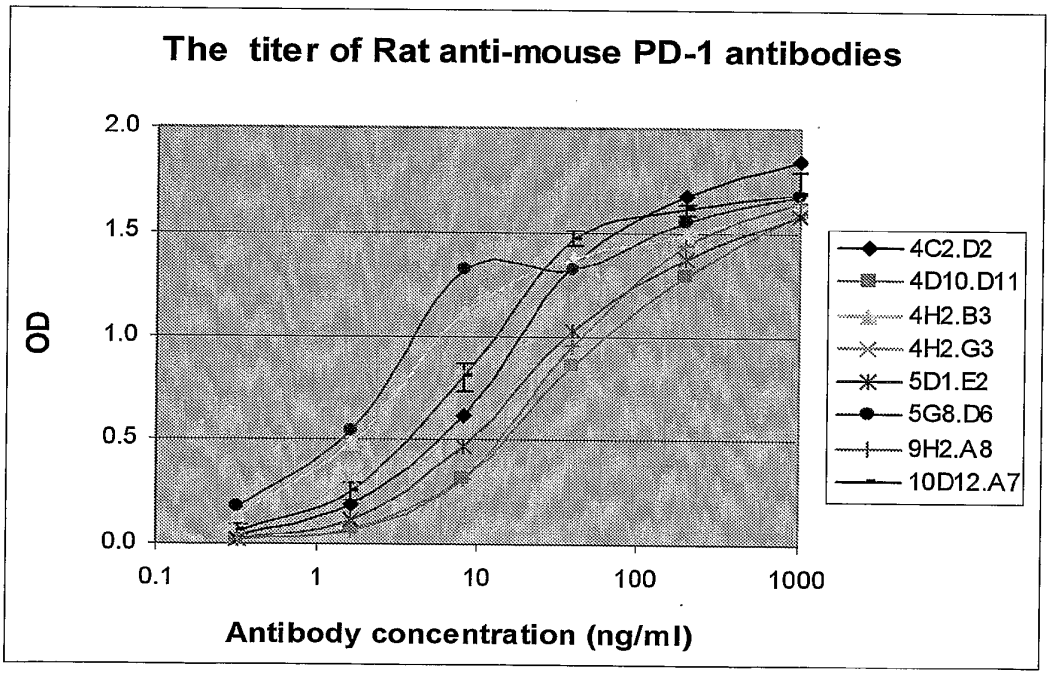


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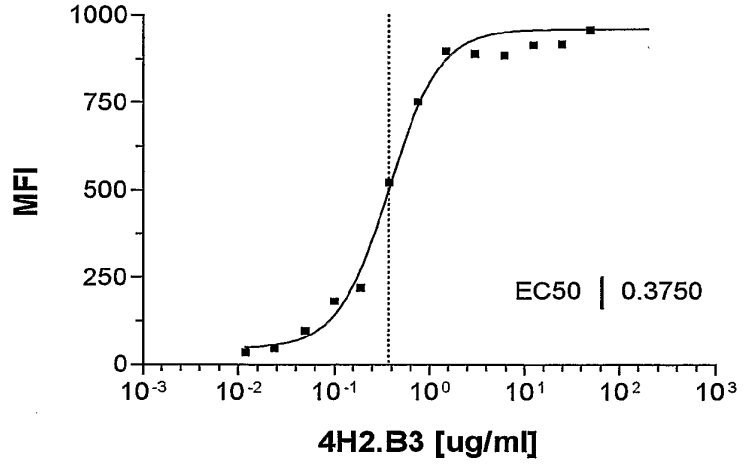


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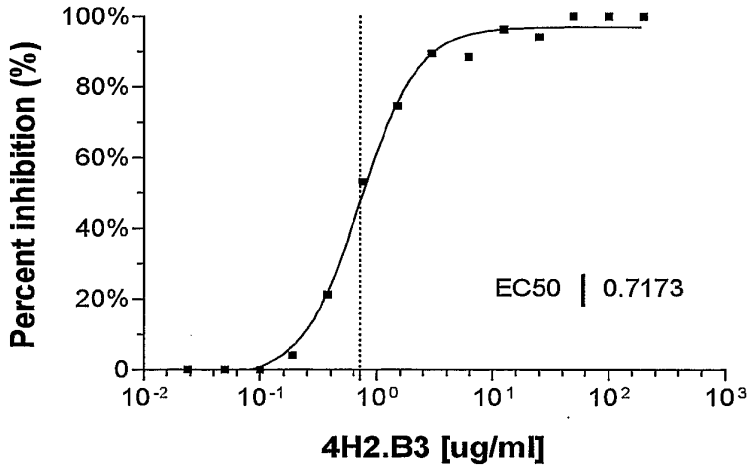


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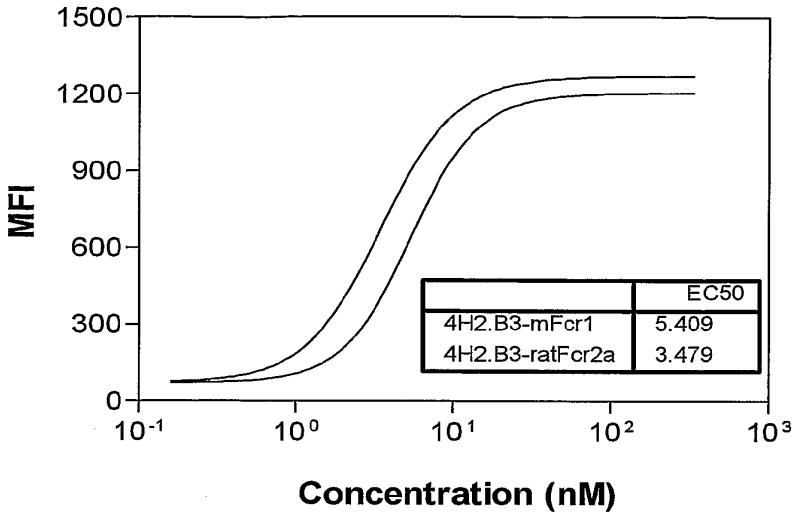


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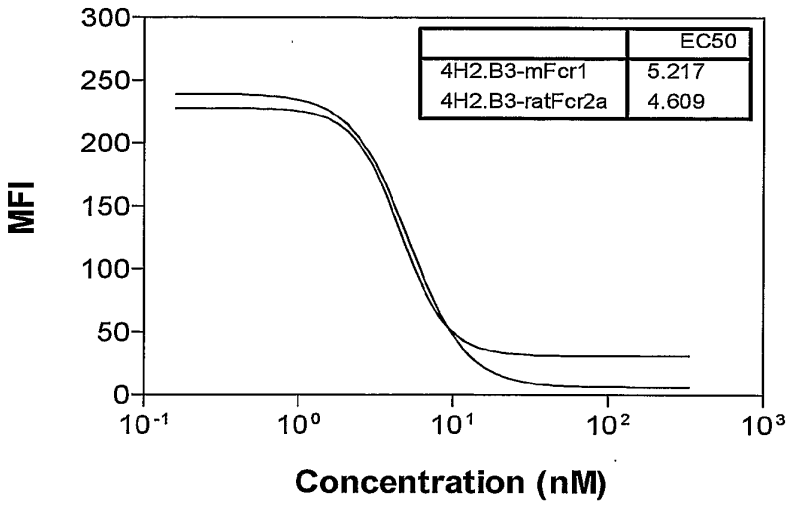


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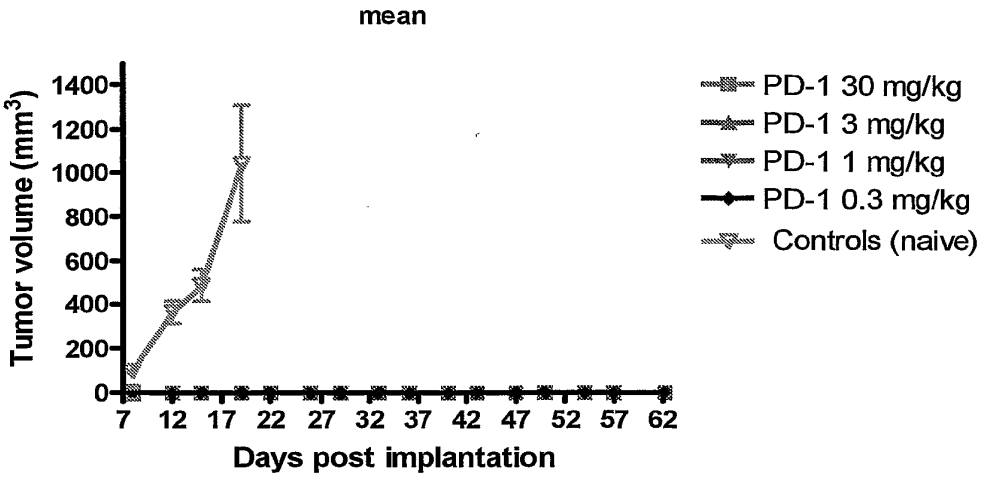


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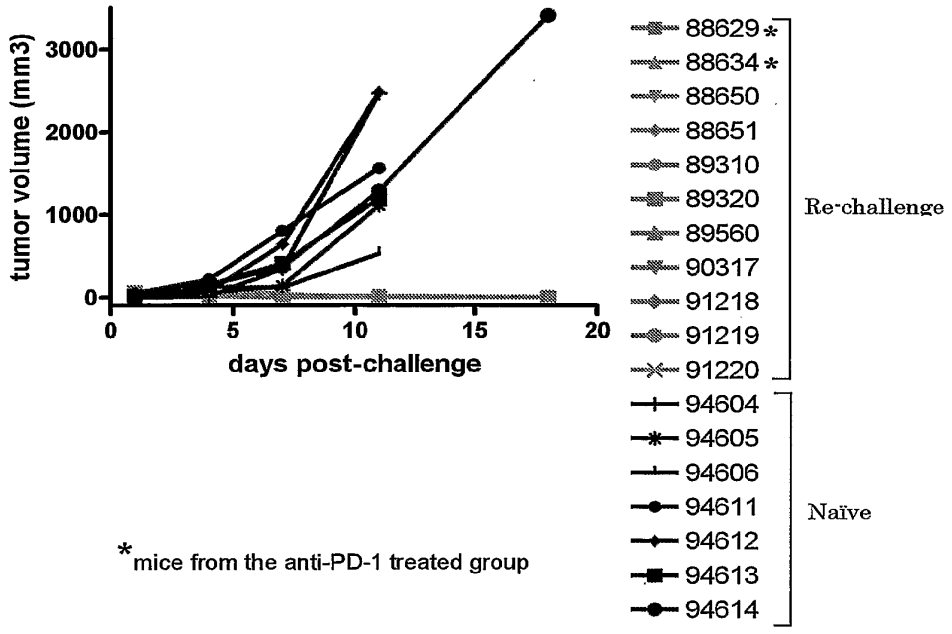


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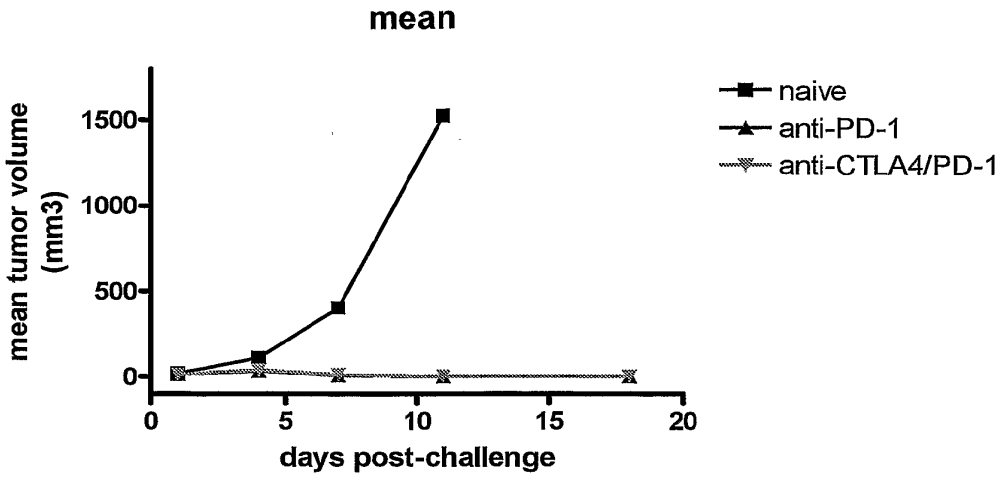


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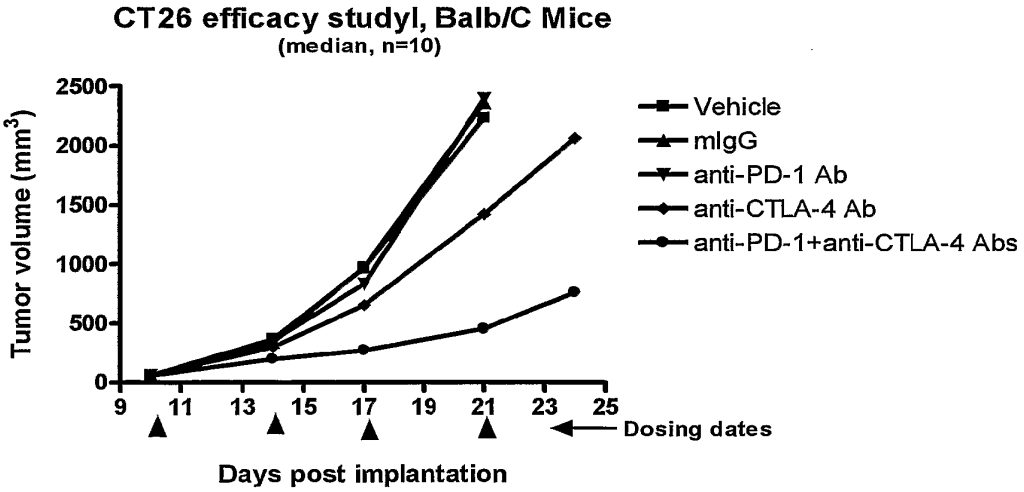


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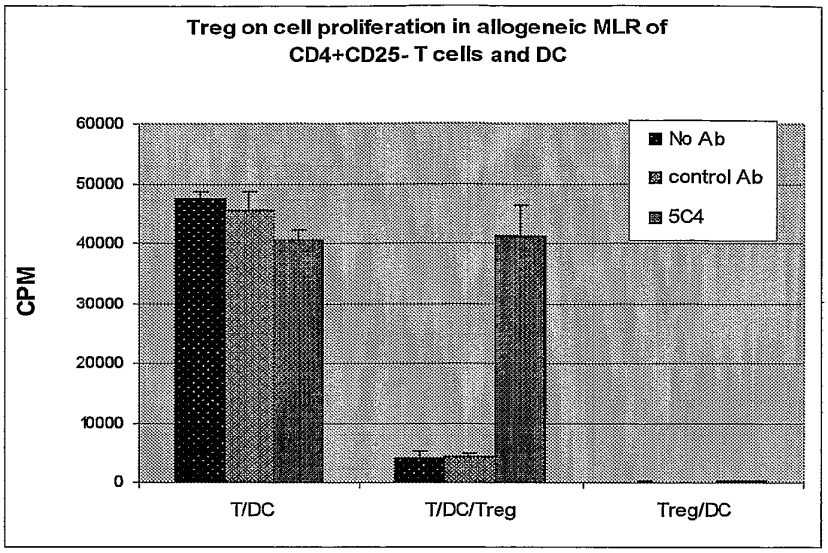


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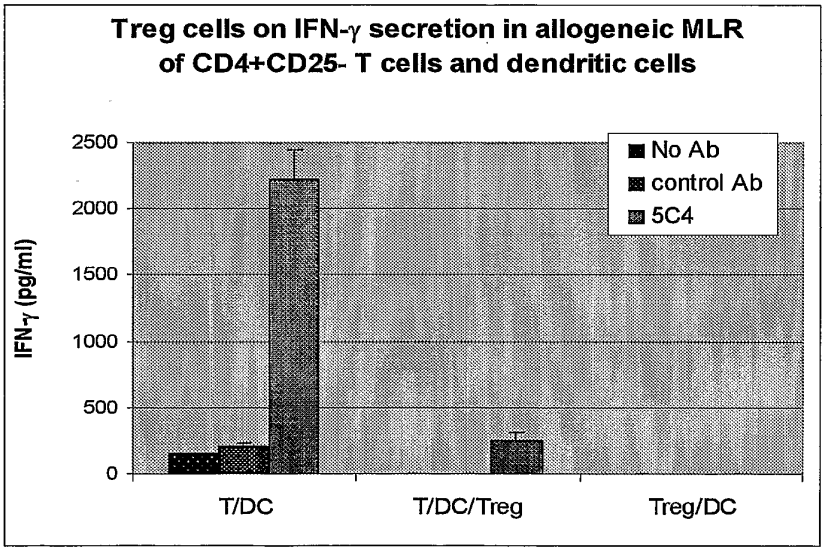


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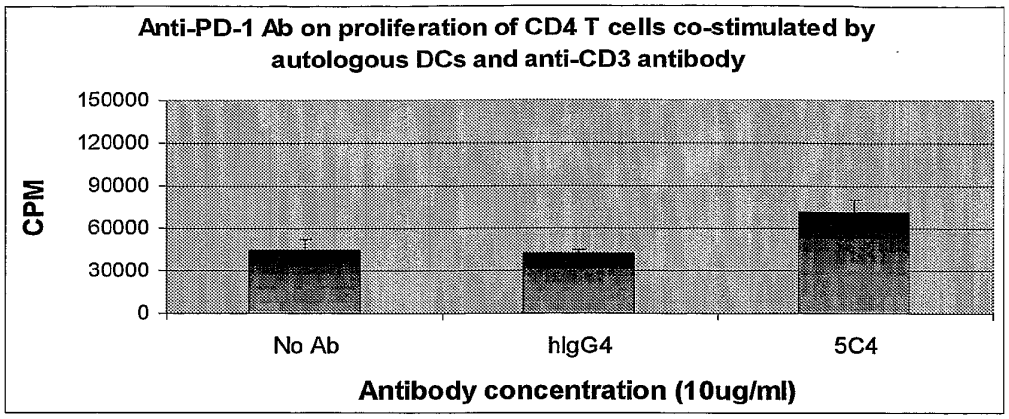


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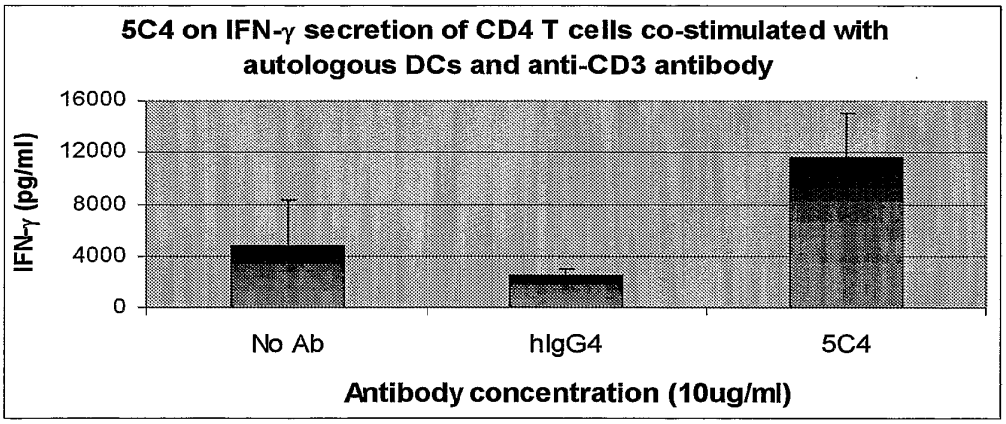


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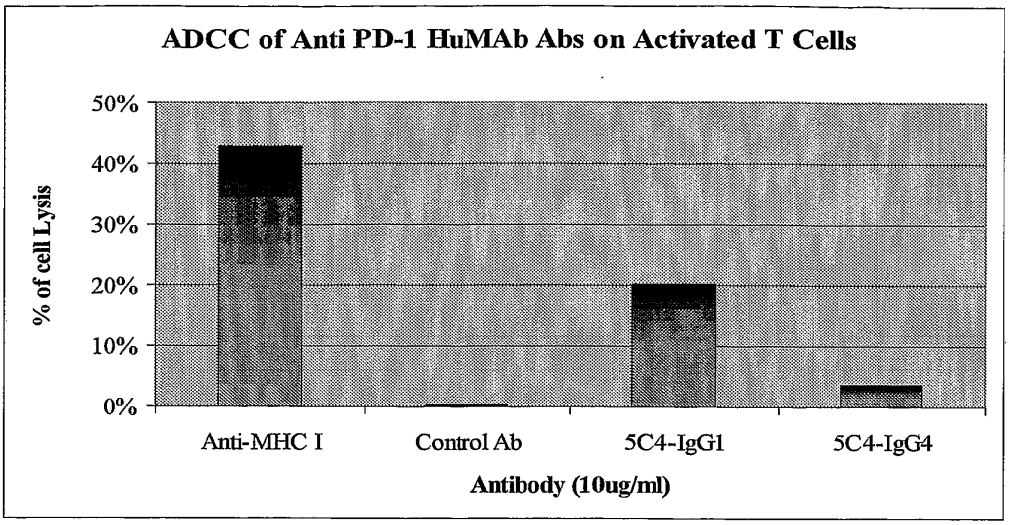


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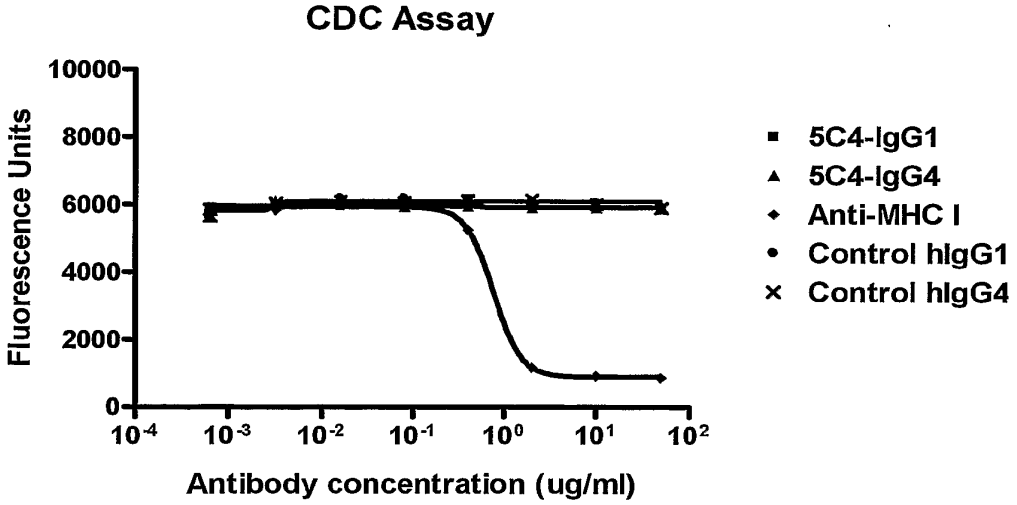


Figure 54

SEQUENCE LISTING

<110> Medarex, Inc.
<110> Ono Pharmaceutical Co., LTD.

<120> HUMAN MONOCLONAL ANTIBODIES TO PROGRAMMED DEATH 1 (PD-1) AND METHODS FOR TREATING CANCER USING ANTI-PD-1 ANTIBODIES ALONE OR IN COMBINATION WITH OTHER IMMUNOTHERAPEUTICS

<130> GEOP-50

<150> US 60/679,466
<151> 2005-05-09

<150> US 60/738,434
<151> 2005-11-21

<150> US 60/748,919
<151> 2005-12-08

<160> 74

<170> PatentIn version 3.1

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20 25 30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Met Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95
Ala Arg Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
100 105 110
Ser

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<211> 113
<212> PRT
<213> Homo sapiens

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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Leu Thr Phe Thr Asn Tyr
20 25 30
Gly Phe His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

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        35                  40                  45
Ala Val Ile Trp Tyr Asp Gly Ser Lys Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80
Leu Gln Met Asn Asn Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Thr Gly Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110
Ser
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<210> 3
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<212> PRT
<213> Homo sapiens

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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
            20                  25                  30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Leu Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80
Leu Gln Met Thr Ser Leu Arg Val Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Ser Asn Val Asp His Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110
Ser
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<212> PRT
<213> Homo sapiens

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Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
            20                  25                  30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
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Ser
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20 25 30
Ser Phe Phe Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
35 40 45
Trp Ile Gly Ser Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
50 55 60
Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65 70 75 80
Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Val Arg Asp Tyr Asp Ile Leu Thr Gly Asp Glu Asp Tyr Trp Gly
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Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

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<212> PRT
<213> Homo sapiens

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20 25 30
Gly Phe His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Val Ile Trp Tyr Asp Gly Ser Lys Lys Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Leu Ser Arg Asp Asp Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Val Thr Gly Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
100 105 110
Ser

<210> 7
<211> 121
<212> PRT
<213> Homo sapiens

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Thr Leu Ser Leu Thr Cys Ser Val Ser Gly Gly Ser Leu Ser Arg Ser

```
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Ser Tyr Phe Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
        35                  40                  45
Trp Ile Ala Ser Ile Phe Tyr Ser Gly Glu Thr Tyr Phe Asn Pro Ser
    50                  55                  60
Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Arg Asn Gln Phe
65                  70                  75                  80
Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
                85                  90                  95
Cys Ala Arg Asp Tyr Asp Ile Leu Thr Gly Asp Glu Asp Tyr Trp Gly
            100                 105                 110
Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120
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<212> PRT
<213> Homo sapiens

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            20                  25                  30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Ile Ile
        35                  40                  45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Leu
                85                  90                  95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105
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<210> 9
<211> 107
<212> PRT
<213> Homo sapiens

<400> 9

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Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
            20                  25                  30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45
Tyr Asp Thr Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Leu
                85                  90                  95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105
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<211> 107
<212> PRT
<213> Homo sapiens

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Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Ser Asn Trp Pro Arg
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 11
<211> 107
<212> PRT
<213> Homo sapiens

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Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Ser Asn Trp Pro Arg
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

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<212> PRT
<213> Homo sapiens

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Asp Arg Val Ser Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys Ser Leu Ile

```
        35                  40                  45
Tyr Ala Ala Ser Asn Leu Arg Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Arg
                85                  90                  95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105
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<211> 107
<212> PRT
<213> Homo sapiens

<400> 13

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Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
            20                  25                  30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Leu
                85                  90                  95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105
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<210> 14
<211> 107
<212> PRT
<213> Homo sapiens

<400> 14

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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
            20                  25                  30
Leu Ala Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys Ser Leu Ile
        35                  40                  45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Arg
                85                  90                  95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105
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Asn Tyr Gly Met His
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Ser Tyr Gly Phe His
1 5

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Gly

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<213> Homo sapiens

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Val Ile Trp Tyr Asp Gly Ser Lys Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15
Gly

<210> 24
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Gly

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1 5 10 15
Gly

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Ser Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser Leu Lys Ser
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1 5 10 15
Gly

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Ser Ile Phe Tyr Ser Gly Glu Thr Tyr Phe Asn Pro Ser Leu Lys Ser
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Gly Asp Asp Tyr
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Asn Asp Asp Tyr
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1 5 10

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1 5 10

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1 5 10

<210> 38
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1 5 10

<210> 39
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1 5 10

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1 5 10

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Gln Gln Ser Ser Asn Trp Pro Arg Thr
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<210> 56
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<400> 56
Gln Gln Tyr Tyr Ser Tyr Pro Arg Thr
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<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(339)

<400> 57
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Gln Val Gln Leu Val Glu Ser Gly Gly Asp Val Val Gln Pro Gly Gly
1 5 10 15

tcc ctg aga ctc tcc tgt gca gcg tct gga gtc gcc ttc agt aac tat 96
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Val Ala Phe Ser Asn Tyr
20 25 30

ggc atg cac tgg gtc cgc cag gct ccc ggc aag ggg ctg gag tgg gtg 144
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

gca gtt atc tgg tat gat gga agt aat aaa tac tat gca gac tcc gtg 192
Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

aag ggc cgg ttc acc atc tcc aga gac aat tcc aag aac atg ctc tat 240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Met Leu Tyr
65 70 75 80

ctg caa atg aac agc ctg aga gcc gag gac acg gct atg tat tac tgt 288
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

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gcg agg aac gat gac tac tgg ggc cag gga acc ctg gtc acc gtc tcc      336
Ala Arg Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110

tca                                                                  339
Ser
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<210> 58
<211> 339
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(339)

<400> 58

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cag gtg cag ctg gtg gaa tct ggg gga gac gtg gtc cag cct ggg agg       48
Gln Val Gln Leu Val Glu Ser Gly Gly Asp Val Val Gln Pro Gly Arg
1               5                   10                  15

tcc ctg aga ctc tcc tgt gca gcg tct gga tta acc ttc act aac tat       96
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Leu Thr Phe Thr Asn Tyr
            20                  25                  30

ggc ttc cac tgg gtc cgc cag gct cca ggc aag ggg ctg gag tgg gtg      144
Gly Phe His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

gct gtt ata tgg tat gat gga agt aag aaa tat tat gca gac tcc gtg      192
Ala Val Ile Trp Tyr Asp Gly Ser Lys Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60

aag ggc cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat      240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

ctg caa atg aac aac ctg aga gcc gag gac acg gct gtg tat tac tgt      288
Leu Gln Met Asn Asn Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

gcg act ggg gat gac tac tgg ggc cag gga acc ctg gtc acc gtc tcc      336
Ala Thr Gly Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110

tca                                                                  339
Ser
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<210> 59
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<212> DNA
<213> Homo sapiens

<220>

<221> CDS
<222> (1)..(339)

<400> 59
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Gln Val Tyr Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

tcc ctg aga ctc tcc tgt gca gcg tct gga ttc acc ttc agt aac tat 96
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30

ggc atg cac tgg gtc cgc cag gct cca ggc aag ggg ctg gag tgg gtg 144
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

gca ctt ata tgg tat gat gga agt aat aaa tac tat gca gac tcc gtg 192
Ala Leu Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

aag ggc cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat 240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

ctg caa atg acc agt ctg aga gtc gag gac acg gct gtg tat tat tgt 288
Leu Gln Met Thr Ser Leu Arg Val Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

gcg agc aac gtt gac cat tgg ggc cag gga acc ctg gtc acc gtc tcc 336
Ala Ser Asn Val Asp His Trp Gly Gln Gly Thr Leu Val Thr Val Ser
100 105 110

tca 339
Ser

<210> 60
<211> 339
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(339)

<400> 60
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Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

tcc ctg aga ctc gac tgt aaa gcg tct gga atc acc ttc agt aac tct 96
Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
20 25 30

ggc atg cac tgg gtc cgc cag gct cca ggc aag ggg ctg gag tgg gtg 144
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

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gca gtt att tgg tat gat gga agt aaa aga tac tat gca gac tcc gtg      192
Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
    50                  55                  60

aag ggc cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg ttt      240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

ctg caa atg aac agc ctg aga gcc gag gac acg gct gtg tat tac tgt      288
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

gcg aca aac gac gac tac tgg ggc cag gga acc ctg gtc acc gtc tcc      336
Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110

tca                                                                 339
Ser
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<210> 61
<211> 363
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(363)

<400> 61

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cag ctg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gag       48
Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1               5                   10                  15

acc ctg tcc ctc acc tgc act gtc tct ggt ggc tcc ctc agc agg agt       96
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Leu Ser Arg Ser
            20                  25                  30

agt ttc ttc tgg ggc tgg atc cgt cag ccc cca ggg aag gga ctg gag      144
Ser Phe Phe Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
        35                  40                  45

tgg att ggg agt atc tat tat agt ggg agc acc tac tac aac ccg tcc      192
Trp Ile Gly Ser Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
    50                  55                  60

ctc aag agt cga gtc acc ata tcc gta gac acg tcc aag aac cag ttc      240
Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65                  70                  75                  80

tcc ctg aag ctg agc tct gtg acc gcc gca gac acg gct gtg tat tac      288
Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
                85                  90                  95

tgt gtg aga gat tac gat att ttg act ggc gac gag gac tac tgg ggc      336
Cys Val Arg Asp Tyr Asp Ile Leu Thr Gly Asp Glu Asp Tyr Trp Gly
```

```
            100                 105                 110

cag gga acc ctg gtc acc gtg tcc tca                                    363
Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120
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<210> 62
<211> 339
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(339)

<400> 62

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cag gtg cag ctg gtg gag tct ggg gga ggc gtg gtc cag cct ggg agg      48
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

tcc ctg aga ctc tcc tgt aca acg tct gga atc acc ttc agt agc tat      96
Ser Leu Arg Leu Ser Cys Thr Thr Ser Gly Ile Thr Phe Ser Ser Tyr
            20                  25                  30

ggc ttt cac tgg gtc cgc cag gct cca ggc aag ggg ctg gag tgg gtg     144
Gly Phe His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

gca gtg ata tgg tat gat gga agt aaa aaa tac tat gca gac tcc gtg     192
Ala Val Ile Trp Tyr Asp Gly Ser Lys Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60

aag ggc cga ttc acc ctc tcc aga gac gat tcc aag aac acg ctg tat     240
Lys Gly Arg Phe Thr Leu Ser Arg Asp Asp Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

ctg caa atg aac agc ctg aga gcc gag gac acg gct gtg tat tac tgt     288
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

gtt act ggg gat gac tac tgg ggc cag gga acc ctg gtc acc gtc tcc     336
Val Thr Gly Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110

tca                                                                 339
Ser
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<210> 63
<211> 363
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(363)

<400> 63
cag ctg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gag 48
Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

acc ctg tcc ctc acc tgc tct gtc tct ggt ggc tcc ctc agc agg agt 96
Thr Leu Ser Leu Thr Cys Ser Val Ser Gly Gly Ser Leu Ser Arg Ser
20 25 30

agt tac ttc tgg ggc tgg atc cgc cag ccc cca ggg aag ggg ctg gag 144
Ser Tyr Phe Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
35 40 45

tgg att gcg agt atc ttt tat agt ggg gaa acc tac ttc aat ccg tcc 192
Trp Ile Ala Ser Ile Phe Tyr Ser Gly Glu Thr Tyr Phe Asn Pro Ser
50 55 60

ctc aag agt cga gtc acc ata tcc gta gac acg tcc agg aac cag ttc 240
Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Arg Asn Gln Phe
65 70 75 80

tcc ctg aag ctg agc tct gtg acc gcc gca gac acg gct gtg tat tac 288
Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
85 90 95

tgt gcg aga gat tac gat att ttg act ggc gac gag gac tac tgg ggc 336
Cys Ala Arg Asp Tyr Asp Ile Leu Thr Gly Asp Glu Asp Tyr Trp Gly
100 105 110

cag gga acc ctg gtc acc gtc tcc tca 363
Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 64
<211> 321
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(321)

<400> 64
gaa att gtg ttg aca cag tct cca gcc acc ctg tct ttg tct cca ggg 48
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

gaa aga gcc acc ctc tcc tgc agg gcc agt cag agt gtt agc agc tac 96
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30

tta gcc tgg tac caa cag aaa cct ggc cag gct ccc agg ctc atc atc 144
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Ile Ile
35 40 45

```
tat gat gca tcc aac agg gcc act ggc atc cca gcc agg ttc agt ggc      192
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60

agt ggg tct ggg aca gac ttc act ctc acc atc agc agc cta gag cct      240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80

gaa gat ttt gca gtt tat tac tgt cag cag cgt agc aac tgg cct ctc      288
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Leu
                85                  90                  95

act ttc ggc gga ggg acc aag gtg gag atc aaa                         321
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105
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<210> 65
<211> 321
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(321)

<400> 65

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gaa att gtg ttg aca cag tct cca gcc acc ctg tct ttg tct cca ggg       48
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1               5                   10                  15

gaa aga gcc acc ctg tcc tgc agg gcc agt cag agt gtt agc agc tac       96
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
            20                  25                  30

tta gcc tgg tac caa cag aaa cct ggc cag gct ccc agg ctc ctc atc      144
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45

tat gat aca tcc aac agg gcc act ggc atc cca gcc agg ttc agt ggc      192
Tyr Asp Thr Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60

agt ggg tct ggg aca gac ttc act ctc acc atc agc agc cta gag cct      240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80

gaa gat ttt gca gtt tat tac tgt cag cag cgt agc aac tgg ccg ctc      288
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Leu
                85                  90                  95

act ttc ggc gga ggg acc aag gtg gag atc aaa                         321
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105
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<210> 66

<211> 324
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(321)

<400> 66
gaa att gtg ttg aca cag tct cca gcc acc ctg tct ttg tct cca ggg 48
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

gaa aga gcc acc ctc tcc tgc agg gcc agt cag agt gtt agt agt tac 96
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30

tta gcc tgg tac caa cag aaa cct ggc cag gct ccc agg ctc ctc atc 144
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

tat gat gca tcc aac agg gcc act ggc atc cca gcc agg ttc agt ggc 192
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

agt ggg tct ggg aca gac ttc act ctc acc atc agc agc cta gag cct 240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

gaa gat ttt gca gtt tat tac tgt cag cag agt agc aac tgg cct cgg 288
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Ser Asn Trp Pro Arg
85 90 95

acg ttc ggc caa ggg acc aag gtg gaa atc aaa 321
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 67
<211> 321
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<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(321)

<400> 67
gaa att gtg ttg aca cag tct cca gcc acc ctg tct ttg tct cca ggg 48
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

gaa aga gcc acc ctc tcc tgc agg gcc agt cag agt gtt agt agt tac 96
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30

tta gcc tgg tac caa cag aaa cct ggc cag gct ccc agg ctc ctc atc 144

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Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45

tat gat gca tcc aac agg gcc act ggc atc cca gcc agg ttc agt ggc      192
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60

agt ggg tct ggg aca gac ttc act ctc acc atc agc agc cta gag cct      240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80

gaa gat ttt gca gtt tat tac tgt cag cag agt agc aac tgg cct cgg      288
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Ser Asn Trp Pro Arg
                85                  90                  95

acg ttc ggc caa ggg acc aag gtg gaa atc aaa                         321
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105
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<210> 68
<211> 321
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(321)

<400> 68

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gac atc cag atg acc cag tct cca tcc tca ctg tct gca tct gtg gga       48
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

gac aga gtc tcc atc act tgt cgg gcg agt cag ggt att agc agc tgg       96
Asp Arg Val Ser Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
            20                  25                  30

tta gcc tgg tat cag cag aaa cca gag aaa gcc cct aag tcc ctg atc      144
Leu Ala Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys Ser Leu Ile
        35                  40                  45

tat gct gca tcc aat tta cga agt ggg gtc cca tca agg ttc agc ggc      192
Tyr Ala Ala Ser Asn Leu Arg Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

agt gga tct ggg aca gat ttc act ctc acc atc agc agc ctg cag cct      240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

gaa gat ttt gca act tat tac tgc caa cag tat tat agt tac cct agg      288
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Arg
                85                  90                  95

acg ttc ggc caa ggg acc aag gtg gaa atc aaa                         321
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105
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<210> 69
<211> 321
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(321)

<400> 69
gaa att gtg ttg aca cag tct cca gcc acc ctg tct ttg tct cca ggg 48
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

gaa aga gcc acc ctc tcc tgc agg gcc agt cag agt gtt agc agc tac 96
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30

tta gcc tgg tac caa cag aaa cct ggc cag gct ccc agg ctc ctc atc 144
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

tat gat gca tcc aac agg gcc act ggc atc cca gcc agg ttc agt ggc 192
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

agt ggg tct ggg aca gac ttc act ctc acc atc agc agc cta gag cct 240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

gaa gat ttt gca gtt tat tac tgt cag cag cgt agc aac tgg cct ctc 288
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Leu
85 90 95

act ttc ggc gga ggg acc aag gtg gag atc aaa 321
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 70
<211> 321
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(321)

<400> 70
gac atc cag atg acc cag tct cca tcc tca ctg tct gca tct gta gga 48
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1 5 10 15

gac aga gtc acc atc act tgt cgg gcg agt cag ggt att agc agc tgg 96
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp

```
            20                  25                  30

tta gcc tgg tat cag cag aaa cca gag aaa gcc cct aag tcc ctg atc      144
Leu Ala Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys Ser Leu Ile
        35                  40                  45

tat gct gca tcc agt ttg caa agt ggg gtc cca tca agg ttc agc ggc      192
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

agt gga tct ggg aca gat ttc act ctc acc atc agc agc ctg cag cct      240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

gaa gat ttt gca act tat tac tgc caa cag tat tat agt tac cct agg      288
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Arg
                85                  90                  95

acg ttc ggc caa ggg acc aag gtg gaa atc aaa                         321
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105
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<210> 71
<211> 98
<212> PRT
<213> Homo sapiens

<400> 71

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Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg
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<210> 72
<211> 95
<212> PRT
<213> Homo sapiens

<400> 72

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Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1               5                   10                  15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
            20                  25                  30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Ile Ile
        35                  40                  45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
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```
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro
                85                  90                  95
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<210> 73
<211> 99
<212> PRT
<213> Homo sapiens

<400> 73

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Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1               5                   10                  15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Ser
            20                  25                  30
Ser Tyr Tyr Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
        35                  40                  45
Trp Ile Gly Ser Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
    50                  55                  60
Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn His Phe
65                  70                  75                  80
Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
                85                  90                  95
Cys Ala Arg
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<210> 74
<211> 95
<212> PRT
<213> Homo sapiens

<400> 74

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
            20                  25                  30
Leu Ala Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys Ser Leu Ile
        35                  40                  45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Tyr Pro
                85                  90                  95
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2006/309606

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl. C12N15/09(2006.01)i, C07K16/28(2006.01)i, C12N5/00(2006.01)i, C12P21/08(2006.01)i, C12Q1/02(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl. C12N15/09, C07K16/28, C12N5/00, C12P21/08, C12Q1/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Published examined utility model applications of Japan 1922-1996
Published unexamined utility model applications of Japan 1971-2006
Registered utility model specifications of Japan 1996-2006
Published registered utility model applications of Japan 1994-2006

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS/WPI(DIALOG)
GenBank/EMBL/DDBJ/GeneSeq
JSTPlus(JDream2)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2001/014557 A1 (Dana-Farber Cancer Institute, Inc. et al.) 2001.03.01. & AU 200070727 A & EP 1210428 A1 & NO 200200859 A & NO 200200861 A & BR 200013581 A & US 2002/0160000 A1 & HU 200202449 A2 & JP 2003-507491 A & ZA 200201512 A & MX 2002001817 A1 & NZ 517955 A & US 6808710 B1.	1-7,43-56
X	WO 2002/079499 A1 (Dana-Farber Cancer Institute, Inc. et al.) 2002.10.10. & US 2003/0044768 A1 & NO 200304387 A & EP 1383912 A1 & AU 2002338272 A1 & JP 2004-533226 A & ZA 200307310 A & BR 200208367 A & MX 2003008959 A1 & CA 2442066 C & NZ 528265 A.	1-7,43-56

☑ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01.08.2006	15.08.2006

Name and mailing address of the ISA/JP	Authorized officer
Japan Patent Office 3-4-3, Kasumigaseki, Chiyoda-ku, Tokyo 100-8915, Japan	Susumu HIKICHI 4N 9549 Telephone No. +81-3-3581-1101 Ext. 3488

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONALSEARCHREPORT

International application No.
PCT/JP2006/309606

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2003/042402 A1 (Dana-Farber Cancer Institute, Inc. et al.) 2003.05.22. & US 2003/0232323 A1 & EP 1456652 A2 & AU 2002360384 A1 & JP 2005-509421 A.	1-7,43-56
X	Yoshiko Iwai et al., "Microanatomical localization of PD-1 in human tonsils," Immunology Letters, 2002, Vol.83, No.3, pp.215-220.	1-7,43-56
X	WO 2004/72286 A1 (Honjo T. et al.) 2004.08.26 & EP 1591527 A1.	1-7,43-56
A	Nils Lonberg et al., "Antigen-specific human antibodies from mice comprising four distinct genetic modifications," Nature, 1994, Vol.368, No.6474, pp.856-859.	1-7,43-56
A	WO 1997/07671 A1 (KIRIN BEER KK et al.) 1997.03.06 & EP 773288 A2 & AU 9668376 A & EP 773288 A3 & JP 9510126 X & EP 843961 A1 & CN 1200014 A & JP 11313576 A & JP 3030092 B & AU 718138 B & KR 99044300 A & TW 448032 A & KR 308764 B & US 6632976 B1 & US 2004/0073957 A1.	1-7,43-56
A	Lawrence R. Einger et al., "The human PD-1 gene: co,plete cDNA, genomic organization, and developmentally regulated expression in B cell progenitors," Gene, 1997, Vol.197, pp.177-187.	1-7,43-56
A	Takashi Shinohara et al.,"Structure and Chromosomal Localization of the Human PD-1 Gene (PDCD1)," Genomics, 1994, Vol.23, pp.704-706.	1-7,43-56
A	JP 07-291996 A (Honjo T. et al.) 1995.09.06. & EP 670369 A2 & CA 2143491 A & US 5629204 A & CN 1113518 A & US 5698520 A & JP 2003-230395 A & KR 391227 B & CN 1080311 C.	1-7,43-56

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

INTERNATIONALSEARCHREPORT

International application No.
PCT/JP2006/309606

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: 57-104
because they relate to subject matter not required to be searched by this Authority, namely:

Claim 57 recites [a method of modulating an immune response in a subject]. Claim 58-68 recites [a method of inhibiting growth of tumor cells in a subject]. Since the word [subject] literally includes a human or animal, these methods are related to treatment practiced on a human or animal body.

Moreover, Claim 69-87 recites [a method for treating a hyperproliferative disease]. Claim 88-104 recites [a method for altering an adverse event associated with treatment of a hyperproliferative disease with an immunostimulatory agent]. Theses methods are also construed as substantially related to treatment practiced on a human or animal body.

Therefore, the subject matter of Claim 57-104 relates to [a method for treatment of the human or animal body by therapy], which does not require an international search by the International Searching Authority in accordance with PCT Article 17(2)(a)(i) and Rule 39.1(iv).

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Invention 1) An isolated human monoclonal antibody, wherein the antibody binds to human with a K_D of 1×10^{-7} M or less;
Invention 2) An isolated human monoclonal antibody, wherein the antibody does not substantially bind to human CD28, CTLA-4 or ICOS;
Invention 3) An isolated human monoclonal antibody, wherein the antibody increases T-cell proliferation in a Mixed Lymphocyte Reaction (MLR) assay;
Invention 4) An isolated human monoclonal antibody, wherein the antibody increases interferon-gamma production in an MLR assay; and
Invention 5) An isolated human monoclonal antibody, wherein the antibody increases interleukin-2 (IL-2) secretion in an MLR assay.
Invention 6) An isolated human monoclonal antibody described in Claim 8(Claim 8-15);
Invention 7) An isolated human monoclonal antibody described in Claim 16(Claim 16);
Invention 8) An isolated human monoclonal antibody described in Claim 17(Claim 17);
Invention 9) An isolated human monoclonal antibody described in Claim 18(Claim 18);
Invention 10) An isolated human monoclonal antibody described in Claim 19(Claim 19);
Invention 11) An isolated human monoclonal antibody described in Claim 20(Claim 20);
Invention 12) An isolated human monoclonal antibody described in Claim 21(Claim 21);
Invention 13) An isolated human monoclonal antibody described in Claim 22(Claim 22-24);
Invention 14) An isolated human monoclonal antibody described in Claim 25(Claim 25-26);
Invention 15) An isolated human monoclonal antibody described in Claim 27(Claim 27-34);
Invention 16) An isolated human monoclonal antibody described in Claim 35(Claim 35-42); and
Invention 17) A method for preparing an anti-PD-1 antibody described in Claim 105(Claim 105)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☑ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-7, 43-56(Invention 1)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

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(71) Applicant *(for all designated States except US)*: **MEDAREX, INC.** [US/US]; 707 State Road, Princeton, NJ 08540 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **KORMAN, Alan, J.** [US/US]; 301 El Cerrito Avenue, Piedmont, CA 94611 (US). **SELBY, Mark, J.** [US/US]; 136 Galewood Circle, San Francisco, CA 94131 (US). **WANG, Changyu** [US/US]; 3803 Darwin Drive, Apt. 203, Fremont, CA 94555 (US). **SRINIVASAN, Mohan** [IN/US]; 1044 Arlington Lane, San Jose, CA 95129 (US). **PASSMORE, David, B.** [US/US]; 1630 Hull Drive, San Carlos, CA 94070 (US). **HUANG, Haichun** [US/US]; 4329 Crestwood Street, Fremont, CA 94538 (US). **CHEN, Haibin** [CN/US]; 125 Connemara Way, Unit 28, Sunnyvale, CA 94087 (US).

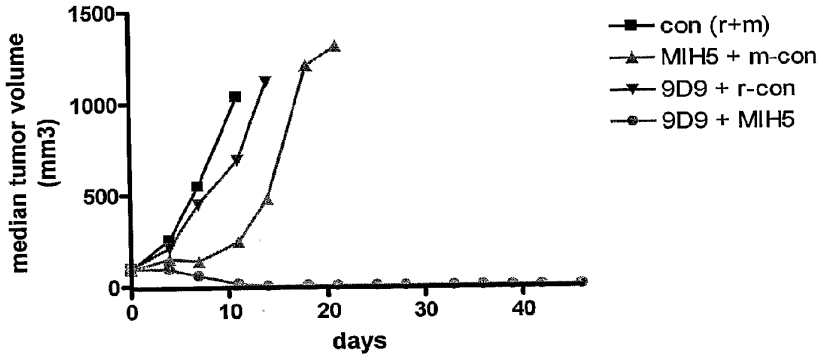
(74) Agents: **PEPE, Jeffrey, C.** et al.; DARBY & DARBY P.C., P.O. Box 5257, New York, NY 10150-5257 (US).

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[Continued on next page]

(54) Title: HUMAN MONOCLONAL ANTIBODIES TO PROGRAMMED DEATH LIGAND 1 (PD-L1)



(57) Abstract: The present disclosure provides isolated monoclonal antibodies, particularly human monoclonal antibodies that specifically bind to PD-L1 with high affinity. Nucleic acid molecules encoding the antibodies of this disclosure, expression vectors, host cells and methods for expressing the antibodies of this disclosure are also provided. Immunoconjugates, bispecific molecules and pharmaceutical compositions comprising the antibodies of the invention are also provided. The disclosure also provides methods for detecting PD-L1, as well as methods for treating various diseases, including cancer and infectious diseases, using anti-PD-L1 antibodies.

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RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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HUMAN MONOCLONAL ANTIBODIES TO PROGRAMMED DEATH LIGAND 1 (PD-L1)

Cross-Reference to Related Applications

This application claims the benefit of U.S. Provisional Patent Application No. 60/696,426, filed July 1, 2005, which is incorporated herein by reference in its entirety.

Background

Programmed death 1 (PD-1) is a member of the CD28 family of receptors, which includes CD28, CTLA-4, ICOS, PD-1, and BTLA. The initial members of the family, CD28 and ICOS, were discovered by functional effect on augmenting T cell proliferation following the addition of monoclonal antibodies (Hutloff *et al.* (1999) *Nature* 397:263-266; Hansen *et al.* (1980) *Immunogenics* 10:247-260). Two cell surface glycoprotein ligands for PD-1 have been identified, PD-L1 and PD-L2, and have been shown to downregulate T cell activation and cytokine secretion upon binding to PD-1 (Freeman *et al.* (2000) *J Exp Med* 192:1027-34; Latchman *et al.* (2001) *Nat Immunol* 2:261-8; Carter *et al.* (2002) *Eur J Immunol* 32:634-43; Ohigashi *et al.*(2005) *Clin Cancer Res* 11:2947-53). Both PD-L1 (B7-H1) and PD-L2 (B7-DC) are B7 homologs that bind to PD-1, but do not bind to other CD28 family members (Blank *et al.* (2004). Expression of PD-L1 on the cell surface has also been shown to be upregulated through IFN-γ stimulation.

PD-L1 expression has been found in several murine and human cancers, including human lung, ovarian and colon carcinoma and various myelomas (Iwai *et al.* (2002) *PNAS* 99:12293-7; Ohigashi *et al.*(2005) *Clin Cancer Res* 11:2947-53). PD-L1 has been suggested to play a role in tumor immunity by increasing apoptosis of antigen-specific T-cell clones (Dong *et al.* (2002) *Nat Med* 8:793-800). It has also been suggested that PD-L1 might be involved in intestinal mucosal inflammation and inhibition of PD-L1 suppresses wasting disease associated with colitis (Kanai *et al.*(2003) *J Immunol* 171:4156-63).

Summary

The present invention provides isolated monoclonal antibodies, in particular human monoclonal antibodies that bind to PD-L1 and exhibit numerous desirable

properties. These properties include high affinity binding to human PD-L1. Still further, antibodies of the invention have been shown to increase T-cell proliferation, IFN-γ secretion, and IL-2 secretion in a mixed lymphocyte reaction.

In one aspect, the invention pertains to an isolated monoclonal antibody, or an antigen-binding portion thereof, wherein the antibody exhibits at least one of the following properties:

(a) binds to human PD-L1 with a K_D of $1x10^{-7}$ M or less;

(b) increases T-cell proliferation in a mixed lymphocyte reaction (MLR) assay;

(c) increases interferon-γ production in an MLR assay;

(d) increases IL-2 secretion in an MLR assay;

(e) stimulates antibody responses; or

(f) reverses the effect of T regulatory cells on T cell effector cells and /or dendritic cells.

Preferably the antibody is a human antibody, although in alternative embodiments the antibody can be, for example, a murine antibody, a chimeric antibody or humanized antibody.

In particular embodiments, the antibody binds to human PD-L1 with a K_D of 5×10^{-8} M or less, binds to human PD-L1 with a K_D of 1×10^{-8} M or less, binds to human PD-L1 with a K_D of $5x10^{-9}$ M or less, binds to human PD-L1 with a K_D of $5x10^{-9}$ M or less, or binds to human PD-L1 with a K_D of between $1x10^{-8}$M and $1x10^{-10}$M.

In another embodiment, the invention provides an isolated monoclonal antibody, or antigen binding portion thereof, wherein the antibody cross-competes for binding to PD-L1 with a reference antibody comprising:

(a) the human heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:1, 2, 3, 4, 5, 6, 7, 8, 9, and 10; and

(b) the human light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:11, 12, 13, 14, 15, 16, 17, 18, 19, and 20.

In various embodiments, the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:1; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:11;

or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:2; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:12;

or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:3; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:13;

or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:4; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:14;

or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:5; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:15;

or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:16;

or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:7; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:17;

or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:18;

or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:9; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:19;

or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:10; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:20.

In another aspect, the invention pertains to an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 1-18 gene, wherein the antibody specifically binds PD-L1. The invention further provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 1-69 gene, wherein the antibody specifically binds PD-L1. The invention further provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 1-3 gene, wherein the antibody specifically binds PD-L1. The invention further provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 3-9 gene, wherein the antibody specifically binds PD-L1. The invention further provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L6 gene, wherein the antibody specifically binds PD-L1. The invention further provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L15 gene, wherein the antibody specifically binds PD-L1. The invention further provides an isolated monoclonal antibody, or an antigen-binding portion thereof,

comprising a light chain variable region that is the product of or derived from a human V_K A27 gene, wherein the antibody specifically binds PD-L1. The invention further provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L18 gene, wherein the antibody specifically binds PD-L1.

In a particularly preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising:

(a) a heavy chain variable region of a human V_H 1-18 gene; and

(b) a light chain variable region of a human V_K L6 gene;

wherein the antibody specifically binds to PD-L1.

In another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising:

(a) a heavy chain variable region of a human V_H 1-69 gene; and

(b) a light chain variable region of a human V_K L6 gene;

wherein the antibody specifically binds to PD-L1.

In another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising:

(a) a heavy chain variable region of a human V_H 1-3 gene; and

(b) a light chain variable region of a human V_K L15 gene;

wherein the antibody specifically binds to PD-L1.

In another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising:

(a) a heavy chain variable region of a human V_H 1-69 gene; and

(b) a light chain variable region of a human V_K A27 gene;

wherein the antibody specifically binds to PD-L1.

In another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising:

(a) a heavy chain variable region of a human V_H 3-9 gene; and

(b) a light chain variable region of a human V_K L15 gene;

wherein the antibody specifically binds to PD-L1.

In another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising:

(a) a heavy chain variable region of a human V_H 3-9 gene; and

(b) a light chain variable region of a human V_K L18 gene;

wherein the antibody specifically binds to PD-L1.

In another aspect, the invention provides an isolated monoclonal antibody, or antigen binding portion thereof, comprising:

a heavy chain variable region that comprises CDR1, CDR2, and CDR3 sequences; and a light chain variable region that comprises CDR1, CDR2, and CDR3 sequences, wherein:

(a) the heavy chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:41, 42, 43, 44, 45, 46, 47, 48, 49, and 50, and conservative modifications thereof;

(b) the light chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:71, 72, 73, 74, 75, 76, 77, 78, 79, and 80, and conservative modifications thereof; and

(c) the antibody specifically binds to human PD-L1.

Preferably, the heavy chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs:31, 32, 33, 34, 35, 36, 37, 38, 39, and 40, and conservative modifications thereof; and the light chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs:61, 62, 63, 64, 65, 66, 67, 68, 69, and 70, and conservative modifications thereof. Preferably, the heavy chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs:21, 22, 23, 24, 25, 26, 27, 28, 29, and 30, and conservative modifications thereof; and the light chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs:51, 52, 53, 54, 55, 56, 57, 58, 59, and 60, and conservative modifications thereof.

In yet another aspect, the invention provides an isolated monoclonal antibody, or antigen binding portion thereof, comprising a heavy chain variable region and a light chain variable region, wherein:

(a) the heavy chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs:1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

(b) the light chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs:11, 12, 13, 14, 15, 16, 17, 18, 19, and 20; and

(c) the antibody binds to human PD-L1 with a K_D of $1x10^{-7}$M or less.

In a preferred embodiment, the antibodies additionally comprise at least one of the following properties:

(a) the antibody increases T-cell proliferation in a mixed lymphocyte reaction (MLR) assay;

(b) the antibody increases interferon-γ production in an MLR assay; or

(c) the antibody increases IL-2 secretion in an MLR assay.

In preferred embodiments, the invention provides an isolated monoclonal antibody, or antigen binding portion thereof, comprising:

(a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:21, 22, 23, 24, 25, 26, 27, 28, 29, and 30;

(b) a heavy chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:31, 32, 33, 34, 35, 36, 37, 38, 39, and 40;

(c) a heavy chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:41, 42, 43, 44, 45, 46, 47, 48, 49, and 50;

(d) a light chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:51, 52, 53, 54, 55, 56, 57, 58, 59, and 60;

(e) a light chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:61, 62, 63, 64, 65, 66, 67, 68, 69, and 70; and

(f) a light chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:71, 72, 73, 74, 75, 76, 77, 78, 79, and 80;

wherein the antibody specifically binds PD-L1.

A preferred combination comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:21;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:31;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:41;

(d) a light chain variable region CDR1 comprising SEQ ID NO:51;

(e) a light chain variable region CDR2 comprising SEQ ID NO:61; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:71.

Another preferred combination comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:22;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:32;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:42;

(d) a light chain variable region CDR1 comprising SEQ ID NO:52;

(e) a light chain variable region CDR2 comprising SEQ ID NO:62; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:72.

Another preferred combination comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:23;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:33;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:43;

(d) a light chain variable region CDR1 comprising SEQ ID NO:53;

(e) a light chain variable region CDR2 comprising SEQ ID NO:63; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:73.

Another preferred combination comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:24;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:34;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:44;

(d) a light chain variable region CDR1 comprising SEQ ID NO:54;

(e) a light chain variable region CDR2 comprising SEQ ID NO:64; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:74.

Another preferred combination comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:25;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:35;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:45;

(d) a light chain variable region CDR1 comprising SEQ ID NO:55;

(e) a light chain variable region CDR2 comprising SEQ ID NO:65; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:75.

Another preferred combination comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:26;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:36;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:46;

(d) a light chain variable region CDR1 comprising SEQ ID NO:56;

(e) a light chain variable region CDR2 comprising SEQ ID NO:66; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:76.

Another preferred combination comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:27;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:37;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:47;

(d) a light chain variable region CDR1 comprising SEQ ID NO:57;

(e) a light chain variable region CDR2 comprising SEQ ID NO:67; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:77.

Another preferred combination comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:28;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:38;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:48;

(d) a light chain variable region CDR1 comprising SEQ ID NO:58;

(e) a light chain variable region CDR2 comprising SEQ ID NO:68; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:78.

Another preferred combination comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:29;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:39;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:49;

(d) a light chain variable region CDR1 comprising SEQ ID NO:59;

(e) a light chain variable region CDR2 comprising SEQ ID NO:69; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:79.

Another preferred combination comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:30;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:40;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:50;

(d) a light chain variable region CDR1 comprising SEQ ID NO:60;

(e) a light chain variable region CDR2 comprising SEQ ID NO:70; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:80.

Other preferred antibodies of the invention, or antigen binding portions thereof comprise:

(a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:1, 2, 3, 4, 5, 6, 7, 8, 9, and 10; and

(b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:11, 12, 13, 14, 15, 16, 17, 18, 19, and 20;

wherein the antibody specifically binds PD-L1.

A preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:1; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:11.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:2; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:12.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:3; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:13.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:4; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:14.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:5; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:15.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:16.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:7; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:17.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:18.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:9; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:19.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:10; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:20.

In another aspect of the instant disclosure, antibodies, or antigen-binding portions thereof, are provided that compete for binding to PD-L1 with any of the aforementioned antibodies.

The antibodies of the instant disclosure can be, for example, full-length antibodies, for example of an IgG1 or IgG4 isotype. Alternatively, the antibodies can be antibody fragments, such as Fab or Fab'2 fragments, or single chain antibodies.

The instant disclosure also provides an immunoconjugate comprising an antibody of the invention, or antigen-binding portion thereof, linked to a therapeutic agent, such as

a cytotoxin or a radioactive isotope. The invention also provides a bispecific molecule comprising an antibody, or antigen-binding portion thereof, of the invention, linked to a second functional moiety having a different binding specificity than said antibody, or antigen binding portion thereof.

Compositions comprising an antibody, or antigen-binding portion thereof, or immunoconjugate or bispecific molecule of the instant disclosure and a pharmaceutically acceptable carrier are also provided.

Nucleic acid molecules encoding the antibodies, or antigen-binding portions thereof, of the invention are also encompassed by the invention, as well as expression vectors comprising such nucleic acids and host cells comprising such expression vectors. Moreover, the invention provides a transgenic mouse comprising human immunoglobulin heavy and light chain transgenes, wherein the mouse expresses an antibody of the invention, as well as hybridomas prepared from such a mouse, wherein the hybridoma produces the antibody of the invention.

In yet another aspect, the invention provides a method of modulating an immune response in a subject comprising administering to the subject the antibody, or antigen-binding portion thereof, of the invention such that the immune response in the subject is modulated. Preferably, the antibody of the invention enhances, stimulates or increases the immune response in the subject.

In a further aspect, the invention provides a method of inhibiting growth of tumor cells in a subject, comprising administering to a subject a therapeutically effective amount of an anti-PD-L1 antibody, or antigen-binding portion thereof. The antibodies of the invention are preferred for use in the method although other anti-PD-L1 antibodies can be used instead (or in combination with an anti-PD-L1 antibody of the invention). For example, a chimeric, humanized or fully human anti-PD-L1 antibody can be used in the method of inhibiting tumor growth.

In a further aspect, the invention provides a method of treating an infectious disease in a subject, comprising administering to a subject a therapeutically effective amount of an anti-PD-L1 antibody, or antigen-binding portion thereof. The antibodies of the invention are preferred for use in the method although other anti-PD-L1 antibodies can be used instead (or in combination with an anti-PD-L1 antibody of the invention). For example, a chimeric, humanized or fully human anti-PD-L1 antibody can be used in the method of treating an infectious disease.

Still further, the invention provides a method of enhancing an immune response to an antigen in a subject, comprising administering to the subject: (i) the antigen; and (ii) an anti-PD-L1 antibody, or antigen-binding portion thereof, such that an immune response to the antigen in the subject is enhanced. The antigen can be, for example, a tumor antigen, a viral antigen, a bacterial antigen or an antigen from a pathogen. The antibodies of the invention are preferred for use in the method although other anti-PD-L1 antibodies can be used instead (or in combination with an anti-PD-L1 antibody of the invention). For example, a chimeric, humanized or fully human anti-PD-L1 antibody can be used in the method of enhancing an immune response to an antigen in a subject.

The invention also provides methods for making "second generation" anti-PD-L1 antibodies based on the sequences of the anti-PD-L1 antibodies provided herein. For example, the invention provides a method for preparing an anti-PD-L1 antibody comprising:

(a) providing: (i) a heavy chain variable region antibody sequence comprising a CDR1 sequence that is selected from the group consisting of SEQ ID NOs:21, 22, 23, 24, 25, 26, 27, 28, 29, and 30, a CDR2 sequence that is selected from the group consisting of SEQ ID NOs:31, 32, 33, 34, 35, 36, 37, 38, 39, and 40; and a CDR3 sequence that is selected from the group consisting of SEQ ID NOs:41, 42, 43, 44, 45, 46, 47, 48, 49, and 50; or (ii) a light chain variable region antibody sequence comprising a CDR1 sequence that is selected from the group consisting of SEQ ID NOs:51, 52, 53, 54, 55, 56, 57, 58, 59, and 60, a CDR2 sequence that is selected from the group consisting of SEQ ID NOs:61, 62, 63, 64, 65, 66, 67, 68, 69, and 70, and a CDR3 sequence that is selected from the group consisting of SEQ ID NOs:71, 72, 73, 74, 75, 76, 77, 78, 79, and 80;

(b) altering at least one amino acid residue within at least one variable region antibody sequence, said sequence being selected from the heavy chain variable region antibody sequence and the light chain variable region antibody sequence, to create at least one altered antibody sequence; and

(c) expressing the altered antibody sequence as a protein.

Other features and advantages of the instant invention will be apparent from the following detailed description and examples which should not be construed as limiting. The contents of all references, Genbank entries, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

Brief Description of the Drawings

Figure 1A shows the nucleotide sequence (SEQ ID NO:81) and amino acid sequence (SEQ ID NO:1) of the heavy chain variable region of the 3G10 human monoclonal antibody. The CDR1 (SEQ ID NO:21), CDR2 (SEQ ID NO:31) and CDR3 (SEQ ID NO:41) regions are delineated and the V, D and J germline derivations are indicated.

Figure 1B shows the nucleotide sequence (SEQ ID NO:91) and amino acid sequence (SEQ ID NO:11) of the light chain variable region of the 3G10 human monoclonal antibody. The CDR1 (SEQ ID NO:51), CDR2 (SEQ ID NO:61) and CDR3 (SEQ ID NO:71) regions are delineated and the V and J germline derivations are indicated.

Figure 2A shows the nucleotide sequence (SEQ ID NO:82) and amino acid sequence (SEQ ID NO:2) of the heavy chain variable region of the 12A4 human monoclonal antibody. The CDR1 (SEQ ID NO:22), CDR2 (SEQ ID NO:32) and CDR3 (SEQ ID NO:42) regions are delineated and the V and J germline derivations are indicated.

Figure 2B shows the nucleotide sequence (SEQ ID NO:92) and amino acid sequence (SEQ ID NO:12) of the light chain variable region of the 12A4 human monoclonal antibody. The CDR1 (SEQ ID NO:52), CDR2 (SEQ ID NO:62) and CDR3 (SEQ ID NO:72) regions are delineated and the V and J germline derivations are indicated.

Figure 3A shows the nucleotide sequence (SEQ ID NO:83) and amino acid sequence (SEQ ID NO:3) of the heavy chain variable region of the 10A5 human monoclonal antibody. The CDR1 (SEQ ID NO:23), CDR2 (SEQ ID NO:33) and CDR3 (SEQ ID NO:43) regions are delineated and the V and J germline derivations are indicated.

Figure 3B shows the nucleotide sequence (SEQ ID NO:93) and amino acid sequence (SEQ ID NO:13) of the light chain variable region of the 10A5 human monoclonal antibody. The CDR1 (SEQ ID NO:53), CDR2 (SEQ ID NO:63) and CDR3 (SEQ ID NO:73) regions are delineated and the V and J germline derivations are indicated.

Figure 4A shows the nucleotide sequence (SEQ ID NO:84) and amino acid sequence (SEQ ID NO:4) of the heavy chain variable region of the 5F8 human

monoclonal antibody. The CDR1 (SEQ ID NO:24), CDR2 (SEQ ID NO:34) and CDR3 (SEQ ID NO:44) regions are delineated and the V and J germline derivations are indicated.

Figure 4B shows the nucleotide sequence (SEQ ID NO:94) and amino acid sequence (SEQ ID NO:14) of the light chain variable region of the 5F8 human monoclonal antibody. The CDR1 (SEQ ID NO:54), CDR2 (SEQ ID NO:64) and CDR3 (SEQ ID NO:74) regions are delineated and the V and J germline derivations are indicated.

Figure 5A shows the nucleotide sequence (SEQ ID NO:85) and amino acid sequence (SEQ ID NO:5) of the heavy chain variable region of the 10H10 human monoclonal antibody. The CDR1 (SEQ ID NO:25), CDR2 (SEQ ID NO:35) and CDR3 (SEQ ID NO:45) regions are delineated and the V and J germline derivations are indicated.

Figure 5B shows the nucleotide sequence (SEQ ID NO:95) and amino acid sequence (SEQ ID NO:15) of the light chain variable region of the 10H10 human monoclonal antibody. The CDR1 (SEQ ID NO:55), CDR2 (SEQ ID NO:65) and CDR3 (SEQ ID NO:75) regions are delineated and the V and J germline derivations are indicated.

Figure 6A shows the nucleotide sequence (SEQ ID NO:86) and amino acid sequence (SEQ ID NO:6) of the heavy chain variable region of the 1B12 human monoclonal antibody. The CDR1 (SEQ ID NO:26), CDR2 (SEQ ID NO:36) and CDR3 (SEQ ID NO:46) regions are delineated and the V and J germline derivations are indicated.

Figure 6B shows the nucleotide sequence (SEQ ID NO:96) and amino acid sequence (SEQ ID NO:16) of the light chain variable region of the 1B12 human monoclonal antibody. The CDR1 (SEQ ID NO:56), CDR2 (SEQ ID NO:66) and CDR3 (SEQ ID NO:76) regions are delineated and the V and J germline derivations are indicated.

Figure 7A shows the nucleotide sequence (SEQ ID NO:87) and amino acid sequence (SEQ ID NO:7) of the heavy chain variable region of the 7H1 human monoclonal antibody. The CDR1 (SEQ ID NO:27), CDR2 (SEQ ID NO:37) and CDR3 (SEQ ID NO:47) regions are delineated and the V and J germline derivations are indicated.

Figure 7B shows the nucleotide sequence (SEQ ID NO:97) and amino acid sequence (SEQ ID NO:17) of the light chain variable region of the 7H1 human monoclonal antibody. The CDR1 (SEQ ID NO:57), CDR2 (SEQ ID NO:67) and CDR3 (SEQ ID NO:77) regions are delineated and the V and J germline derivations are indicated.

Figure 8A shows the nucleotide sequence (SEQ ID NO:88) and amino acid sequence (SEQ ID NO:8) of the heavy chain variable region of the 11E6 human monoclonal antibody. The CDR1 (SEQ ID NO:28), CDR2 (SEQ ID NO:38) and CDR3 (SEQ ID NO:48) regions are delineated and the V and J germline derivations are indicated.

Figure 8B shows the nucleotide sequence (SEQ ID NO:98) and amino acid sequence (SEQ ID NO:18) of the light chain variable region of the 11E6 human monoclonal antibody. The CDR1 (SEQ ID NO:58), CDR2 (SEQ ID NO:68) and CDR3 (SEQ ID NO:78) regions are delineated and the V and J germline derivations are indicated.

Figure 9A shows the nucleotide sequence (SEQ ID NO:89) and amino acid sequence (SEQ ID NO:9) of the heavy chain variable region of the 12B7 human monoclonal antibody. The CDR1 (SEQ ID NO:29), CDR2 (SEQ ID NO:39) and CDR3 (SEQ ID NO:49) regions are delineated and the V and J germline derivations are indicated.

Figure 9B shows the nucleotide sequence (SEQ ID NO:99) and amino acid sequence (SEQ ID NO:19) of the light chain variable region of the 12B7 human monoclonal antibody. The CDR1 (SEQ ID NO:59), CDR2 (SEQ ID NO:69) and CDR3 (SEQ ID NO:79) regions are delineated and the V and J germline derivations are indicated.

Figure 10A shows the nucleotide sequence (SEQ ID NO:90) and amino acid sequence (SEQ ID NO:10) of the heavy chain variable region of the 13G4 human monoclonal antibody. The CDR1 (SEQ ID NO:30), CDR2 (SEQ ID NO:40) and CDR3 (SEQ ID NO:50) regions are delineated and the V and J germline derivations are indicated.

Figure 10B shows the nucleotide sequence (SEQ ID NO:100) and amino acid sequence (SEQ ID NO:20) of the light chain variable region of the 13G4 human monoclonal antibody. The CDR1 (SEQ ID NO:60), CDR2 (SEQ ID NO:70) and CDR3

(SEQ ID NO:80) regions are delineated and the V and J germline derivations are indicated.

Figure 11 shows the alignment of the amino acid sequence of the heavy chain variable region of 3G10 with the human germline V_H 1-18 amino acid sequence (SEQ ID NO:101).

Figure 12 shows the alignment of the amino acid sequence of the heavy chain variable region of 12A4 with the human germline V_H 1-69 amino acid sequence (SEQ ID NO:102).

Figure 13 shows the alignment of the amino acid sequence of the heavy chain variable region of 10A5 with the human germline V_H 1-3 amino acid sequence (SEQ ID NO:103).

Figure 14 shows the alignment of the amino acid sequence of the heavy chain variable region of 5F8 with the human germline V_H 1-69 amino acid sequence (SEQ ID NO:102).

Figure 15 shows the alignment of the amino acid sequence of the heavy chain variable region of 10H10 with the human germline V_H 3-9 amino acid sequence (SEQ ID NO:104).

Figure 16 shows the alignment of the amino acid sequence of the heavy chain variable region of 1B12 with the human germline V_H 1-69 amino acid sequence (SEQ ID NO:102).

Figure 17 shows the alignment of the amino acid sequence of the heavy chain variable region of 7H1 with the human germline V_H 1-69 amino acid sequence (SEQ ID NO:102).

Figure 18 shows the alignment of the amino acid sequence of the heavy chain variable region of 11E6 with the human germline V_H 1-69 amino acid sequence (SEQ ID NO:102).

Figure 19 shows the alignment of the amino acid sequence of the heavy chain variable region of 12B7 with the human germline V_H 1-69 amino acid sequence (SEQ ID NO:102).

Figure 20 shows the alignment of the amino acid sequence of the heavy chain variable region of 13G4 with the human germline V_H 3-9 amino acid sequence (SEQ ID NO:104).

Figure 21 shows the alignment of the amino acid sequence of the light chain variable region of 3G10 with the human germline V_k L6 amino acid sequence (SEQ ID NO:105).

Figure 22 shows the alignment of the amino acid sequence of the light chain variable region of 12A4 with the human germline V_k L6 amino acid sequence (SEQ ID NO:105).

Figure 23 shows the alignment of the amino acid sequence of the light chain variable region of 10A5 with the human germline V_k L15 amino acid sequence (SEQ ID NO:106).

Figure 24 shows the alignment of the amino acid sequence of the light chain variable region of 5F8 with the human germline V_k A27 amino acid sequence (SEQ ID NO:107).

Figure 25 shows the alignment of the amino acid sequence of the light chain variable region of 10H10 with the human germline V_k L15 amino acid sequence (SEQ ID NO:106).

Figure 26 shows the alignment of the amino acid sequence of the light chain variable region of 1B12 with the human germline V_k L6 amino acid sequence (SEQ ID NO:105).

Figure 27 shows the alignment of the amino acid sequence of the light chain variable region of 7H1 with the human germline V_k L6 amino acid sequence (SEQ ID NO:105).

Figure 28 shows the alignment of the amino acid sequence of the light chain variable region of 11E6 with the human germline V_k A27 amino acid sequence (SEQ ID NO:107).

Figure 29 shows the alignment of the amino acid sequence of the light chain variable region of 11E6a (SEQ ID NO:109) with the human germline V_k A27 amino acid sequence (SEQ ID NO:107).

Figure 30 shows the alignment of the amino acid sequence of the light chain variable region of 12B7 with the human germline V_k L6 amino acid sequence (SEQ ID NO:105).

Figure 31 shows the alignment of the amino acid sequence of the light chain variable region of 13G4 with the human germline V_k L18 amino acid sequence (SEQ ID NO:108).

Figures 32A-C show the results of flow cytometry experiments demonstrating that the human monoclonal antibodies 3G10, 10A5, and 12A4, directed against human PD-L1, binds the cell surface of CHO cells transfected with full-length human PD-L1. (A) Flow cytometry plot for 3G10 (B) Flow cytometry plot for 10A5 (C) Flow cytometry plot for 12A4.

Figure 33 shows the results of flow cytometry experiments demonstrating that the human monoclonal antibodies 3G10, 10A5, and 12A4, directed against human PD-L1, binds the cell surface of CHO cells transfected with full-length human PD-L1 in a concentration dependent manner.

Figure 34 shows the results of ELISA experiments demonstrating that the human monoclonal antibodies 3G10, 10A5, and 12A4, directed against human PD-L1, binds to PD-L1-Fc fusion protein.

Figure 35 shows the results of experiments demonstrating HuMab titration on stimulated human CD4+ T cells.

Figure 36 shows the results of experiments demonstrating HuMab titration on stimulated cynomolgus PBMC.

Figures 37A-C shows the results of flow cytometry experiments demonstrating that the human monoclonal antibodies 3G10, 10A5, and 12A4, directed against human PD-L1, binds to PD-L1 on the cell surface of activated T cells. (A) Flow cytometry plot for 3G10 (B) Flow cytometry plot for 10A5 (C) Flow cytometry plot for 12A4.

Figure 38 demonstrates binding of HuMabs to ES-2 cells.

Figures 39A-D shows the results of experiments demonstrating that human monoclonal antibodies against human PD-L1 promote T-cell proliferation, IFN-γ secretion and IL-2 secretion in a mixed lymphocyte reaction assay. Figure 39A is a bar graph showing concentration dependent T-cell proliferation using HuMAb 10A5; Figure 39B is a bar graph showing concentration dependent IFN-γ secretion using HuMAb 10A5; Figure 39C is a bar graph showing IFN-γ secretion using HuMAbs 3G10 and 12A4; Figure 39D is a bar graph showing concentration dependent IL-2 secretion using HuMAb 10A5.

Figure 40 demonstrates the effect of human anti-PD-L1 antibody on proliferation and IFN-γ secretion in the MLR using allogeneic dendritic cells and T cells (CD4+ effector T cells) Dendritic Cells.

Figures 41A-B shows the results of experiments demonstrating that human monoclonal antibodies against human PD-L1 promote T-cell proliferation and IFN-γ secretion in MLR containing T regulatory cells. Figure 41A is a bar graph showing concentration dependent T-cell proliferation using HuMAb 10A5; Figure 41B is a bar graph showing concentration dependent IFN-γ secretion using HuMAb 10A5.

Figure 42 demonstrates the results of anti-PD-L1 antibodies on cell proliferation in a Mixed Lymphocyte Reaction in the presence of regulatory T cells.

Figure 43 demonstrates the results of anti-PD-L1 antibodies on cytokine production in a Mixed Lymphocyte Reaction in the presence of regulatory T cells.

Figure 44 demonstrates the results of anti-PD-L1 antibodies on CMV lysate stimulated human PBMC IFN-γ secretion.

Figure 45 shows the results of flow cytometry experiments demonstrating that human monoclonal antibodies against human PD-L1 block the binding of PD-L1 to CHO transfected cells expressing PD-1.

Figure 46 shows that anti-PD-L1 antibodies block binding of PD-1 to IFNγ treated ES-2 cells.

Figure 47 shows the effect of anti-PD-L1 antibodies on tumor growth *in vivo*.

Detailed Description

In one aspect, the present disclosure relates to isolated monoclonal antibodies, particularly human monoclonal antibodies that bind specifically to PD-L1. In certain embodiments, the antibodies of the invention exhibit one or more desirable functional properties, such as high affinity binding to PD-L1, the ability to augment T cell proliferation, IFN-γ and/or IL-2 secretion in mixed lymphocyte reactions, the ability to inhibit binding of PD-L1 to the PD-1 receptor, the ability to stimulate antibody responses and/or the ability to reverse the suppressive function of T regulatory cells. Additionally or alternatively, the antibodies of the invention are derived from particular heavy and light chain germline sequences and/or comprise particular structural features such as CDR regions comprising particular amino acid sequences.

The instant disclosure provides, for example, isolated antibodies, methods of making such antibodies, immunoconjugates and bispecific molecules comprising such antibodies and pharmaceutical compositions containing the antibodies, immunconjugates or bispecific molecules of the invention.

In another aspect, the disclosure pertains to methods of inhibiting growth of tumor cells in a subject using anti-PD-L1 antibodies. The invention also relates to methods of using the antibodies to modify an immune response, as well as to treat diseases such as cancer or infectious disease, or to stimulate a protective autoimmune response or to stimulate antigen-specific immune responses (e.g., by coadministration of anti-PD-L1 with an antigen of interest).

In order that the present disclosure may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

The term "immune response" refers to the action of, for example, lymphocytes, antigen presenting cells, phagocytic cells, granulocytes, and soluble macromolecules produced by the above cells or the liver (including antibodies, cytokines, and complement) that results in selective damage to, destruction of, or elimination from the human body of invading pathogens, cells or tissues infected with pathogens, cancerous cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

A "signal transduction pathway" refers to the biochemical relationship between a variety of signal transduction molecules that play a role in the transmission of a signal from one portion of a cell to another portion of a cell. As used herein, the phrase "cell surface receptor" includes, for example, molecules and complexes of molecules capable of receiving a signal and the transmission of such a signal across the plasma membrane of a cell. An example of a "cell surface receptor" of the present invention is the PD-L1 receptor.

The term "antibody" as referred to herein includes whole antibodies and any antigen binding fragment (*i.e.*, "antigen-binding portion") or single chains thereof. An "antibody" refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, C_{H1}, C_{H2} and C_{H3}. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions

(FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system.

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, PD-L1). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_{H1} domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_{H1} domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a V_H domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); *see e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

An "isolated antibody," as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds PD-L1 is substantially free of antibodies that specifically bind antigens other than PD-L1). An isolated antibody that specifically binds PD-L1 may, however, have cross-reactivity to other antigens, such as PD-L1 molecules

from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

The term "human antibody," as used herein, is intended to include antibodies having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody," as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "human monoclonal antibody" refers to antibodies displaying a single binding specificity which have variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. In one embodiment, the human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic nonhuman animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

The term "recombinant human antibody," as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom (described further below), (b) antibodies isolated from a host cell transformed to express the human antibody, *e.g.*, from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial human antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable regions in which the framework and CDR regions are derived

from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

As used herein, "isotype" refers to the antibody class (*e.g.*, IgM or IgGl) that is encoded by the heavy chain constant region genes.

The phrases "an antibody recognizing an antigen" and "an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen."

The term "human antibody derivatives" refers to any modified form of the human antibody, *e.g.*, a conjugate of the antibody and another agent or antibody.

The term "humanized antibody" is intended to refer to antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Additional framework region modifications may be made within the human framework sequences.

The term "chimeric antibody" is intended to refer to antibodies in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

As used herein, an antibody that "specifically binds to human PD-L1" is intended to refer to an antibody that binds to human PD-L1 with a K_D of 1×10^{-7} M or less, more preferably 5×10^{-8} M or less, more preferably 1×10^{-8} M or less, more preferably 5×10^{-9} M or less, even more preferably between 1×10^{-8} M and 1×10^{-10} M or less.

The term "K_{assoc}" or "K_a," as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term "K_{dis}" or "K_d," as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term "K_D," as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of K_d to K_a (*i.e.*, K_d/K_a) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art. A preferred method for determining the K_D of an antibody is by

using surface plasmon resonance, preferably using a biosensor system such as a Biacore® system.

As used herein, the term "high affinity" for an IgG antibody refers to an antibody having a K_D of 10^{-8} M or less, more preferably 10^{-9} M or less and even more preferably 10^{-10} M or less for a target antigen. However, "high affinity" binding can vary for other antibody isotypes. For example, "high affinity" binding for an IgM isotype refers to an antibody having a K_D of 10^{-7} M or less, more preferably 10^{-8} M or less, even more preferably 10^{-9} M or less.

As used herein, the term "subject" includes any human or nonhuman animal. The term "nonhuman animal" includes all vertebrates, *e.g.*, mammals and non-mammals, such as nonhuman primates, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, *etc.*

Various aspects of the disclosure are described in further detail in the following subsections.

Anti-PD-L1 Antibodies

The antibodies of the invention are characterized by particular functional features or properties of the antibodies. For example, the antibodies bind specifically to human PD-L1. Preferably, an antibody of the invention binds to PD-L1 with high affinity, for example with a K_D of 1 x 10^{-7} M or less. The anti-PD-L1 antibodies of the invention preferably exhibit one or more of the following characteristics:

(a) binds to human PD-L1 with a K_D of 1x10^{-7} M or less;

(b) increases T-cell proliferation in a mixed lymphocyte reaction (MLR) assay;

(c) increases interferon-γ production in an MLR assay;

(d) increases IL-2 secretion in an MLR assay

(e) stimulates antibody responses; and/or

(f) reverses the effect of T regulatory cells on T cell effector cells and /or dendritic cells..

Preferably, the antibody binds to human PD-L1 with a K_D of 5 x 10^{-8} M or less, binds to human PD-L1 with a K_D of 1 x 10^{-8} M or less, binds to human PD-L1 with a K_D of 5 x 10^{-9} M or less, binds to human PD-L1 with a K_D of 4 x 10^{-9} M or less, binds to human PD-L1 with a K_D of 2 x 10^{-9} M or less, or binds to human PD-L1 with a K_D of between 1x10^{-9}M and 1x10^{-10} M or less.

Standard assays to evaluate the binding ability of the antibodies toward PD-L1 are known in the art, including for example, ELISAs, Western blots and RIAs. Suitable assays are described in detail in the Examples. The binding kinetics (*e.g.*, binding affinity) of the antibodies also can be assessed by standard assays known in the art, such as by Biacore® analysis.

Monoclonal Antibodies 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4.

Preferred antibodies of the invention are the human monoclonal antibodies 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4, isolated and structurally characterized as described in Examples 1 and 2. The V_H amino acid sequences of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4 are shown in SEQ ID NOs:1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, respectively. The V_L amino acid sequences of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4 are shown in SEQ ID NOs:11, 12, 13, 14, 15, 16, 17, 18, 19, and 20, respectively.

Given that each of these antibodies can bind to PD-L1, the V_H and V_L sequences can be "mixed and matched" to create other anti-PD-L1 binding molecules of the invention. PD-L1 binding of such "mixed and matched" antibodies can be tested using the binding assays described above and in the Examples (*e.g.*, ELISAs). Preferably, when V_H and V_L chains are mixed and matched, a V_H sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_H sequence. Likewise, preferably a V_L sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_L sequence.

Accordingly, in one aspect, the invention provides an isolated monoclonal antibody, or antigen binding portion thereof comprising:

(a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:1, 2, 3, 4, 5, 6, 7, 8, 9, and 10; and

(b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:11, 12, 13, 14, 15, 16, 17, 18, 19, and 20;

wherein the antibody specifically binds PD-L1, preferably human PD-L1.

Preferred heavy and light chain combinations include:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:1; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:11; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:2; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:12; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:3; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:13; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:4; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:14; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:5; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:15; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:16; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:7; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:17; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:8; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:18; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:9; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:19; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:10; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:20.

In another aspect, the invention provides antibodies that comprise the heavy chain and light chain CDR1s, CDR2s and CDR3s of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4, or combinations thereof. The amino acid sequences of the V_H CDR1s of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4 are shown in SEQ ID NOs:21, 22, 23, 24, 25, 26, 27, 28, 29, and 30, respectively. The amino acid sequences of the V_H CDR2s of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6,

12B7, and 13G4 are shown in SEQ ID NOs:31, 32, 33, 34, 35, 36, 37, 38, 39, and 40, respectively. The amino acid sequences of the V_H CDR3s of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4 are shown in SEQ ID NOs:41, 42, 43, 44, 45, 46, 47, 48, 49, and 50, respectively. The amino acid sequences of the V_k CDR1s of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4 are shown in SEQ ID NOs:51, 52, 53, 54, 55, 56, 57, 58, 59, and 60, respectively. The amino acid sequences of the V_k CDR2s of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4 are shown in SEQ ID NOs:61, 62, 63, 64, 65, 66, 67, 68, 69, and 70, respectively. The amino acid sequences of the V_k CDR3s of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4 are shown in SEQ ID NOs:71, 72, 73, 74, 75, 76, 77, 78, 79, and 80, respectively. The CDR regions are delineated using the Kabat system (Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242).

Given that each of these antibodies can bind to PD-L1 and that antigen-binding specificity is provided primarily by the CDR1, CDR2, and CDR3 regions, the V_H CDR1, CDR2, and CDR3 sequences and V_k CDR1, CDR2, and CDR3 sequences can be "mixed and matched" (*i.e.*, CDRs from different antibodies can be mixed and match, although each antibody must contain a V_H CDR1, CDR2, and CDR3 and a V_k CDR1, CDR2, and CDR3) to create other anti-PD-L1 binding molecules of the invention. PD-L1 binding of such "mixed and matched" antibodies can be tested using the binding assays described above and in the Examples (*e.g.*, ELISAs, Biacore analysis). Preferably, when V_H CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular V_H sequence is replaced with a structurally similar CDR sequence(s). Likewise, when V_k CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular V_k sequence preferably is replaced with a structurally similar CDR sequence(s). It will be readily apparent to the ordinarily skilled artisan that novel V_H and V_L sequences can be created by substituting one or more V_H and/or V_L CDR region sequences with structurally similar sequences from the CDR sequences disclosed herein for monoclonal antibodies antibodies 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4.

Accordingly, in another aspect, the invention provides an isolated monoclonal antibody, or antigen binding portion thereof comprising:

(a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:21, 22, 23, 24, 25, 26, 27, 28, 29, and 30;

(b) a heavy chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:31, 32, 33, 34, 35, 36, 37, 38, 39, and 40;

(c) a heavy chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:41, 42, 43, 44, 45, 46, 47, 48, 49, and 50;

(d) a light chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:51, 52, 53, 54, 55, 56, 57, 58, 59, and 60;

(e) a light chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:61, 62, 63, 64, 65, 66, 67, 68, 69, and 70; and

(f) a light chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:71, 72, 73, 74, 75, 76, 77, 78, 79, and 80;

wherein the antibody specifically binds PD-L1, preferably human PD-L1.

In a preferred embodiment, the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:21;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:31;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:41;

(d) a light chain variable region CDR1 comprising SEQ ID NO:51;

(e) a light chain variable region CDR2 comprising SEQ ID NO:61; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:71.

In another preferred embodiment the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:22;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:32;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:42;

(d) a light chain variable region CDR1 comprising SEQ ID NO:52;

(e) a light chain variable region CDR2 comprising SEQ ID NO:62; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:72.

In another preferred embodiment the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:23;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:33;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:43;

(d) a light chain variable region CDR1 comprising SEQ ID NO:53;

(e) a light chain variable region CDR2 comprising SEQ ID NO:63; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:73.

In another preferred embodiment the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:24;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:34;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:44;

(d) a light chain variable region CDR1 comprising SEQ ID NO:54;

(e) a light chain variable region CDR2 comprising SEQ ID NO:64; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:74.

In another preferred embodiment the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:25;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:35;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:45;

(d) a light chain variable region CDR1 comprising SEQ ID NO:55;

(e) a light chain variable region CDR2 comprising SEQ ID NO:65; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:75.

In another preferred embodiment the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:26;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:36;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:46;

(d) a light chain variable region CDR1 comprising SEQ ID NO:56;

(e) a light chain variable region CDR2 comprising SEQ ID NO:66; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:76.

In another preferred embodiment the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:27;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:37;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:47;

(d) a light chain variable region CDR1 comprising SEQ ID NO:57;

(e) a light chain variable region CDR2 comprising SEQ ID NO:67; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:77.

In another preferred embodiment the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:28;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:38;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:48;

(d) a light chain variable region CDR1 comprising SEQ ID NO:58;

(e) a light chain variable region CDR2 comprising SEQ ID NO:68; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:78.

In another preferred embodiment the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:29;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:39;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:49;

(d) a light chain variable region CDR1 comprising SEQ ID NO:59;

(e) a light chain variable region CDR2 comprising SEQ ID NO:69; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:79.

In another preferred embodiment the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:30;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:40;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:50;

(d) a light chain variable region CDR1 comprising SEQ ID NO:60;

(e) a light chain variable region CDR2 comprising SEQ ID NO:70; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:80.

It is well known in the art that the CDR3 domain, independently from the CDR1 and/or CDR2 domain(s), alone can determine the binding specificity of an antibody for a cognate antigen and that multiple antibodies can predictably be generated having the same binding specificity based on a common CDR3 sequence. *See*, for example, Klimka *et al.*, *British J. of Cancer* 83(2):252-260 (2000) (describing the production of a humanized anti-CD30 antibody using only the heavy chain variable domain CDR3 of murine anti-CD30 antibody Ki-4); Beiboer *et al.*, *J. Mol. Biol.* 296:833-849 (2000) (describing recombinant epithelial glycoprotein-2 (EGP-2) antibodies using only the heavy chain CDR3 sequence of the parental murine MOC-31 anti-EGP-2 antibody); Rader *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 95:8910-8915 (1998) (describing a panel of

humanized anti-integrin $\alpha_v\beta_3$ antibodies using a heavy and light chain variable CDR3 domain of a murine anti-integrin $\alpha_v\beta_3$ antibody LM609 wherein each member antibody comprises a distinct sequence outside the CDR3 domain and capable of binding the same epitope as the parent muring antibody with affinities as high or higher than the parent murine antibody); Barbas *et al.*, *J. Am. Chem. Soc.* 116:2161-2162 (1994) (disclosing that the CDR3 domain provides the most significant contribution to antigen binding); Barbas *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 92:2529-2533 (1995) (describing the grafting of heavy chain CDR3 seqeunces of three Fabs (SI-1, SI-40, and SI-32) against human placental DNA onto the heavy chain of an anti-tetanus toxoid Fab thereby replacing the existing heavy chain CDR3 and demonstrating that the CDR3 domain alone conferred binding specificity); and Ditzel *et al.*, *J. Immunol.* 157:739-749 (1996) (describing grafting studies wherein transfer of only the heavy chain CDR3 of a parent polyspecific Fab LNA3 to a heavy chain of a monospecific IgG tetanus toxoid-binding Fab p313 antibody was sufficient to retain binding specificity of the parent Fab). Each of these references is hereby incorporated by reference in its entirety.

Accordingly, within certain aspects, the present invention provides monoclonal antibodies comprising one or more heavy and/or light chain CDR3 domain from a non-human antibody, such as a mouse or rat antibody, wherein the monoclonal antibody is capable of specifically binding to PD-L1. Within some embodiments, such inventive antibodies comprising one or more heavy and/or light chain CDR3 domain from a non-human antibody (a) are capable of competing for binding with; (b) retain the functional characteristics; (c) bind to the same epitope; and/or (d) have a similar binding affinity as the corresponding parental non-human antibody.

Within other aspects, the present invention provides monoclonal antibodies comprising one or more heavy and/or light chain CDR3 domain from a first human antibody, such as, for example, a human antibody obtained from a non-human animal, wherein the first human antibody is capable of specifically binding to PD-L1 and wherein the CDR3 domain from the first human antibody replaces a CDR3 domain in a human antibody that is lacking binding specificity for PD-L1 to generate a second human antibody that is capable of specifically binding to PD-L1. Within some embodiments, antibodies of the instant disclosure comprising one or more heavy and/or light chain CDR3 domain from the first human antibody (a) are capable of competing for

binding with; (b) retain the functional characteristics; (c) bind to the same epitope; and/or (d) have a similar binding affinity as the corresponding parental first human antibody.

Antibodies Having Particular Germline Sequences

In certain embodiments, an antibody of the invention comprises a heavy chain variable region from a particular germline heavy chain immunoglobulin gene and/or a light chain variable region from a particular germline light chain immunoglobulin gene.

For example, in a preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 1-18 gene, wherein the antibody specifically binds PD-L1. In another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 1-69 gene, wherein the antibody specifically binds PD-L1. In another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 1-3 gene, wherein the antibody specifically binds PD-L1. In another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 3-9 gene, wherein the antibody specifically binds PD-L1. In yet another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L6 gene, wherein the antibody specifically binds PD-L1. In yet another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L15 gene, wherein the antibody specifically binds PD-L1. In yet another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K A27 gene, wherein the antibody specifically binds PD-L1. In yet another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L18 gene, wherein the antibody

specifically binds PD-L1. In yet another preferred embodiment, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, wherein the antibody:

(a) comprises a heavy chain variable region that is the product of or derived from a human V_H 1-18, 1-69, 1-3 or 3-9 gene (which encodes the amino acid sequences set forth in SEQ ID NOs:101, 102, 103 and 104, respectively);

(b) comprises a light chain variable region that is the product of or derived from a human V_K L6, L15, A27 or L18 gene (which encodes the amino acid sequences set forth in SEQ ID NOs:105, 106, 107 and 108, respectively); and

(c) specifically binds to PD-L1, preferably human PD-L1.

An example of an antibody having V_H and V_K of V_H 1-18 and V_K L6, respectively, is 3G10. Examples of antibodies having V_H and V_K of V_H 1-69 and V_K L6, respectively, 12A4, 1B12, 7H1, and 12B7. An example of an antibody having V_H and V_K of V_H 1-3 and V_K L15, respectively, is 10A5. Examples of antibodies having V_H and V_K of V_H 1-69 and V_K A27, respectively, are 5F8, 11E6 and 11E6a. An example of an antibody having V_H and V_K of V_H 3-9 and V_K L15, respectively, is 10H10. An example of an antibody having V_H and V_K of V_H 1-3 and V_K L15, respectively, is 10A5. An example of an antibody having V_H and V_K of V_H 3-9 and V_K L18, respectively, is 13G4.

As used herein, a human antibody comprises heavy or light chain variable regions that is "the product of" or "derived from" a particular germline sequence if the variable regions of the antibody are obtained from a system that uses human germline immunoglobulin genes. Such systems include immunizing a transgenic mouse carrying human immunoglobulin genes with the antigen of interest or screening a human immunoglobulin gene library displayed on phage with the antigen of interest. A human antibody that is "the product of" or "derived from" a human germline immunoglobulin sequence can be identified as such by comparing the amino acid sequence of the human antibody to the amino acid sequences of human germline immunoglobulins and selecting the human germline immunoglobulin sequence that is closest in sequence (*i.e.*, greatest % identity) to the sequence of the human antibody. A human antibody that is "the product of" or "derived from" a particular human germline immunoglobulin sequence may contain amino acid differences as compared to the germline sequence, due to, for example, naturally-occurring somatic mutations or intentional introduction of site-directed mutation. However, a selected human antibody is generally at least 90% identical in amino acids sequence to an amino acid sequence encoded by a human

germline immunoglobulin gene and contains amino acid residues that identify the human antibody as being human when compared to the germline immunoglobulin amino acid sequences of other species (*e.g.*, murine germline sequences). In certain cases, a human antibody may be at least 95%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. In certain embodiments, a human antibody derived from a particular human germline sequence will display no more than 10 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene. In certain other embodiments, the human antibody may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

Homologous Antibodies

In yet another embodiment, an antibody of the invention comprises heavy and light chain variable regions comprising amino acid sequences that are homologous to the amino acid sequences of the preferred antibodies described herein, and wherein the antibodies retain the desired functional properties of the anti-PD-L1 antibodies of the invention.

For example, the invention provides an isolated monoclonal antibody, or antigen binding portion thereof, comprising a heavy chain variable region and a light chain variable region, wherein:

(a) the heavy chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs:1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

(b) the light chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs:11, 12, 13, 14, 15, 16, 17, 18, 19, and 20;

(c) the antibody binds to human PD-L1 with a K_D of $1x10^{-7}$ M or less;

(d) the antibody increases T-cell proliferation in a mixed lymphocyte reaction (MLR) assay;

(e) the antibody increases interferon-γ production in an MLR assay;

(f) the antibody increases IL-2 secretion in an MLR assay,

(g) the antibody stimulates antibody responses; and

(h) reverses the effect of T regulatory cells on T cell effector cells and /or dendritic cells.

In other embodiments, the V_H and/or V_L amino acid sequences may be 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to the sequences set forth above. An antibody having V_H and V_L regions having high (*i.e.*, 80% or greater) homology to the V_H and V_L regions of the sequences set forth above, can be obtained by mutagenesis (*e.g.*, site-directed or PCR-mediated mutagenesis) of nucleic acid molecules encoding SEQ ID NOs:25, 26, 27, 28, 29, and 30, followed by testing of the encoded altered antibody for retained function (*i.e.*, the functions set forth in (c) through (h) above) using the functional assays described herein.

As used herein, the percent homology between two amino acid sequences is equivalent to the percent identity between the two sequences. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

The percent identity between two amino acid sequences can be determined using the algorithm of E. Meyers and W. Miller (*Comput. Appl. Biosci.*, 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (*J. Mol. Biol.* 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

In certain instances, the protein sequences of the present disclosure can be further used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the XBLAST program (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to the antibody molecules of the invention. To obtain gapped

alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See www.ncbi.nlm.nih.gov.

Antibodies with Conservative Modifications

In certain embodiments, an antibody of the invention comprises a heavy chain variable region comprising CDR1, CDR2 and CDR3 sequences and a light chain variable region comprising CDR1, CDR2 and CDR3 sequences, wherein one or more of these CDR sequences comprise specified amino acid sequences based on the preferred antibodies described herein (*e.g.*, 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 or 13G4), or conservative modifications thereof, and wherein the antibodies retain the desired functional properties of the anti-PD-L1 antibodies of the invention. Accordingly, the invention provides an isolated monoclonal antibody, or antigen binding portion thereof, comprising a heavy chain variable region comprising CDR1, CDR2, and CDR3 sequences and a light chain variable region comprising CDR1, CDR2, and CDR3 sequences, wherein:

(a) the heavy chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs:41, 42, 43, 44, 45, 46, 47, 48, 49, and 50, and conservative modifications thereof;

(b) the light chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequence of SEQ ID NOs:71, 72, 73, 74, 75, 76, 77, 78, 79, and 80, and conservative modifications thereof;

(c) the antibody binds to human PD-L1 with a K_D of $1x10^{-7}$ M or less;

(d) the antibody increases T-cell proliferation in a mixed lymphocyte reaction (MLR) assay;

(e) the antibody increases interferon-γ production in an MLR assay;

(f) the antibody increases IL-2 secretion in an MLR assay

(g) the antibody stimulates antibody responses; and

(h) reverses the effect of T regulatory cells on T cell effector cells and /or dendritic cells.

In a preferred embodiment, the heavy chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid

sequences of SEQ ID NOs:31, 32, 33, 34, 35, 36, 37, 38, 39, and 40, and conservative modifications thereof; and the light chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs:61, 62, 63, 64, 65, 66, 67, 68, 69, and 70, and conservative modifications thereof. In another preferred embodiment, the heavy chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs:21, 22, 23, 24, 25, 26, 27, 28, 29, and 30, and conservative modifications thereof; and the light chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs:51, 52, 53, 54, 55, 56, 57, 58, 59, and 60, and conservative modifications thereof.

As used herein, the term "conservative sequence modifications" is intended to refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into an antibody of the invention by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within the CDR regions of an antibody of the invention can be replaced with other amino acid residues from the same side chain family and the altered antibody can be tested for retained function (*i.e.*, the functions set forth in (c) through (h) above) using the functional assays described herein.

Antibodies that Bind to the Same Epitope as Anti-PD-L1 Antibodies of the Invention

In another embodiment, the invention provides antibodies that bind to the same epitope on human PD-L1 as any of the PD-L1 monoclonal antibodies of the invention

(*i.e.*, antibodies that have the ability to cross-compete for binding to PD-L1 with any of the monoclonal antibodies of the invention). In preferred embodiments, the reference antibody for cross-competition studies can be the monoclonal antibody 3G10 (having V_H and V_L sequences as shown in SEQ ID NOs:1 and 11, respectively), or the monoclonal antibody 12A4 (having V_H and V_L sequences as shown in SEQ ID NOs:2 and 12, respectively), or the monoclonal antibody 10A5 (having V_H and V_L sequences as shown in SEQ ID NOs:3 and 13, respectively), or the monoclonal antibody 10A5 (having V_H and V_L sequences as shown in SEQ ID NOs:3 and 13,respectively) , or the monoclonal antibody 5F8 (having V_H and V_L sequences as shown in SEQ ID NOs:4 and 14, respectively) , or the monoclonal antibody 10H10 (having V_H and V_L sequences as shown in SEQ ID NOs:5 and 15, respectively) , or the monoclonal antibody 1B12 (having V_H and V_L sequences as shown in SEQ ID NOs:6 and 16, respectively) , or the monoclonal antibody 7H1 (having V_H and V_L sequences as shown in SEQ ID NOs:7 and 17, respectively) , or the monoclonal antibody 11E6 (having V_H and V_L sequences as shown in SEQ ID NOs:8 and 18, respectively) , or the monoclonal antibody 12B7 (having V_H and V_L sequences as shown in SEQ ID NOs:9 and 19, respectively) , or the monoclonal antibody 13G4 (having V_H and V_L sequences as shown in SEQ ID NOs:10 and 20,respectively). Such cross-competing antibodies can be identified based on their ability to cross-compete with 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 or 13G4 in standard PD-L1 binding assays. For example, BIAcore analysis, ELISA assays or flow cytometry may be used to demonstrate cross-competition with the antibodies of the current invention. The ability of a test antibody to inhibit the binding of, for example, 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 or 13G4, to human PD-L1 demonstrates that the test antibody can compete with 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 or 13G4 for binding to human PD-L1 and thus binds to the same epitope on human PD-L1 as 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 or 13G4. In a preferred embodiment, the antibody that binds to the same epitope on human PD-L1 as 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 or 13G4 is a human monoclonal antibody. Such human monoclonal antibodies can be prepared and isolated as described in the Examples.

Engineered and Modified Antibodies

An antibody of the invention further can be prepared using an antibody having one or more of the V_H and/or V_L sequences disclosed herein as starting material to engineer a modified antibody, which modified antibody may have altered properties from the starting antibody. An antibody can be engineered by modifying one or more residues within one or both variable regions (*i.e.*, V_H and/or V_L), for example within one or more CDR regions and/or within one or more framework regions. Additionally or alternatively, an antibody can be engineered by modifying residues within the constant region(s), for example to alter the effector function(s) of the antibody.

One type of variable region engineering that can be performed is CDR grafting. Antibodies interact with target antigens predominantly through amino acid residues that are located in the six heavy and light chain complementarity determining regions (CDRs). For this reason, the amino acid sequences within CDRs are more diverse between individual antibodies than sequences outside of CDRs. Because CDR sequences are responsible for most antibody-antigen interactions, it is possible to express recombinant antibodies that mimic the properties of specific naturally occurring antibodies by constructing expression vectors that include CDR sequences from the specific naturally occurring antibody grafted onto framework sequences from a different antibody with different properties (see, *e.g.*, Riechmann, L. *et al.* (1998) *Nature* 332:323-327; Jones, P. *et al.* (1986) *Nature* 321:522-525; Queen, C. *et al.* (1989) *Proc. Natl. Acad. See. U.S.A.* 86:10029-10033; U.S. Patent No. 5,225,539 to Winter, and U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen *et al.*)

Accordingly, another embodiment of the invention pertains to an isolated monoclonal antibody, or antigen binding portion thereof, comprising a heavy chain variable region comprising CDR1, CDR2, and CDR3 sequences comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:21, 22, 23, 24, 25, 26, 27, 28, 29, and 30, SEQ ID NOs:31, 32, 33, 34, 35, 36, 37, 38, 39, and 40, and SEQ ID NOs:41, 42, 43, 44, 45, 46, 47, 48, 49, and 50, respectively, and a light chain variable region comprising CDR1, CDR2, and CDR3 sequences comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:51, 52, 53, 54, 55, 56, 57, 58, 59, and 60, SEQ ID NOs:61, 62, 63, 64, 65, 66, 67, 68, 69, and 70, and SEQ ID NOs:71, 72, 73, 74, 75, 76, 77, 78, 79, and 80, respectively. Thus, such antibodies contain the V_H and V_L CDR sequences of monoclonal antibodies 3G10, 12A4, 10A5,

5F8, 10H10, 1B12, 7H1, 11E6, 12B7 or 13G4 yet may contain different framework sequences from these antibodies.

Such framework sequences can be obtained from public DNA databases or published references that include germline antibody gene sequences. For example, germline DNA sequences for human heavy and light chain variable region genes can be found in the "VBase" human germline sequence database (available on the Internet at www.mrc-cpe.cam.ac.uk/vbase), as well as in Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Tomlinson, I. M., *et al.* (1992) "The Repertoire of Human Germline V_H Sequences Reveals about Fifty Groups of V_H Segments with Different Hypervariable Loops" *J. Mol. Biol.* 227:776-798; and Cox, J. P. L. *et al.* (1994) "A Directory of Human Germ-line V_H Segments Reveals a Strong Bias in their Usage" *Eur. J. Immunol.* 24:827-836; the contents of each of which are expressly incorporated herein by reference.

Antibody protein sequences are compared against a compiled protein sequence database using one of the sequence similarity searching methods called the Gapped BLAST (Altschul *et al.* (1997) *Nucleic Acids Research* 25:3389-3402), which is well known to those skilled in the art. BLAST is a heuristic algorithm in that a statistically significant alignment between the antibody sequence and the database sequence is likely to contain high-scoring segment pairs (HSP) of aligned words. Segment pairs whose scores cannot be improved by extension or trimming is called a hit. Briefly, the nucleotide sequences of VBASE origin (*vbase.mrc-cpe.cam.ac.uk/vbase1/list2.php*) are translated and the region between and including FR1 through FR3 framework region is retained. The database sequences have an average length of 98 residues. Duplicate sequences which are exact matches over the entire length of the protein are removed. A BLAST search for proteins using the program blastp with default, standard parameters except the low complexity filter which is turned off and the substitution matrix of BLOSUM62, filters for top 5 hits yielding sequence matches. The nucleotide sequences are translated in all six frames and the frame with no stop codons in the matching segment of the database sequence is considered the potential hit. This is in turn confirmed using the BLAST program tblastx. This translates the antibody sequence in all six frames and compares those translations to the VBASE nucleotide sequences dynamically translated in all six frames.

The identities are exact amino acid matches between the antibody sequence and the protein database over the entire length of the sequence. The positives (identities + substitution match) are not identical but amino acid substitutions guided by the BLOSUM62 substitution matrix. If the antibody sequence matches two of the database sequences with same identity, the hit with most positives would be decided to be the matching sequence hit.

Preferred framework sequences for use in the antibodies of the invention are those that are structurally similar to the framework sequences used by selected antibodies of the invention, *e.g.*, similar to the V_H 1-18 framework sequences (SEQ ID NO:101) and/or the V_H 1-69 framework sequences (SEQ ID NO:102) and/or the V_H 1-3 framework sequences (SEQ ID NO:103) and/or the V_H 3-9 framework sequences (SEQ ID NO:104) and/or the V_K L6 framework sequences (SEQ ID NO:105) and/or the V_K L15 framework sequences (SEQ ID NO:106) and/or the V_K A27 framework sequences (SEQ ID NO:107) and/or the V_K L18 framework sequences (SEQ ID NO:107) used by preferred monoclonal antibodies of the invention. The V_H CDR1, CDR2, and CDR3 sequences, and the V_K CDR1, CDR2, and CDR3 sequences, can be grafted onto framework regions that have the identical sequence as that found in the germline immunoglobulin gene from which the framework sequence derive, or the CDR sequences can be grafted onto framework regions that contain one or more mutations as compared to the germline sequences. For example, it has been found that in certain instances it is beneficial to mutate residues within the framework regions to maintain or enhance the antigen binding ability of the antibody (see *e.g.*, U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen *et al*).

Another type of variable region modification is to mutate amino acid residues within the V_H and/or V_K CDR1, CDR2 and/or CDR3 regions to thereby improve one or more binding properties (*e.g.*, affinity) of the antibody of interest. Site-directed mutagenesis or PCR-mediated mutagenesis can be performed to introduce the mutation(s) and the effect on antibody binding, or other functional property of interest, can be evaluated in *in vitro* or *in vivo* assays as described herein and provided in the Examples. Preferably conservative modifications (as discussed above) are introduced. The mutations may be amino acid substitutions, additions or deletions, but are preferably substitutions. Moreover, typically no more than one, two, three, four or five residues within a CDR region are altered.

Accordingly, in another embodiment, the invention provides isolated anti-PD-L1 monoclonal antibodies, or antigen binding portions thereof, comprising a heavy chain variable region comprising: (a) a V_H CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:21, 22, 23, 24, 25, 26, 27, 28, 29, and 30, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs:21, 22, 23, 24, 25, 26, 27, 28, 29, and 30; (b) a V_H CDR2 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:31, 32, 33, 34, 35, 36, 37, 38, 39, and 40, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs:31, 32, 33, 34, 35, 36, 37, 38, 39, and 40; (c) a V_H CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:41, 42, 43, 44, 45, 46, 47, 48, 49, and 50, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs:41, 42, 43, 44, 45, 46, 47, 48, 49, and 50; (d) a V_K CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:51, 52, 53, 54, 55, 56, 57, 58, 59, and 60, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs:51, 52, 53, 54, 55, 56, 57, 58, 59, and 60; (e) a V_K CDR2 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:61, 62, 63, 64, 65, 66, 67, 68, 69, and 70, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs:61, 62, 63, 64, 65, 66, 67, 68, 69, and 70; and (f) a V_K CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:71, 72, 73, 74, 75, 76, 77, 78, 79, and 80, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs:71, 72, 73, 74, 75, 76, 77, 78, 79, and 80.

Engineered antibodies of the invention include those in which modifications have been made to framework residues within V_H and/or V_K, *e.g.* to improve the properties of the antibody. Typically such framework modifications are made to decrease the immunogenicity of the antibody. For example, one approach is to "backmutate" one or more framework residues to the corresponding germline sequence. More specifically, an antibody that has undergone somatic mutation may contain framework residues that differ from the germline sequence from which the antibody is derived. Such residues can be

identified by comparing the antibody framework sequences to the germline sequences from which the antibody is derived. For example, as described below, a number of amino acid changes in the framework regions of the anti-PD-L1 antibodies 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 and 13G4 that differ from the parent germline sequence. To return the framework region sequences to their germline configuration, the somatic mutations can be "backmutated" to the germline sequence by, for example, site-directed mutagenesis or PCR-mediated mutagenesis. The alignment of the V_H region for 3G10 against the parent germline V_H 1-18 sequence is shown in Figure 11. The alignment of the V_H region for 12A4 against the parent germline V_H 1-69 sequence is shown in Figure 12. The alignment of the V_H region for 10A5 against the parent germline V_H 1-3 sequence is shown in Figure 13. The alignment of the V_H region for 5F8 against the parent germline V_H 1-69 sequence is shown in Figure 14. The alignment of the V_H region for 10H10 against the parent germline V_H 3-9 sequence is shown in Figure 15. The alignment of the V_H region for 1B12 against the parent germline V_H 1-69 sequence is shown in Figure 16. The alignment of the V_H region for 7H1 against the parent germline V_H 1-69 sequence is shown in Figure 17. The alignment of the V_H region for 11E6 against the parent germline V_H 1-69 sequence is shown in Figure 18. The alignment of the V_H region for 12B7 against the parent germline V_H 1-69 sequence is shown in Figure 19. The alignment of the V_H region for 13G4 against the parent germline V_H 3-9 sequence is shown in Figure 20.

For example, for 3G10, amino acid residue #79 (within FR3) of V_H is a valine whereas this residue in the corresponding V_H 1-18 germline sequence is an alanine. To return the framework region sequences to their germline configuration, the somatic mutations can be "backmutated" to the germline sequence by, for example, site-directed mutagenesis or PCR-mediated mutagenesis (*e.g.*, residue #79 (residue #13 of FR3) of the V_H of 3G10 can be "backmutated" from valine to alanine).

As another example, for 12A4, amino acid residue #24 (within FR1) of V_H is a threonine whereas this residue in the corresponding V_H 1-69 germline sequence is an alanine. To return the framework region sequences to their germline configuration, for example, residue #24 of the V_H of 12A4 can be "backmutated" from threonine to alanine. Such "backmutated" antibodies are also intended to be encompassed by the invention.

As another example, for 12A4, amino acid residue #27 (within FR1) of V_H is an aspartic acid whereas this residue in the corresponding V_H 1-69 germline sequence is a

glycine. To return the framework region sequences to their germline configuration, for example, residue #27 of the V_H of 12A4 can be "backmutated" from aspartic acid to glycine. Such "backmutated" antibodies are also intended to be encompassed by the invention.

As another example, for 12A4, amino acid residue #95 (within FR3) of V_H is a phenylalanine whereas this residue in the corresponding V_H 1-69 germline sequence is a tyrosine. To return the framework region sequences to their germline configuration, for example, residue #95 (residue #29 of FR3) of the V_H of 12A4 can be "backmutated" from phenylalanine to tyrosine. Such "backmutated" antibodies are also intended to be encompassed by the invention.

As another example, for 5F8, amino acid residue #24 (within FR1) is a valine whereas this residue in the corresponding V_H 1-69 germline sequence is an alanine. To return the framework region sequences to their germline configuration, for example, residue #24 of the V_H of 5F8 can be "backmutated" from valine to alanine. Such "backmutated" antibodies are also intended to be encompassed by the invention.

As another example, for 5F8, amino acid residue #28 (within FR1) is an isoleucine whereas this residue in the corresponding V_H 1-69 germline sequence is an threonine. To return the framework region sequences to their germline configuration, for example, residue #28 of the V_H of 5F8 can be "backmutated" from isoleucine to threonine. Such "backmutated" antibodies are also intended to be encompassed by the invention.

As another example, for 10H10, amino acid residue #24 (within FR1) is a valine whereas this residue in the corresponding V_H 3-9 germline sequence is an alanine. To return the framework region sequences to their germline configuration, for example, residue #24 of the V_H of 10H10 can be "backmutated" from valine to alanine. Such "backmutated" antibodies are also intended to be encompassed by the invention.

As another example, for 10H10, an amino acid can be inserted following amino acid residue #97 (within FR3). This amino acid is a valine. To return the framework region sequences to their germline configuration, for example, the inserted amino acid following residue #97 of the V_H of 10H10 can be "backmutated" to delete this valine. Such "backmutated" antibodies are also intended to be encompassed by the invention.

As another example, for 1B12, amino acid residue #24 (within FR1) is a threonine whereas this residue in the corresponding V_H 1-69 germline sequence is an alanine. To

return the framework region sequences to their germline configuration, for example, residue #24 of the V_H of 1B12 can be "backmutated" from threonine to alanine. Such "backmutated" antibodies are also intended to be encompassed by the invention.

As another example, for 1B12, amino acid residue #27 (within FR1) is an aspartic acid whereas this residue in the corresponding V_H 1-69 germline sequence is an glycine. To return the framework region sequences to their germline configuration, for example, residue #27 of the V_H of 1B12 can be "backmutated" from aspartic acid to glycine. Such "backmutated" antibodies are also intended to be encompassed by the invention.

As another example, for 1B12, amino acid residue #95 (within FR3) is a phenylalanine whereas this residue in the corresponding V_H 1-69 germline sequence is an tyrosine. To return the framework region sequences to their germline configuration, for example, residue #95 (residue #29 of FR3) of the V_H of 1B12 can be "backmutated" from phenylalanine to tyrosine. Such "backmutated" antibodies are also intended to be encompassed by the invention.

As another example, for 7H1, amino acid residue #24 (within FR1) is a threonine whereas this residue in the corresponding V_H 1-69 germline sequence is an alanine. To return the framework region sequences to their germline configuration, for example, residue #24 of the V_H of 7H1 can be "backmutated" from threonine to alanine. Such "backmutated" antibodies are also intended to be encompassed by the invention.

As another example, for 7H1, amino acid residue #77 (within FR3) is a threonine whereas this residue in the corresponding V_H 1-69 germline sequence is a serine. To return the framework region sequences to their germline configuration, for example, residue #72 (residue #11 of FR3) of the V_H of 7H1 can be "backmutated" from threonine to serine. Such "backmutated" antibodies are also intended to be encompassed by the invention.

As another example, for 11E6, amino acid residue #78 (within FR3) is an alanine whereas this residue in the corresponding V_H 1-69 germline sequence is a threonine. To return the framework region sequences to their germline configuration, for example, residue #78 (residue 12 of FR3) of the V_H of 11E6 can be "backmutated" from alanine to threonine. Such "backmutated" antibodies are also intended to be encompassed by the invention.

As another example, for 12B7, amino acid residue #13 (within FR1) is a glutamic acid whereas this residue in the corresponding V_H 1-69 germline sequence is an lysine. To

return the framework region sequences to their germline configuration, for example, residue #13 of the V_H of 12B7 can be "backmutated" glutamic acid to lysine. Such "backmutated" antibodies are also intended to be encompassed by the invention.

As another example, for 12B7, amino acid residue #30 (within FR1) is an asparagine whereas this residue in the corresponding V_H 1-69 germline sequence is an serine. To return the framework region sequences to their germline configuration, for example, residue #30 of the V_H of 12B7 can be "backmutated" from asparagine to serine. Such "backmutated" antibodies are also intended to be encompassed by the invention.

As another example, for 12B7, amino acid residue #77 (within FR3) is an asparagine whereas this residue in the corresponding V_H 1-69 germline sequence is an serine. To return the framework region sequences to their germline configuration, for example, residue #377 (residue 11 of FR3) of the V_H of 12B7 can be "backmutated" from asparagine to serine. Such "backmutated" antibodies are also intended to be encompassed by the invention.

As another example, for 12B7, amino acid residue #82 (within FR3) is an aspartic acid whereas this residue in the corresponding V_H 1-69 germline sequence is a glutamic acid. To return the framework region sequences to their germline configuration, for example, residue #82 (residue #16 of FR3) of the V_H of 12B7 can be "backmutated" from aspartic acid to glutamic acid. Such "backmutated" antibodies are also intended to be encompassed by the invention.

As another example, for 13G4, amino acid residue #27 (within FR1) is an isoleucine whereas this residue in the corresponding V_H 1-69 germline sequence is an phenylalanine. To return the framework region sequences to their germline configuration, for example, residue #27 of the V_H of 12B7 can be "backmutated" from isoleucine to phenylalanine. Such "backmutated" antibodies are also intended to be encompassed by the invention.

Another type of framework modification involves mutating one or more residues within the framework region, or even within one or more CDR regions, to remove T cell epitopes to thereby reduce the potential immunogenicity of the antibody. This approach is also referred to as "deimmunization" and is described in further detail in U.S. Patent Publication No. 20030153043 by Carr *et al.*

In addition or alternative to modifications made within the framework or CDR regions, antibodies of the invention may be engineered to include modifications within

the Fc region, typically to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity. Furthermore, an antibody of the invention may be chemically modified (*e.g.*, one or more chemical moieties can be attached to the antibody) or be modified to alter its glycosylation, again to alter one or more functional properties of the antibody. Each of these embodiments is described in further detail below. The numbering of residues in the Fc region is that of the EU index of Kabat.

In one embodiment, the hinge region of CH1 is modified such that the number of cysteine residues in the hinge region is altered, *e.g.*, increased or decreased. This approach is described further in U.S. Patent No. 5,677,425 by Bodmer *et al.* The number of cysteine residues in the hinge region of CH1 is altered to, for example, facilitate assembly of the light and heavy chains or to increase or decrease the stability of the antibody.

In another embodiment, the Fc hinge region of an antibody is mutated to decrease the biological half life of the antibody. More specifically, one or more amino acid mutations are introduced into the CH2-CH3 domain interface region of the Fc-hinge fragment such that the antibody has impaired Staphylococcyl protein A (SpA) binding relative to native Fc-hinge domain SpA binding. This approach is described in further detail in U.S. Patent No. 6,165,745 by Ward *et al.*

In another embodiment, the antibody is modified to increase its biological half life. Various approaches are possible. For example, one or more of the following mutations can be introduced: T252L, T254S, T256F, as described in U.S. Patent No. 6,277,375 to Ward. Alternatively, to increase the biological half life, the antibody can be altered within the CH1 or CL region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Patent Nos. 5,869,046 and 6,121,022 by Presta *et al.*

In yet other embodiments, the Fc region is altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector function(s) of the antibody. For example, one or more amino acids selected from amino acid residues 234, 235, 236, 237, 297, 318, 320 and 322 can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is

described in further detail in U.S. Patent Nos. 5,624,821 and 5,648,260, both by Winter *et al.*

In another example, one or more amino acids selected from amino acid residues 329, 331 and 322 can be replaced with a different amino acid residue such that the antibody has altered C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in further detail in U.S. Patent Nos. 6,194,551 by Idusogie *et al.*

In another example, one or more amino acid residues within amino acid positions 231 and 239 are altered to thereby alter the ability of the antibody to fix complement. This approach is described further in PCT Publication WO 94/29351 by Bodmer *et al.*

In yet another example, the Fc region is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fcγ receptor by modifying one or more amino acids at the following positions: 238, 239, 248, 249, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 301, 303, 305, 307, 309, 312, 315, 320, 322, 324, 326, 327, 329, 330, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 378, 382, 388, 389, 398, 414, 416, 419, 430, 434, 435, 437, 438 or 439. This approach is described further in PCT Publication WO 00/42072 by Presta. Moreover, the binding sites on human IgG1 for FcγR1, FcγRII, FcγRIII and FcRn have been mapped and variants with improved binding have been described (see Shields, R.L. *et al.* (2001) *J. Biol. Chem.* 276:6591-6604). Specific mutations at positions 256, 290, 298, 333, 334 and 339 were shown to improve binding to FcγRIII. Additionally, the following combination mutants were shown to improve FcγRIII binding: T256A/S298A, S298A/E333A, S298A/K224A and S298A/E333A/K334A.

In still another embodiment, the glycosylation of an antibody is modified. For example, an aglycoslated antibody can be made (*i.e.*, the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. Such an

approach is described in further detail in U.S. Patent Nos. 5,714,350 and 6,350,861 by Co *et al.*

In certain other embodiments, an antibody can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNac structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation. For example, the cell lines Ms704, Ms705, and Ms709 lack the fucosyltransferase gene, FUT8 (alpha (1,6) fucosyltransferase), such that antibodies expressed in the Ms704, Ms705, and Ms709 cell lines lack fucose on their carbohydrates. The Ms704, Ms705, and Ms709 $FUT8^{-/-}$ cell lines were created by the targeted disruption of the FUT8 gene in CHO/DG44 cells using two replacement vectors (see U.S. Patent Publication No. 20040110704 by Yamane *et al.* and Yamane-Ohnuki *et al.* (2004) *Biotechnol Bioeng* 87:614-22). As another example, EP 1,176,195 by Hanai *et al.* describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, such that antibodies expressed in such a cell line exhibit hypofucosylation by reducing or eliminating the alpha 1,6 bond-related enzyme. Hanai *et al.* also describe cell lines which have a low enzyme activity for adding fucose to the N-acetylglucosamine that binds to the Fc region of the antibody or does not have the enzyme activity, for example the rat myeloma cell line YB2/0 (ATCC CRL 1662). PCT Publication WO 03/035835 by Presta describes a variant CHO cell line, Lec13 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields, R.L. *et al.* (2002) *J. Biol. Chem.* 277:26733-26740). PCT Publication WO 99/54342 by Umana *et al.* describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (*e.g.*, beta(1,4)-N-acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNac structures which results in increased ADCC activity of the antibodies (see also Umana *et al.* (1999) *Nat. Biotech.* 17:176-180). Alternatively, the fucose residues of the antibody may be cleaved off using

a fucosidase enzyme. For example, the fucosidase alpha-L-fucosidase removes fucosyl residues from antibodies (Tarentino, A.L. *et al.* (1975) *Biochem.* 14:5516-23).

Another modification of the antibodies herein that is contemplated by the invention is pegylation. An antibody can be pegylated to, for example, increase the biological (*e.g.*, serum) half life of the antibody. To pegylate an antibody, the antibody, or fragment thereof, typically is reacted with polyethylene glycol (PEG), such as a reactive ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the antibody or antibody fragment. Preferably, the pegylation is carried out via an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive water-soluble polymer). As used herein, the term "polyethylene glycol" is intended to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono (C1-C10) alkoxy- or aryloxy-polyethylene glycol or polyethylene glycol-maleimide. In certain embodiments, the antibody to be pegylated is an aglycosylated antibody. Methods for pegylating proteins are known in the art and can be applied to the antibodies of the invention. See for example, EP 0 154 316 by Nishimura *et al.* and EP 0 401 384 by Ishikawa *et al.*

Methods of Engineering Antibodies

As discussed above, the anti-PD-L1 antibodies having V_H and V_K sequences disclosed herein can be used to create new anti-PD-L1 antibodies by modifying the VH and/or V_K sequences, or the constant region(s) attached thereto. Thus, in another aspect of the invention, the structural features of an anti-PD-L1 antibody of the invention, *e.g.* 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, or 13G4,, are used to create structurally related anti-PD-L1 antibodies that retain at least one functional property of the antibodies of the invention, such as binding to human PD-L1. For example, one or more CDR regions of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, or 13G4 or mutations thereof, can be combined recombinantly with known framework regions and/or other CDRs to create additional, recombinantly-engineered, anti-PD-L1 antibodies of the invention, as discussed above. Other types of modifications include those described in the previous section. The starting material for the engineering method is one or more of the V_H and/or V_K sequences provided herein, or one or more CDR regions thereof. To create the engineered antibody, it is not necessary to actually prepare (*i.e.*, express as a protein) an antibody having one or more of the V_H and/or V_K sequences

provided herein, or one or more CDR regions thereof. Rather, the information contained in the sequence(s) is used as the starting material to create a "second generation" sequence(s) derived from the original sequence(s) and then the "second generation" sequence(s) is prepared and expressed as a protein.

Accordingly, in another embodiment, the invention provides a method for preparing an anti-PD-L1 antibody comprising:

(a) providing: (i) a heavy chain variable region antibody sequence comprising a CDR1 sequence selected from the group consisting of SEQ ID NOs:21, 22, 23, 24, 25, 26, 27, 28, 29, and 30, a CDR2 sequence selected from the group consisting of SEQ ID NOs:31, 32, 33, 34, 35, 36, 37, 38, 39, and 40, and/or a CDR3 sequence selected from the group consisting of SEQ ID NOs:41, 42, 43, 44, 45, 46, 47, 48, 49, and 50; and/or (ii) a light chain variable region antibody sequence comprising a CDR1 sequence selected from the group consisting of SEQ ID NOs:51, 52, 53, 54, 55, 56, 57, 58, 59, and 60, a CDR2 sequence selected from the group consisting of SEQ ID NOs:61, 62, 63, 64, 65, 66, 67, 68, 69, and 70, and/or a CDR3 sequence selected from the group consisting of SEQ ID NOs:71, 72, 73, 74, 75, 76, 77, 78, 79, and 80;

(b) altering at least one amino acid residue within the heavy chain variable region antibody sequence and/or the light chain variable region antibody sequence to create at least one altered antibody sequence; and

(c) expressing the altered antibody sequence as a protein.

Standard molecular biology techniques can be used to prepare and express the altered antibody sequence.

Preferably, the antibody encoded by the altered antibody sequence(s) is one that retains one, some or all of the functional properties of the anti-PD-L1 antibodies described herein, which functional properties include, but are not limited to:

(i) binds to human PD-L1 with a K_D of $1x10^{-7}$ M or less;

(ii) increases T-cell proliferation in a mixed lymphocyte reaction (MLR) assay;

(iii) increases interferon-γ production in an MLR assay;

(iv) increases IL-2 secretion in an MLR assay;

(v) stimulates antibody responses; and/or

(vi) reverses the effect of T regulatory cells on T cell effector cells and /or dendritic cells.

The functional properties of the altered antibodies can be assessed using standard assays available in the art and/or described herein, such as those set forth in the Examples (*e.g.*, flow cytometry, binding assays).

In certain embodiments of the methods of engineering antibodies of the invention, mutations can be introduced randomly or selectively along all or part of an anti-PD-L1 antibody coding sequence and the resulting modified anti-PD-L1 antibodies can be screened for binding activity and/or other functional properties as described herein. Mutational methods have been described in the art. For example, PCT Publication WO 02/092780 by Short describes methods for creating and screening antibody mutations using saturation mutagenesis, synthetic ligation assembly, or a combination thereof. Alternatively, PCT Publication WO 03/074679 by Lazar *et al.* describes methods of using computational screening methods to optimize physiochemical properties of antibodies.

Nucleic Acid Molecules Encoding Antibodies of the Disclosure

Another aspect of the disclosure pertains to nucleic acid molecules that encode the antibodies of the invention. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid is "isolated" or "rendered substantially pure" when purified away from other cellular components or other contaminants, *e.g.*, other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis and others well known in the art. *See*, F. Ausubel, *et al.*, ed. (1987) Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York. A nucleic acid of the invention can be, for example, DNA or RNA and may or may not contain intronic sequences. In a preferred embodiment, the nucleic acid is a cDNA molecule.

Nucleic acids of the invention can be obtained using standard molecular biology techniques. For antibodies expressed by hybridomas (*e.g.*, hybridomas prepared from transgenic mice carrying human immunoglobulin genes as described further below), cDNAs encoding the light and heavy chains of the antibody made by the hybridoma can be obtained by standard PCR amplification or cDNA cloning techniques. For antibodies obtained from an immunoglobulin gene library (*e.g.*, using phage display techniques), nucleic acid encoding the antibody can be recovered from the library.

Preferred nucleic acids molecules of the invention are those encoding the VH and VL sequences of the 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4, monoclonal antibodies. DNA sequences encoding the VH sequences of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4, are shown in SEQ ID NOs:81, 82, 83, 84, 85, 86, 87, 88, 89 and 90, respectively. DNA sequences encoding the VL sequences of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4, are shown in SEQ ID NOs:91, 92, 93, 94, 95, 96, 97, 98, 99 and 100, respectively.

Once DNA fragments encoding VH and VL segments are obtained, these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene. In these manipulations, a VL- or VH-encoding DNA fragment is operatively linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term "operatively linked," as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

The isolated DNA encoding the VH region can be converted to a full-length heavy chain gene by operatively linking the VH-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are known in the art (see *e.g.*, Kabat, E. A., el al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region, but most preferably is an IgG1 or IgG4 constant region. For a Fab fragment heavy chain gene, the VH-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region.

The isolated DNA encoding the VL region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the VL-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art (see *e.g.*, Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification.

The light chain constant region can be a kappa or lambda constant region, but most preferably is a kappa constant region.

To create a scFv gene, the VH- and VL-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, *e.g.*, encoding the amino acid sequence $(Gly_4\text{-}Ser)_3$, such that the VH and VL sequences can be expressed as a contiguous single-chain protein, with the VL and VH regions joined by the flexible linker (see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883; McCafferty *et al.*, (1990) *Nature* 348:552-554).

Production of Monoclonal Antibodies of the Invention

Monoclonal antibodies (mAbs) of the present invention can be produced by a variety of techniques, including conventional monoclonal antibody methodology *e.g.*, the standard somatic cell hybridization technique of Kohler and Milstein (1975) *Nature* 256: 495. Although somatic cell hybridization procedures are preferred, in principle, other techniques for producing monoclonal antibody can be employed *e.g.*, viral or oncogenic transformation of B lymphocytes.

The preferred animal system for preparing hybridomas is the murine system. Hybridoma production in the mouse is a very well-established procedure. Immunization protocols and techniques for isolation of immunized splenocytes for fusion are known in the art. Fusion partners (*e.g.*, murine myeloma cells) and fusion procedures are also known.

Chimeric or humanized antibodies of the present invention can be prepared based on the sequence of a murine monoclonal antibody prepared as described above. DNA encoding the heavy and light chain immunoglobulins can be obtained from the murine hybridoma of interest and engineered to contain non-murine (*e.g.*, human) immunoglobulin sequences using standard molecular biology techniques. For example, to create a chimeric antibody, the murine variable regions can be linked to human constant regions using methods known in the art (see *e.g.*, U.S. Patent No. 4,816,567 to Cabilly *et al.*). To create a humanized antibody, the murine CDR regions can be inserted into a human framework using methods known in the art (see *e.g.*, U.S. Patent No. 5,225,539 to Winter, and U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen *et al.*).

In a preferred embodiment, the antibodies of the invention are human monoclonal antibodies. Such human monoclonal antibodies directed against PD-L1 can be generated using transgenic or transchromosomic mice carrying parts of the human immune system rather than the mouse system. These transgenic and transchromosomic mice include mice referred to herein as HuMAb mice and KM mice™, respectively, and are collectively referred to herein as "human Ig mice."

The HuMAb mouse® (Medarex, Inc.) contains human immunoglobulin gene miniloci that encode unrearranged human heavy (μ and γ) and κ light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (see *e.g.*, Lonberg, *et al.* (1994) Nature 368(6474): 856-859). Accordingly, the mice exhibit reduced expression of mouse IgM or κ, and in response to immunization, the introduced human heavy and light chain transgenes undergo class switching and somatic mutation to generate high affinity human IgGκ monoclonal (Lonberg, N. *et al.* (1994), *supra*; reviewed in Lonberg, N. (1994) *Handbook of Experimental Pharmacology* 113:49-101; Lonberg, N. and Huszar, D. (1995) *Intern. Rev. Immunol.* 13: 65-93, and Harding, F. and Lonberg, N. (1995) *Ann. N.Y. Acad. Sci.* 764:536-546). The preparation and use of HuMab mice, and the genomic modifications carried by such mice, is further described in Taylor, L. *et al.* (1992) *Nucleic Acids Research* 20:6287-6295; Chen, J. *et al.* (1993) *International Immunology* 5: 647-656; Tuaillon *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:3720-3724; Choi *et al.* (1993) *Nature Genetics* 4:117-123; Chen, J. *et al.* (1993) *EMBO J.* 12: 821-830; Tuaillon *et al.* (1994) *J. Immunol.* 152:2912-2920; Taylor, L. *et al.* (1994) *International Immunology* 6: 579-591; and Fishwild, D. *et al.* (1996) *Nature Biotechnology* 14: 845-851, the contents of all of which are hereby specifically incorporated by reference in their entirety. See further, U.S. Patent Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661,016; 5,814,318; 5,874,299; and 5,770,429; all to Lonberg and Kay; U.S. Patent No. 5,545,807 to Surani *et al.*; PCT Publication Nos. WO 92/03918, WO 93/12227, WO 94/25585, WO 97/13852, WO 98/24884 and WO 99/45962, all to Lonberg and Kay; and PCT Publication No. WO 01/14424 to Korman *et al.*

In another embodiment, human antibodies of the invention can be raised using a mouse that carries human immunoglobulin sequences on transgenes and transchomosomes, such as a mouse that carries a human heavy chain transgene and a

human light chain transchromosome. Such mice, referred to herein as "KM mice™," are described in detail in PCT Publication WO 02/43478 to Ishida *et al.*

Still further, alternative transgenic animal systems expressing human immunoglobulin genes are available in the art and can be used to raise anti-PD-L1 antibodies of the invention. For example, an alternative transgenic system referred to as the Xenomouse (Abgenix, Inc.) can be used; such mice are described in, for example, U.S. Patent Nos. 5,939,598; 6,075,181; 6,114,598; 6, 150,584 and 6,162,963 to Kucherlapati *et al.*

Moreover, alternative transchromosomic animal systems expressing human immunoglobulin genes are available in the art and can be used to raise anti-PD-L1 antibodies of the invention. For example, mice carrying both a human heavy chain transchromosome and a human light chain tranchromosome, referred to as "TC mice" can be used; such mice are described in Tomizuka *et al.* (2000) *Proc. Natl. Acad. Sci. USA* 97:722-727. Furthermore, cows carrying human heavy and light chain transchromosomes have been described in the art (Kuroiwa *et al.* (2002) *Nature Biotechnology* 20:889-894) and can be used to raise anti-PD-L1 antibodies of the invention.

Human monoclonal antibodies of the invention can also be prepared using phage display methods for screening libraries of human immunoglobulin genes. Such phage display methods for isolating human antibodies are established in the art. See for example: U.S. Patent Nos. 5,223,409; 5,403,484; and 5,571,698 to Ladner *et al.*; U.S. Patent Nos. 5,427,908 and 5,580,717 to Dower *et al.*; U.S. Patent Nos. 5,969,108 and 6,172,197 to McCafferty *et al.*; and U.S. Patent Nos. 5,885,793; 6,521,404; 6,544,731; 6,555,313; 6,582,915 and 6,593,081 to Griffiths *et al.*

Human monoclonal antibodies of the invention can also be prepared using SCID mice into which human immune cells have been reconstituted such that a human antibody response can be generated upon immunization. Such mice are described in, for example, U.S. Patent Nos. 5,476,996 and 5,698,767 to Wilson *et al.*

Immunization of Human Ig Mice

When human Ig mice are used to raise human antibodies of the invention, such mice can be immunized with a purified or enriched preparation of PD-L1 antigen and/or recombinant PD-L1, or an PD-L1 fusion protein, as described by Lonberg, N. *et al.* (1994) *Nature* 368(6474): 856-859; Fishwild, D. *et al.* (1996) *Nature Biotechnology* 14:

845-851; and PCT Publication WO 98/24884 and WO 01/14424. Preferably, the mice will be 6-16 weeks of age upon the first infusion. For example, a purified or recombinant preparation (5-50 μg) of PD-L1 antigen can be used to immunize the human Ig mice intraperitoneally.

Detailed procedures to generate fully human monoclonal antibodies to PD-L1 are described in Example 1 below. Cumulative experience with various antigens has shown that the transgenic mice respond when initially immunized intraperitoneally (IP) with antigen in complete Freund's adjuvant, followed by every other week IP immunizations (up to a total of 6) with antigen in incomplete Freund's adjuvant. However, adjuvants other than Freund's are also found to be effective. In addition, whole cells in the absence of adjuvant are found to be highly immunogenic. The immune response can be monitored over the course of the immunization protocol with plasma samples being obtained by retroorbital bleeds. The plasma can be screened by ELISA (as described below), and mice with sufficient titers of anti-PD-L1 human immunoglobulin can be used for fusions. Mice can be boosted intravenously with antigen 3 days before sacrifice and removal of the spleen. It is expected that 2-3 fusions for each immunization may need to be performed. Between 6 and 24 mice are typically immunized for each antigen. Usually both HCo7 and HCo12 strains are used. In addition, both HCo7 and HCo12 transgene can be bred together into a single mouse having two different human heavy chain transgenes (HCo7/HCo12). Alternatively or additionally, the KM mouse™ strain can be used, as described in Example 1.

Generation of Hybridomas Producing Human Monoclonal Antibodies of the Disclosure

To generate hybridomas producing human monoclonal antibodies of the invention, splenocytes and/or lymph node cells from immunized mice can be isolated and fused to an appropriate immortalized cell line, such as a mouse myeloma cell line. The resulting hybridomas can be screened for the production of antigen-specific antibodies. For example, single cell suspensions of splenic lymphocytes from immunized mice can be fused to one-sixth the number of P3X63-Ag8.653 nonsecreting mouse myeloma cells (ATCC, CRL 1580) with 50% PEG. Cells are plated at approximately 2 x 10^5 in flat bottom microtiter plate, followed by a two week incubation in selective medium containing 20% fetal Clone Serum, 18% "653" conditioned media, 5% origen (IGEN), 4 mM L-glutamine, 1 mM sodium pyruvate, 5mM HEPES, 0.055 mM 2-mercaptoethanol,

50 units/ml penicillin, 50 mg/ml streptomycin, 50 mg/ml gentamycin and 1X HAT (Sigma; the HAT is added 24 hours after the fusion). After approximately two weeks, cells can be cultured in medium in which the HAT is replaced with HT. Individual wells can then be screened by ELISA for human monoclonal IgM and IgG antibodies. Once extensive hybridoma growth occurs, medium can be observed usually after 10-14 days. The antibody secreting hybridomas can be re-plated, screened again, and if still positive for human IgG, the monoclonal antibodies can be subcloned at least twice by limiting dilution. The stable subclones can then be cultured *in vitro* to generate small amounts of antibody in tissue culture medium for characterization.

To purify human monoclonal antibodies, selected hybridomas can be grown in two-liter spinner-flasks for monoclonal antibody purification. Supernatants can be filtered and concentrated before affinity chromatography with protein A-sepharose (Pharmacia, Piscataway, N.J.). Eluted IgG can be checked by gel electrophoresis and high performance liquid chromatography to ensure purity. The buffer solution can be exchanged into PBS, and the concentration can be determined by OD280 using 1.43 extinction coefficient. The monoclonal antibodies can be aliquoted and stored at -80° C.

Generation of Transfectomas Producing Monoclonal Antibodies of the Disclosure

Antibodies of the invention also can be produced in a host cell transfectoma using, for example, a combination of recombinant DNA techniques and gene transfection methods as is well known in the art (*e.g.*, Morrison, S. (1985) Science 229:1202).

For example, to express the antibodies, or antibody fragments thereof, DNAs encoding partial or full-length light and heavy chains, can be obtained by standard molecular biology techniques (*e.g.*, PCR amplification or cDNA cloning using a hybridoma that expresses the antibody of interest) and the DNAs can be inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vector or, more typically, both genes are inserted into the same

expression vector. The antibody genes are inserted into the expression vector by standard methods (*e.g.*, ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). The light and heavy chain variable regions of the antibodies described herein can be used to create full-length antibody genes of any antibody isotype by inserting them into expression vectors already encoding heavy chain constant and light chain constant regions of the desired isotype such that the V_H segment is operatively linked to the C_H segment(s) within the vector and the V_K segment is operatively linked to the C_L segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (*i.e.*, a signal peptide from a non-immunoglobulin protein).

In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel (Gene Expression Technology. Methods in Enzymology 185, Academic Press, San Diego, CA (1990)). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences, may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, *etc.* Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV), Simian Virus 40 (SV40), adenovirus, (*e.g.*, the adenovirus major late promoter (AdMLP) and polyoma. Alternatively, nonviral regulatory sequences may be used, such as the ubiquitin promoter or β-globin promoter. Still further, regulatory elements composed of sequences from different sources, such as the SRα promoter system, which contains sequences from the SV40 early promoter and the long terminal repeat of human T cell leukemia virus type 1 (Takebe, Y. *et al.* (1988) *Mol. Cell. Biol.* 8:466-472).

In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (*e.g.*, origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see, *e.g.*, U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel *et al.*). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr- host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is theoretically possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, and most preferably mammalian host cells, is the most preferred because such eukaryotic cells, and in particular mammalian cells, are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. Prokaryotic expression of antibody genes has been reported to be ineffective for production of high yields of active antibody (Boss, M. A. and Wood, C. R. (1985) *Immunology Today* 6:12-13).

Preferred mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in R. J. Kaufman and P. A. Sharp (1982) *Mol. Biol. 159*:601-621), NSO myeloma cells, COS cells and SP2 cells. In particular, for use with NSO myeloma cells, another preferred expression system is the GS gene expression system disclosed in WO 87/04462, WO 89/01036 and EP 338,841. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more

preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Characterization of Antibody Binding to Antigen

Antibodies of the invention can be tested for binding to PD-L1 by, for example, standard ELISA. Briefly, microtiter plates are coated with purified PD-L1 at 0.25 μg/ml in PBS, and then blocked with 5% bovine serum albumin in PBS. Dilutions of antibody (*e.g.*, dilutions of plasma from PD-L1-immunized mice) are added to each well and incubated for 1-2 hours at 37°C. The plates are washed with PBS/Tween and then incubated with secondary reagent (*e.g.*, for human antibodies, a goat-anti-human IgG Fc-specific polyclonal reagent) conjugated to alkaline phosphatase for 1 hour at 37°C. After washing, the plates are developed with pNPP substrate (1 mg/ml), and analyzed at OD of 405-650. Preferably, mice which develop the highest titers will be used for fusions.

An ELISA assay as described above can also be used to screen for hybridomas that show positive reactivity with PD-L1 immunogen. Hybridomas that bind with high avidity to PD-L1 are subcloned and further characterized. One clone from each hybridoma, which retains the reactivity of the parent cells (by ELISA), can be chosen for making a 5-10 vial cell bank stored at -140 °C, and for antibody purification.

To purify anti-PD-L1 antibodies, selected hybridomas can be grown in two-liter spinner-flasks for monoclonal antibody purification. Supernatants can be filtered and concentrated before affinity chromatography with protein A-sepharose (Pharmacia, Piscataway, NJ). Eluted IgG can be checked by gel electrophoresis and high performance liquid chromatography to ensure purity. The buffer solution can be exchanged into PBS, and the concentration can be determined by OD_{280} using 1.43 extinction coefficient. The monoclonal antibodies can be aliquoted and stored at -80 °C.

To determine if the selected anti-PD-L1 monoclonal antibodies bind to unique epitopes, each antibody can be biotinylated using commercially available reagents (Pierce, Rockford, IL). Competition studies using unlabeled monoclonal antibodies and biotinylated monoclonal antibodies can be performed using PD-L1 coated-ELISA plates as described above. Biotinylated mAb binding can be detected with a strep-avidin-alkaline phosphatase probe.

To determine the isotype of purified antibodies, isotype ELISAs can be performed using reagents specific for antibodies of a particular isotype. For example, to determine the isotype of a human monoclonal antibody, wells of microtiter plates can be coated with 1 μg/ml of anti-human immunoglobulin overnight at 4° C. After blocking with 1% BSA, the plates are reacted with 1 μg /ml or less of test monoclonal antibodies or purified isotype controls, at ambient temperature for one to two hours. The wells can then be reacted with either human IgG1 or human IgM-specific alkaline phosphatase-conjugated probes. Plates are developed and analyzed as described above.

Anti-PD-L1 human IgGs can be further tested for reactivity with PD-L1 antigen by Western blotting. Briefly, PD-L1 can be prepared and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. After electrophoresis, the separated antigens are transferred to nitrocellulose membranes, blocked with 10% fetal calf serum, and probed with the monoclonal antibodies to be tested. Human IgG binding can be detected using anti-human IgG alkaline phosphatase and developed with BCIP/NBT substrate tablets (Sigma Chem. Co., St. Louis, Mo.).

Antibody Physical Properties

The antibodies of the present invention may be further characterized by the various physical properties of the anti-PD-L1 antibodies. Various assays may be used to detect and/or differentiate different classes of antibodies based on these physical properties.

In some embodiments, antibodies of the present invention may contain one or more glycosylation sites in either the light or heavy chain variable region. The presence of one or more glycosylation sites in the variable region may result in increased immunogenicity of the antibody or an alteration of the pK of the antibody due to altered antigen binding (Marshall *et al* (1972) *Annu Rev Biochem* 41:673-702; Gala FA and Morrison SL (2004) *J Immunol* 172:5489-94; Wallick *et al* (1988) *J Exp Med* 168:1099-109; Spiro RG (2002) *Glycobiology* 12:43R-56R; Parekh *et al* (1985) *Nature* 316:452-7; Mimura *et al.* (2000) *Mol Immunol* 37:697-706). Glycosylation has been known to occur at motifs containing an N-X-S/T sequence. Variable region glycosylation may be tested using a Glycoblot assay, which cleaves the antibody to produce a Fab, and then tests for glycosylation using an assay that measures periodate oxidation and Schiff base formation. Alternatively, variable region glycosylation may be tested using Dionex light

chromatography (Dionex-LC), which cleaves saccharides from a Fab into monosaccharides and analyzes the individual saccharide content. In some instances, it is preferred to have an anti- PD-L1 antibody that does not contain variable region glycosylation. This can be achieved either by selecting antibodies that do not contain the glycosylation motif in the variable region or by mutating residues within the glycosylation motif using standard techniques well known in the art.

In a preferred embodiment, the antibodies of the present invention do not contain asparagine isomerism sites. A deamidation or isoaspartic acid effect may occur on N-G or D-G sequences, respectively. The deamidation or isoaspartic acid effect results in the creation of isoaspartic acid which decreases the stability of an antibody by creating a kinked structure off a side chain carboxy terminus rather than the main chain. The creation of isoaspartic acid can be measured using an iso-quant assay, which uses a reverse-phase HPLC to test for isoaspartic acid.

Each antibody will have a unique isoelectric point (pI), but generally antibodies will fall in the pH range of between 6 and 9.5. The pI for an IgG1 antibody typically falls within the pH range of 7-9.5 and the pI for an IgG4 antibody typically falls within the pH range of 6-8. Antibodies may have a pI that is outside this range. Although the effects are generally unknown, there is speculation that antibodies with a pI outside the normal range may have some unfolding and instability under *in vivo* conditions. The isoelectric point may be tested using a capillary isoelectric focusing assay, which creates a pH gradient and may utilize laser focusing for increased accuracy (Janini *et al* (2002) *Electrophoresis* 23:1605-11; Ma *et al.* (2001) *Chromatographia* 53:S75-89; Hunt *et al* (1998) *J Chromatogr A* 800:355-67). In some instances, it is preferred to have an anti-PD-L1 antibody that contains a pI value that falls in the normal range. This can be achieved either by selecting antibodies with a pI in the normal range, or by mutating charged surface residues using standard techniques well known in the art.

Each antibody will have a melting temperature that is indicative of thermal stability (Krishnamurthy R and Manning MC (2002) *Curr Pharm Biotechnol* 3:361-71). A higher thermal stability indicates greater overall antibody stability *in vivo*. The melting point of an antibody may be measure using techniques such as differential scanning calorimetry (Chen *et al* (2003) *Pharm Res* 20:1952-60; Ghirlando *et al* (1999) *Immunol Lett* 68:47-52). T_{M1} indicates the temperature of the initial unfolding of the antibody. T_{M2} indicates the temperature of complete unfolding of the antibody. Generally, it is

preferred that the T_{M1} of an antibody of the present invention is greater than 60°C, preferably greater than 65°C, even more preferably greater than 70°C. Alternatively, the thermal stability of an antibody may be measure using circular dichroism (Murray *et al.* (2002) *J. Chromatogr Sci* 40:343-9). The thermal stability of anti-PD-L1 antibodies disclosed herein is summarized in Table 1.

Table 1.

mAb	Tm1 (°C)	Tm2 (°C)
3G10	70	75
5F8	72	74
11E6	64	73
1B12	69	72
12A4	68	72
10A5		71
12B7		70
13G4	66	69
10H10		69

In a preferred embodiment, antibodies are selected that do not rapidly degrade. Fragmentation of an anti- PD-L1 antibody may be measured using capillary electrophoresis (CE) and MALDI-MS, as is well understood in the art (Alexander AJ and Hughes DE (1995) *Anal Chem* 67:3626-32).

In another preferred embodiment, antibodies are selected that have minimal aggregation effects. Aggregation may lead to triggering of an unwanted immune response and/or altered or unfavorable pharmacokinetic properties. Generally, antibodies are acceptable with aggregation of 25% or less, preferably 20% or less, even more preferably 15% or less, even more preferably 10% or less and even more preferably 5% or less. Aggregation may be measured by several techniques well known in the art, including size-exclusion column (SEC) high performance liquid chromatography (HPLC), and light scattering to identify monomers, dimers, trimers or multimers.

Immunoconjugates

In another aspect, the present invention features an anti-PD-L1 antibody, or a fragment thereof, conjugated to a therapeutic moiety, such as a cytotoxin, a drug (*e.g.*, an immunosuppressant) or a radiotoxin. Such conjugates are referred to herein as "immunoconjugates". Immunoconjugates that include one or more cytotoxins are referred to as "immunotoxins." A cytotoxin or cytotoxic agent includes any agent that is detrimental to (*e.g.*, kills) cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents also include, for example, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

Other preferred examples of therapeutic cytotoxins that can be conjugated to an antibody of the invention include duocarmycins, calicheamicins, maytansines and auristatins, and derivatives thereof. An example of a calicheamicin antibody conjugate is commercially available (Mylotarg™; Wyeth-Ayerst).

Cytotoxins can be conjugated to antibodies of the invention using linker technology available in the art. Examples of linker types that have been used to conjugate a cytotoxin to an antibody include, but are not limited to, hydrazones, thioethers, esters, disulfides and peptide-containing linkers. A linker can be chosen that is, for example, susceptible to cleavage by low pH within the lysosomal compartment or susceptible to cleavage by proteases, such as proteases preferentially expressed in tumor tissue such as cathepsins (*e.g.*, cathepsins B, C, D).

For further discussion of types of cytotoxins, linkers and methods for conjugating therapeutic agents to antibodies, see also Saito, G. *et al.* (2003) *Adv. Drug Deliv. Rev.* 55:199-215; Trail, P.A. *et al.* (2003) *Cancer Immunol. Immunother.* 52:328-337; Payne,

G. (2003) *Cancer Cell* 3:207-212; Allen, T.M. (2002) *Nat. Rev. Cancer* 2:750-763; Pastan, I. and Kreitman, R. J. (2002) *Curr. Opin. Investig. Drugs* 3:1089-1091; Senter, P.D. and Springer, C.J. (2001) *Adv. Drug Deliv. Rev.* 53:247-264.

Antibodies of the present invention also can be conjugated to a radioactive isotope to generate cytotoxic radiopharmaceuticals, also referred to as radioimmunoconjugates. Examples of radioactive isotopes that can be conjugated to antibodies for use diagnostically or therapeutically include, but are not limited to, iodine^{131}, indium^{111}, yttrium^{90} and lutetium^{177}. Method for preparing radioimmunconjugates are established in the art. Examples of radioimmunoconjugates are commercially available, including Zevalin™ (IDEC Pharmaceuticals) and Bexxar™ (Corixa Pharmaceuticals), and similar methods can be used to prepare radioimmunoconjugates using the antibodies of the invention.

The antibody conjugates of the invention can be used to modify a given biological response, and the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, an enzymatically active toxin, or active fragment thereof, such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor or interferon-γ; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy," in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery," in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy," in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16

(Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates," Immunol. Rev., 62:119-58 (1982).

Bispecific Molecules

In another aspect, the present invention features bispecific molecules comprising an anti-PD-L1 antibody, or a fragment thereof, of the invention. An antibody of the invention, or antigen-binding portions thereof, can be derivatized or linked to another functional molecule, *e.g.*, another peptide or protein (*e.g.*, another antibody or ligand for a receptor) to generate a bispecific molecule that binds to at least two different binding sites or target molecules. The antibody of the invention may in fact be derivatized or linked to more than one other functional molecule to generate multispecific molecules that bind to more than two different binding sites and/or target molecules; such multispecific molecules are also intended to be encompassed by the term "bispecific molecule" as used herein. To create a bispecific molecule of the invention, an antibody of the invention can be functionally linked (*e.g.*, by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other binding molecules, such as another antibody, antibody fragment, peptide or binding mimetic, such that a bispecific molecule results.

Accordingly, the present invention includes bispecific molecules comprising at least one first binding specificity for PD-L1 and a second binding specificity for a second target epitope. In a particular embodiment of the invention, the second target epitope is an Fc receptor, *e.g.*, human FcγRI (CD64) or a human Fcα receptor (CD89). Therefore, the invention includes bispecific molecules capable of binding both to FcγR or FcαR expressing effector cells (*e.g.*, monocytes, macrophages or polymorphonuclear cells (PMNs)), and to target cells expressing PD-L1. These bispecific molecules target PD-L1 expressing cells to effector cell and trigger Fc receptor-mediated effector cell activities, such as phagocytosis of an PD-L1 expressing cells, antibody dependent cell-mediated cytotoxicity (ADCC), cytokine release, or generation of superoxide anion.

In an embodiment of the invention in which the bispecific molecule is multispecific, the molecule can further include a third binding specificity, in addition to an anti-Fc binding specificity and an anti-PD-L1 binding specificity. In one embodiment, the third binding specificity is an anti-enhancement factor (EF) portion, *e.g.*, a molecule which binds to a surface protein involved in cytotoxic activity and thereby increases the

immune response against the target cell. The "anti-enhancement factor portion" can be an antibody, functional antibody fragment or a ligand that binds to a given molecule, *e.g.*, an antigen or a receptor, and thereby results in an enhancement of the effect of the binding determinants for the F_c receptor or target cell antigen. The "anti-enhancement factor portion" can bind an F_c receptor or a target cell antigen. Alternatively, the anti-enhancement factor portion can bind to an entity that is different from the entity to which the first and second binding specificities bind. For example, the anti-enhancement factor portion can bind a cytotoxic T-cell (*e.g.* via CD2, CD3, CD8, CD28, CD4, CD40, ICAM-1 or other immune cell that results in an increased immune response against the target cell).

In one embodiment, the bispecific molecules of the invention comprise as a binding specificity at least one antibody, or an antibody fragment thereof, including, *e.g.*, an Fab, Fab', $F(ab')_2$, Fv, or a single chain Fv. The antibody may also be a light chain or heavy chain dimer, or any minimal fragment thereof such as a Fv or a single chain construct as described in Ladner *et al.* U.S. Patent No. 4,946,778, the contents of which is expressly incorporated by reference.

In one embodiment, the binding specificity for an Fcγ receptor is provided by a monoclonal antibody, the binding of which is not blocked by human immunoglobulin G (IgG). As used herein, the term "IgG receptor" refers to any of the eight γ-chain genes located on chromosome 1. These genes encode a total of twelve transmembrane or soluble receptor isoforms which are grouped into three Fcγ receptor classes: FcγRI (CD64), FcγRII(CD32), and FcγRIII (CD16). In one preferred embodiment, the Fcγ receptor a human high affinity FcγRI. The human FcγRI is a 72 kDa molecule, which shows high affinity for monomeric IgG (10^8 - $10^9 M^{-1}$).

The production and characterization of certain preferred anti-Fcγ monoclonal antibodies are described by Fanger *et al.* in PCT Publication WO 88/00052 and in U.S. Patent No. 4,954,617, the teachings of which are fully incorporated by reference herein. These antibodies bind to an epitope of FcγRI, FcγRII or FcγRIII at a site which is distinct from the Fcγ binding site of the receptor and, thus, their binding is not blocked substantially by physiological levels of IgG. Specific anti-FcγRI antibodies useful in this invention are mAb 22, mAb 32, mAb 44, mAb 62 and mAb 197. The hybridoma producing mAb 32 is available from the American Type Culture Collection, ATCC

Accession No. HB9469. In other embodiments, the anti-Fcγ receptor antibody is a humanized form of monoclonal antibody 22 (H22). The production and characterization of the H22 antibody is described in Graziano, R.F. *et al.* (1995) *J. Immunol* 155 (10): 4996-5002 and PCT Publication WO 94/10332. The H22 antibody producing cell line was deposited at the American Type Culture Collection under the designation HA022CL1 and has the accession no. CRL 11177.

In still other preferred embodiments, the binding specificity for an Fc receptor is provided by an antibody that binds to a human IgA receptor, *e.g.*, an Fc-alpha receptor (FcαRI (CD89)), the binding of which is preferably not blocked by human immunoglobulin A (IgA). The term "IgA receptor" is intended to include the gene product of one α-gene (FcαRI) located on chromosome 19. This gene is known to encode several alternatively spliced transmembrane isoforms of 55 to 110 kDa. FcαRI (CD89) is constitutively expressed on monocytes/macrophages, eosinophilic and neutrophilic granulocytes, but not on non-effector cell populations. FcαRI has medium affinity ($\approx 5 \times 10^7\ M^{-1}$) for both IgA1 and IgA2, which is increased upon exposure to cytokines such as G-CSF or GM-CSF (Morton, H.C. *et al.* (1996) *Critical Reviews in Immunology* 16:423-440). Four FcαRI-specific monoclonal antibodies, identified as A3, A59, A62 and A77, which bind FcαRI outside the IgA ligand binding domain, have been described (Monteiro, R.C. *et al.* (1992) *J. Immunol.* 148:1764).

FcαRI and FcγRI are preferred trigger receptors for use in the bispecific molecules of the invention because they are (1) expressed primarily on immune effector cells, *e.g.*, monocytes, PMNs, macrophages and dendritic cells; (2) expressed at high levels (*e.g.*, 5,000-100,000 per cell); (3) mediators of cytotoxic activities (*e.g.*, ADCC, phagocytosis); (4) mediate enhanced antigen presentation of antigens, including self-antigens, targeted to them.

While human monoclonal antibodies are preferred, other antibodies which can be employed in the bispecific molecules of the invention are murine, chimeric and humanized monoclonal antibodies.

The bispecific molecules of the present invention can be prepared by conjugating the constituent binding specificities, *e.g.*, the anti-FcR and anti-PD-L1 binding specificities, using methods known in the art. For example, each binding specificity of the bispecific molecule can be generated separately and then conjugated to one another. When the binding specificities are proteins or peptides, a variety of coupling or cross-

linking agents can be used for covalent conjugation. Examples of cross-linking agents include protein A, carbodiimide, N-succinimidyl-S-acetyl-thioacetate (SATA), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), o-phenylenedimaleimide (oPDM), N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), and sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohaxane-1-carboxylate (sulfo-SMCC) (see *e.g.*, Karpovsky *et al.* (1984) *J. Exp. Med.* 160:1686; Liu, MA *et al.* (1985) *Proc. Natl. Acad. Sci. USA* 82:8648). Other methods include those described in Paulus (1985) Behring Ins. Mitt. No. 78, 118-132; Brennan *et al.* (1985) *Science* 229:81-83), and Glennie *et al.* (1987) *J. Immunol.* 139: 2367-2375). Preferred conjugating agents are SATA and sulfo-SMCC, both available from Pierce Chemical Co. (Rockford, IL).

When the binding specificities are antibodies, they can be conjugated via sulfhydryl bonding of the C-terminus hinge regions of the two heavy chains. In a particularly preferred embodiment, the hinge region is modified to contain an odd number of sulfhydryl residues, preferably one, prior to conjugation.

Alternatively, both binding specificities can be encoded in the same vector and expressed and assembled in the same host cell. This method is particularly useful where the bispecific molecule is a mAb x mAb, mAb x Fab, Fab x $F(ab')_2$ or ligand x Fab fusion protein. A bispecific molecule of the invention can be a single chain molecule comprising one single chain antibody and a binding determinant, or a single chain bispecific molecule comprising two binding determinants. Bispecific molecules may comprise at least two single chain molecules. Methods for preparing bispecific molecules are described for example in U.S. Patent Number 5,260,203; U.S. Patent Number 5,455,030; U.S. Patent Number 4,881,175; U.S. Patent Number 5,132,405; U.S. Patent Number 5,091,513; U.S. Patent Number 5,476,786; U.S. Patent Number 5,013,653; U.S. Patent Number 5,258,498; and U.S. Patent Number 5,482,858.

Binding of the bispecific molecules to their specific targets can be confirmed by, for example, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), FACS analysis, bioassay (*e.g.*, growth inhibition), or Western Blot assay. Each of these assays generally detects the presence of protein-antibody complexes of particular interest by employing a labeled reagent (*e.g.*, an antibody) specific for the complex of interest. For example, the FcR-antibody complexes can be detected using *e.g.*, an enzyme-linked antibody or antibody fragment which recognizes and specifically binds to the antibody-FcR complexes. Alternatively, the complexes can be detected using any of a variety of

other immunoassays. For example, the antibody can be radioactively labeled and used in a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by such means as the use of a γ counter or a scintillation counter or by autoradiography.

Pharmaceutical Compositions

In another aspect, the present invention provides a composition, *e.g.*, a pharmaceutical composition, containing one or a combination of monoclonal antibodies, or antigen-binding portion(s) thereof, of the present invention, formulated together with a pharmaceutically acceptable carrier. Such compositions may include one or a combination of (*e.g.*, two or more different) antibodies, or immunoconjugates or bispecific molecules of the invention. For example, a pharmaceutical composition of the invention can comprise a combination of antibodies (or immunoconjugates or bispecifics) that bind to different epitopes on the target antigen or that have complementary activities.

Pharmaceutical compositions of the invention also can be administered in combination therapy, *i.e.*, combined with other agents. For example, the combination therapy can include an anti-PD-L1 antibody of the present invention combined with at least one other anti-inflammatory or immunosuppressant agent. Examples of therapeutic agents that can be used in combination therapy are described in greater detail below in the section on uses of the antibodies of the invention.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (*e.g.*, by injection or infusion). Depending on the route of administration, the active compound, *i.e.*, antibody, immunoconjuage, or bispecific molecule, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

The pharmaceutical compounds of the invention may include one or more pharmaceutically acceptable salts. A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart

any undesired toxicological effects (see *e.g.*, Berge, S.M., *et al.* (1977) *J. Pharm. Sci.* 66:1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

A pharmaceutical composition of the invention also may include a pharmaceutically acceptable anti-oxidant. Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, *supra,* and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought

about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be

combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 0.01 per cent to about ninety-nine percent of active ingredient, preferably from about 0.1 per cent to about 70 per cent, most preferably from about 1 per cent to about 30 per cent of active ingredient in combination with a pharmaceutically acceptable carrier.

Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

For administration of the antibody, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight. For example dosages can be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg body weight, 5 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. An exemplary treatment regime entails administration once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months. Preferred dosage regimens for an anti-PD-L1 antibody of the invention include 1 mg/kg body weight or 3 mg/kg body weight via intravenous administration, with the antibody being given using one of the following dosing schedules: (i) every four weeks for six dosages, then every three months; (ii) every three weeks; (iii) 3 mg/kg body weight once followed by 1 mg/kg body weight every three weeks.

In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody

administered falls within the ranges indicated. Antibody is usually administered on multiple occasions. Intervals between single dosages can be, for example, weekly, monthly, every three months or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody to the target antigen in the patient. In some methods, dosage is adjusted to achieve a plasma antibody concentration of about 1-1000 μg /ml and in some methods about 25-300 μg /ml.

Alternatively, antibody can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, human antibodies show the longest half life, followed by humanized antibodies, chimeric antibodies, and nonhuman antibodies. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A "therapeutically effective dosage" of an anti-PD-L1 antibody of the invention preferably results in a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. For example, for the treatment of PD-L1+

tumors, a "therapeutically effective dosage" preferably inhibits cell growth or tumor growth by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to inhibit tumor growth can be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit, such inhibition *in vitro* by assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound can decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

A composition of the present invention can be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Preferred routes of administration for antibodies of the invention include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

Alternatively, an antibody of the invention can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically.

The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the

art. *See, e.g., Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

Therapeutic compositions can be administered with medical devices known in the art. For example, in a preferred embodiment, a therapeutic composition of the invention can be administered with a needleless hypodermic injection device, such as the devices disclosed in U.S. Patent Nos. 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824; or 4,596,556. Examples of well-known implants and modules useful in the present invention include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,486,194, which discloses a therapeutic device for administering medicants through the skin; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Patent No. 4,475,196, which discloses an osmotic drug delivery system. These patents are incorporated herein by reference. Many other such implants, delivery systems, and modules are known to those skilled in the art.

In certain embodiments, the human monoclonal antibodies of the invention can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the invention cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, *e.g.*, U.S. Patents 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (*see, e.g.*, V.V. Ranade (1989) *J. Clin. Pharmacol.* 29:685). Exemplary targeting moieties include folate or biotin (see, *e.g.*, U.S. Patent 5,416,016 to Low *et al.*); mannosides (Umezawa *et al.*, (1988) *Biochem. Biophys. Res. Commun.* 153:1038); antibodies (P.G. Bloeman *et al.* (1995) *FEBS Lett.* 357:140; M. Owais *et al.* (1995) *Antimicrob. Agents Chemother.* 39:180); surfactant protein A receptor (Briscoe *et al.* (1995) *Am. J. Physiol.* 1233:134); p120 (Schreier *et al.* (1994) *J. Biol. Chem.* 269:9090); see also K. Keinanen; M.L. Laukkanen (1994) *FEBS Lett.* 346:123; J.J. Killion; I.J. Fidler (1994) *Immunomethods* 4:273.

Uses and Methods of the Invention

The antibodies, antibody compositions and methods of the present invention have numerous *in vitro* and *in vivo* utilities involving, for example, detection of PD-L1 or enhancement of immune response by blockade of PD-L1. In a preferred embodiment, the antibodies of the present invention are human antibodies. For example, these molecules can be administered to cells in culture, *in vitro* or *ex vivo*, or to human subjects, *e.g.*, *in vivo*, to enhance immunity in a variety of situations. Accordingly, in one aspect, the invention provides a method of modifying an immune response in a subject comprising administering to the subject the antibody, or antigen-binding portion thereof, of the invention such that the immune response in the subject is modified. Preferably, the response is enhanced, stimulated or up-regulated.

As used herein, the term "subject" is intended to include human and non-human animals. Non-human animals includes all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, sheep, dogs, cats, cows, horses, chickens, amphibians, and reptiles, although mammals are preferred, such as non-human primates, sheep, dogs, cats, cows and horses. Preferred subjects include human patients in need of enhancement of an immune response. The methods are particularly suitable for treating human patients having a disorder that can be treated by augmenting the T-cell mediated immune response. In a particular embodiment, the methods are particularly suitable for treatment of cancer cells *in vivo*. To achieve antigen-specific enhancement of immunity, the anti-PD-L1 antibodies can be administered together with an antigen of interest. When antibodies to PD-L1 are administered together with another agent, the two can be administered in either order or simultaneously.

The invention further provides methods for detecting the presence of human PD-L1 antigen in a sample, or measuring the amount of human PD-L1 antigen, comprising contacting the sample, and a control sample, with a human monoclonal antibody, or an antigen binding portion thereof, which specifically binds to human PD-L1, under conditions that allow for formation of a complex between the antibody or portion thereof and human PD-L1. The formation of a complex is then detected, wherein a difference complex formation between the sample compared to the control sample is indicative the presence of human PD-L1 antigen in the sample.

Cancer

Blockade of PD-L1 by antibodies can enhance the immune response to cancerous cells in the patient. PD-L1 is not expressed in normal human cells, but is abundant in a variety of human cancers (Dong *et al.* (2002) *Nat Med* 8:787-9). The interaction between PD-1 and PD-L1 results in a decrease in tumor infiltrating lymphocytes, a decrease in T-cell receptor mediated proliferation, and immune evasion by the cancerous cells (Dong *et al.* (2003) *J Mol Med* 81:281-7; Blank *et al.* (2004) *Cancer Immunol. Immunother.* [epub]; Konishi *et al.* (2004) *Clin. Cancer Res.* 10:5094-100). Immune suppression can be reversed by inhibiting the local interaction of PD-L1 to PD-1 and the effect is additive when the interaction of PD-L2 to PD-1 is blocked as well (Iwai *et al.* (2002) *PNAS* 99:12293-7; Brown *et al.* (2003) *J. Immunol.* 170:1257-66). An anti-PD-L1 antibody may be used alone to inhibit the growth of cancerous tumors. Alternatively, an anti-PD-L1 antibody may be used in conjunction with other immunogenic agents, standard cancer treatments, or other antibodies, as described below.

Accordingly, in one embodiment, the invention provides a method of inhibiting growth of tumor cells in a subject, comprising administering to the subject a therapeutically effective amount of an anti-PD-L1 antibody, or antigen-binding portion thereof. Preferably, the antibody is a human anti-PD-L1 antibody (such as any of the human anti-human PD-L1 antibodies described herein). Additionally or alternatively, the antibody may be a chimeric or humanized anti-PD-L1 antibody.

Preferred cancers whose growth may be inhibited using the antibodies of the invention include cancers typically responsive to immunotherapy. Non-limiting examples of preferred cancers for treatment include melanoma (e.g., metastatic malignant melanoma), renal cancer, prostate cancer, breast cancer, colon cancer and lung cancer. Examples of other cancers that may be treated using the methods of the invention include bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including

acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers. The present invention is also useful for treatment of metastatic cancers, especially metastatic cancers that express PD-L1 (Iwai *et al.* (2005) *Int. Immunol.* 17:133-144).

Optionally, antibodies to PD-L1 can be combined with an immunogenic agent, such as cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), cells, and cells transfected with genes encoding immune stimulating cytokines (He et al (2004) *J. Immunol.* 173:4919-28). Non-limiting examples of tumor vaccines that can be used include peptides of melanoma antigens, such as peptides of gp100, MAGE antigens, Trp-2, MART1 and/or tyrosinase, or tumor cells transfected to express the cytokine GM-CSF (discussed further below).

In humans, some tumors have been shown to be immunogenic such as melanomas. It is anticipated that by raising the threshold of T cell activation by PD-L1 blockade, we may expect to activate tumor responses in the host.

PD-L1 blockade is likely to be most effective when combined with a vaccination protocol. Many experimental strategies for vaccination against tumors have been devised (see Rosenberg, S., 2000, Development of Cancer Vaccines, ASCO Educational Book Spring: 60-62; Logothetis, C., 2000, ASCO Educational Book Spring: 300-302; Khayat, D. 2000, ASCO Educational Book Spring: 414-428; Foon, K. 2000, ASCO Educational Book Spring: 730-738; see also Restifo, N. and Sznol, M., Cancer Vaccines, Ch. 61, pp. 3023-3043 in DeVita, V. *et al.* (eds.), 1997, Cancer: Principles and Practice of Oncology. Fifth Edition). In one of these strategies, a vaccine is prepared using autologous or allogeneic tumor cells. These cellular vaccines have been shown to be most effective when the tumor cells are transduced to express GM-CSF. GM-CSF has been shown to be a potent activator of antigen presentation for tumor vaccination (Dranoff *et al.* (1993) *Proc. Natl. Acad. Sci U.S.A.* 90: 3539-43).

The study of gene expression and large scale gene expression patterns in various tumors has led to the definition of so-called tumor specific antigens (Rosenberg, SA

(1999) Immunity 10: 281-7). In many cases, these tumor specific antigens are differentiation antigens expressed in the tumors and in the cell from which the tumor arose, for example melanocyte antigens gp100, MAGE antigens, and Trp-2. More importantly, many of these antigens can be shown to be the targets of tumor specific T cells found in the host. PD-L1 blockade may be used in conjunction with a collection of recombinant proteins and/or peptides expressed in a tumor in order to generate an immune response to these proteins. These proteins are normally viewed by the immune system as self antigens and are therefore tolerant to them. The tumor antigen may also include the protein telomerase, which is required for the synthesis of telomeres of chromosomes and which is expressed in more than 85% of human cancers and in only a limited number of somatic tissues (Kim, N *et al.* (1994) *Science* 266: 2011-2013). (These somatic tissues may be protected from immune attack by various means). Tumor antigen may also be "neo-antigens" expressed in cancer cells because of somatic mutations that alter protein sequence or create fusion proteins between two unrelated sequences (i.e. bcr-abl in the Philadelphia chromosome), or idiotype from B cell tumors.

Other tumor vaccines may include the proteins from viruses implicated in human cancers such a Human Papilloma Viruses (HPV), Hepatitis Viruses (HBV and HCV) and Kaposi's Herpes Sarcoma Virus (KHSV). Another form of tumor specific antigen which may be used in conjunction with PD-L1 blockade is purified heat shock proteins (HSP) isolated from the tumor tissue itself. These heat shock proteins contain fragments of proteins from the tumor cells and these HSPs are highly efficient at delivery to antigen presenting cells for eliciting tumor immunity (Suot, R & Srivastava, P (1995) *Science* 269:1585-1588; Tamura, Y. *et al.* (1997) *Science* 278:117-120).

Dendritic cells (DC) are potent antigen presenting cells that can be used to prime antigen-specific responses. DC's can be produced *ex vivo* and loaded with various protein and peptide antigens as well as tumor cell extracts (Nestle, F. *et al.* (1998) *Nature Medicine* 4: 328-332). DCs may also be transduced by genetic means to express these tumor antigens as well. DCs have also been fused directly to tumor cells for the purposes of immunization (Kugler, A. *et al.* (2000) *Nature Medicine* 6:332-336). As a method of vaccination, DC immunization may be effectively combined with PD-L1 blockade to activate more potent anti-tumor responses.

PD-L1 blockade may also be combined with standard cancer treatments. PD-L1 blockade may be effectively combined with chemotherapeutic regimes. In these

instances, it may be possible to reduce the dose of chemotherapeutic reagent administered (Mokyr, M. *et al.* (1998) *Cancer Research* 58: 5301-5304). An example of such a combination is an anti-PD-L1 antibody in combination with decarbazine for the treatment of melanoma. Another example of such a combination is an anti-PD-L1 antibody in combination with interleukin-2 (IL-2) for the treatment of melanoma. The scientific rationale behind the combined use of PD-L1 blockade and chemotherapy is that cell death, that is a consequence of the cytotoxic action of most chemotherapeutic compounds, should result in increased levels of tumor antigen in the antigen presentation pathway. Other combination therapies that may result in synergy with PD-L1 blockade through cell death are radiation, surgery, and hormone deprivation. Each of these protocols creates a source of tumor antigen in the host. Angiogenesis inhibitors may also be combined with PD-L1 blockade. Inhibition of angiogenesis leads to tumor cell death which may feed tumor antigen into host antigen presentation pathways.

PD-L1 blocking antibodies can also be used in combination with bispecific antibodies that target Fc alpha or Fc γ receptor-expressing effectors cells to tumor cells (see, *e.g.*, U.S. Pat. Nos. 5,922,845 and 5,837,243). Bispecific antibodies can be used to target two separate antigens. For example anti-Fc receptor/anti tumor antigen (*e.g.*, Her-2/neu) bispecific antibodies have been used to target macrophages to sites of tumor. This targeting may more effectively activate tumor specific responses. The T cell arm of these responses would by augmented by the use of PD-L1 blockade. Alternatively, antigen may be delivered directly to DCs by the use of bispecific antibodies which bind to tumor antigen and a dendritic cell specific cell surface marker.

Tumors evade host immune surveillance by a large variety of mechanisms. Many of these mechanisms may be overcome by the inactivation of proteins which are expressed by the tumors and which are immunosuppressive. These include among others TGF-beta (Kehrl, J. *et al.* (1986) *J. Exp. Med.* 163: 1037-1050), IL-10 (Howard, M. & O'Garra, A. (1992) *Immunology Today* 13: 198-200), and Fas ligand (Hahne, M. *et al.* (1996) *Science* 274: 1363-1365). Antibodies to each of these entities may be used in combination with anti-PD-L1 to counteract the effects of the immunosuppressive agent and favor tumor immune responses by the host.

Other antibodies which may be used to activate host immune responsiveness can be used in combination with anti-PD-L1. These include molecules on the surface of dendritic cells which activate DC function and antigen presentation. Anti-CD40

antibodies are able to substitute effectively for T cell helper activity (Ridge, J. *et al.* (1998) Nature 393: 474-478) and can be used in conjunction with PD-L1 antibodies (Ito, N. *et al.* (2000) *Immunobiology* 201 (5) 527-40). Activating antibodies to T cell costimulatory molecules such as OX-40 (Weinberg, A. *et al.* (2000) *Immunol* 164: 2160-2169), 4-1BB (Melero, I. *et al.* (1997) *Nature Medicine* 3: 682-685 (1997), and ICOS (Hutloff, A. *et al.* (1999) *Nature* 397: 262-266) as well as antibodies which block the activity of negative costimulatory molecules such as CTLA-4 (e.g., US Patent No. 5,811,097) or BTLA (Watanabe, N. *et al.* (2003) *Nat Immunol* 4:670-9), B7-H4 (Sica, GL *et al.* (2003) *Immunity* 18:849-61) may also provide for increased levels of T cell activation.

Bone marrow transplantation is currently being used to treat a variety of tumors of hematopoietic origin. While graft versus host disease is a consequence of this treatment, therapeutic benefit may be obtained from graft vs. tumor responses. PD-L1 blockade can be used to increase the effectiveness of the donor engrafted tumor specific T cells.

There are also several experimental treatment protocols that involve *ex vivo* activation and expansion of antigen specific T cells and adoptive transfer of these cells into recipients in order to antigen-specific T cells against tumor (Greenberg, R. & Riddell, S. (1999) *Science* 285: 546-51). These methods may also be used to activate T cell responses to infectious agents such as CMV. *Ex vivo* activation in the presence of anti-PD-L1 antibodies may be expected to increase the frequency and activity of the adoptively transferred T cells.

Infectious Diseases

Other methods of the invention are used to treat patients that have been exposed to particular toxins or pathogens. Accordingly, another aspect of the invention provides a method of treating an infectious disease in a subject comprising administering to the subject an anti-PD-L1 antibody, or antigen-binding portion thereof, such that the subject is treated for the infectious disease. Preferably, the antibody is a human anti-human PD-L1 antibody (such as any of the human anti-PD-L1 antibodies described herein). Additionally or alternatively, the antibody can be a chimeric or humanized antibody.

Similar to its application to tumors as discussed above, antibody mediated PD-L1 blockade can be used alone, or as an adjuvant, in combination with vaccines, to stimulate the immune response to pathogens, toxins, and self-antigens. Examples of pathogens for which this therapeutic approach may be particularly useful, include pathogens for which

there is currently no effective vaccine, or pathogens for which conventional vaccines are less than completely effective. These include, but are not limited to HIV, Hepatitis (A, B, & C), Influenza, Herpes, Giardia, Malaria, Leishmania, Staphylococcus aureus, Pseudomonas Aeruginosa. PD-L1 blockade is particularly useful against established infections by agents such as HIV that present altered antigens over the course of the infections. These novel epitopes are recognized as foreign at the time of anti-human PD-L1 administration, thus provoking a strong T cell response that is not dampened by negative signals through PD-L1.

Some examples of pathogenic viruses causing infections treatable by methods of the invention include HIV, hepatitis (A, B, or C), herpes virus (*e.g.*, VZV, HSV-1, HAV-6, HSV-II, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus.

Some examples of pathogenic bacteria causing infections treatable by methods of the invention include chlamydia, rickettsial bacteria, mycobacteria, staphylococci, streptococci, pneumonococci, meningococci and conococci, klebsiella, proteus, serratia, pseudomonas, legionella, diphtheria, salmonella, bacilli, cholera, tetanus, botulism, anthrax, plague, leptospirosis, and Lyme's disease bacteria.

Some examples of pathogenic fungi causing infections treatable by methods of the invention include Candida (albicans, krusei, glabrata, tropicalis, etc.), Cryptococcus neoformans, Aspergillus (fumigatus, niger, etc.), Genus Mucorales (mucor, absidia, rhizophus), Sporothrix schenkii, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Coccidioides immitis and Histoplasma capsulatum.

Some examples of pathogenic parasites causing infections treatable by methods of the invention include Entamoeba histolytica, Balantidium coli, Naegleriafowleri, Acanthamoeba sp., Giardia lambia, Cryptosporidium sp., Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondi, Nippostrongylus brasiliensis.

In all of the above methods, PD-L1 blockade can be combined with other forms of immunotherapy such as cytokine treatment (*e.g.*, interferons, GM-CSF, G-CSF, IL-2), or bispecific antibody therapy, which provides for enhanced presentation of tumor antigens

(see, *e.g.*, Holliger (1993) *Proc. Natl. Acad. Sci.* USA 90:6444-6448; Poljak (1994) *Structure* 2:1121-1123).

Autoimmune reactions

Anti-PD-L1 antibodies may provoke and amplify autoimmune responses. Indeed, induction of anti-tumor responses using tumor cell and peptide vaccines reveals that many anti-tumor responses involve anti-self reactivities (depigmentation observed in anti-CTLA-4 + GM-CSF-modified B16 melanoma in van Elsas *et al.* supra; depigmentation in Trp-2 vaccinated mice (Overwijk, W. *et al.* (1999) *Proc. Natl. Acad. Sci. U.S.A.* 96: 2982-2987); autoimmune prostatitis evoked by TRAMP tumor cell vaccines (Hurwitz, A. (2000) *supra*), melanoma peptide antigen vaccination and vitilago observed in human clinical trials (Rosenberg, SA and White, DE (1996) *J. Immunother Emphasis Tumor Immunol* 19 (1): 81-4).

Therefore, it is possible to consider using anti-PD-L1 blockade in conjunction with various self proteins in order to devise vaccination protocols to efficiently generate immune responses against these self proteins for disease treatment. For example, Alzheimer's disease involves inappropriate accumulation of Aβ peptide in amyloid deposits in the brain; antibody responses against amyloid are able to clear these amyloid deposits (Schenk *et al.*, (1999) *Nature* 400: 173-177).

Other self proteins may also be used as targets such as IgE for the treatment of allergy and asthma, and TNFα for rheumatoid arthritis. Finally, antibody responses to various hormones may be induced by the use of anti-PD-L1 antibody. Neutralizing antibody responses to reproductive hormones may be used for contraception. Neutralizing antibody response to hormones and other soluble factors that are required for the growth of particular tumors may also be considered as possible vaccination targets.

Analogous methods as described above for the use of anti-PD-L1 antibody can be used for induction of therapeutic autoimmune responses to treat patients having an inappropriate accumulation of other self-antigens, such as amyloid deposits, including Aβ in Alzheimer's disease, cytokines such as TNFα, and IgE.

Vaccines

Anti-PD-L1 antibodies may be used to stimulate antigen-specific immune responses by coadministration of an anti-PD-L1 antibody with an antigen of interest (*e.g.*, a vaccine). Accordingly, in another aspect the invention provides a method of enhancing

an immune response to an antigen in a subject, comprising administering to the subject: (i) the antigen; and (ii) an anti-PD-L1 antibody, or antigen-binding portion thereof, such that an immune response to the antigen in the subject is enhanced. Preferably, the antibody is a human anti-human PD-L1 antibody (such as any of the human anti-PD-L1 antibodies described herein). Additionally or alternatively, the antibody can be a chimeric or humanized antibody. The antigen can be, for example, a tumor antigen, a viral antigen, a bacterial antigen or an antigen from a pathogen. Non-limiting examples of such antigens include those discussed in the sections above, such as the tumor antigens (or tumor vaccines) discussed above, or antigens from the viruses, bacteria or other pathogens described above.

Anti-PD-L1 antibodies may also be used to abrogate secondary effects associated with diseases such as T cell suppressed wasting disease with colitis (Kanai *et al.* (2003) *J. Immunol.* 171:4156-63). Accordingly, in another aspect the invention provides a method of abrogating leukocyte infiltration, decreasing production of IFN-γ, IL-2, and IFN-α by T cells. Preferably, the antibody is a human anti-human PD-L1 antibody (such as any of the human anti-PD-L1 antibodies described herein). Additionally or alternatively, the antibody can be a chimeric or humanized antibody.

Anti-PD-L1 antibodies may also be used to treat diseases such as chronic inflammatory diseases, such as lichen planus, a T-cell mediated chronic inflammatory mucocutaneous disease (Youngnak-Piboonratanakit *et al.* (2004) *Immunol Letters* 94:215-22). Accordingly, in another aspect the invention provides a method of abrogating chronic inflammatory disease by T cells. Preferably, the antibody is a human anti-human PD-L1 antibody (such as any of the human anti-PD-L1 antibodies described herein). Additionally or alternatively, the antibody can be a chimeric or humanized antibody.

Suitable routes of administering the antibody compositions (*e.g.*, human monoclonal antibodies, multispecific and bispecific molecules and immunoconjugates) of the invention *in vivo* and *in vitro* are well known in the art and can be selected by those of ordinary skill. For example, the antibody compositions can be administered by injection (*e.g.*, intravenous or subcutaneous). Suitable dosages of the molecules used will depend on the age and weight of the subject and the concentration and/or formulation of the antibody composition.

As previously described, human anti-PD-L1 antibodies of the invention can be co-administered with one or other more therapeutic agents, *e.g.*, a cytotoxic agent, a radiotoxic agent or an immunosuppressive agent. The antibody can be linked to the agent (as an immunocomplex) or can be administered separate from the agent. In the latter case (separate administration), the antibody can be administered before, after or concurrently with the agent or can be co-administered with other known therapies, *e.g.*, an anti-cancer therapy, *e.g.*, radiation. Such therapeutic agents include, among others, anti-neoplastic agents such as doxorubicin (adriamycin), cisplatin bleomycin sulfate, carmustine, chlorambucil, and cyclophosphamide hydroxyurea which, by themselves, are only effective at levels which are toxic or subtoxic to a patient. Cisplatin is intravenously administered as a 100 mg/ dose once every four weeks and adriamycin is intravenously administered as a 60-75 mg/ml dose once every 21 days. Co-administration of the human anti-PD-L1 antibodies, or antigen binding fragments thereof, of the present invention with chemotherapeutic agents provides two anti-cancer agents which operate via different mechanisms which yield a cytotoxic effect to human tumor cells. Such co-administration can solve problems due to development of resistance to drugs or a change in the antigenicity of the tumor cells which would render them unreactive with the antibody.

Also within the scope of the present invention are kits comprising the antibody compositions of the invention (*e.g.*, human antibodies, bispecific or multispecific molecules, or immunoconjugates) and instructions for use. The kit can further contain a least one additional reagent, or one or more additional human antibodies of the invention (e.g., a human antibody having a complementary activity which binds to an epitope in PD-L1 antigen distinct from the first human antibody). Kits typically include a label indicating the intended use of the contents of the kit. The term label includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit.

The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of all figures and all references, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

Examples

Example 1: Generation of Human Monoclonal Antibodies Against PD-L1

Antigen

Immunization protocols utilized as antigen both (i) a recombinant fusion protein comprising the extracellular portion of PD-L1, and (ii) membrane bound full-length PD-L1. Both antigens were generated by recombinant transfection methods in a CHO cell line.

Transgenic mice (KM-Mouse® colony)

Fully human monoclonal antibodies to PD-L1 were prepared using the KM strain of transgenic transchromosomic mice, which expresses human antibody genes. In this mouse strain, the endogenous mouse kappa light chain gene has been homozygously disrupted as described in Chen *et al.* (1993) *EMBO J.* 12:811-820 and the endogenous mouse heavy chain gene has been homozygously disrupted as described in Example 1 of PCT Publication WO 01/09187. Furthermore, this mouse strain carries a human kappa light chain transgene, KCo5, as described in Fishwild *et al.* (1996) *Nature Biotechnology* 14:845-851, and a SC20 transchromosome as described in PCT Publication WO 02/43478.

KM-Mouse® Immunizations

To generate fully human monoclonal antibodies to PD-L1, a cohort of mice of the KM-Mouse® strain were immunized with purified recombinant PD-L1-Ig and PD-L1-transfected CHO cells as antigen. General immunization schemes for HuMab mice are described in Lonberg, N. *et al* (1994*) Nature* 368(6474): 856-859; Fishwild, D. *et al.* (1996) *Nature Biotechnology* 14: 845-851 and PCT Publication WO 98/24884. The mice were 6-16 weeks of age upon the first infusion of antigen. A purified recombinant preparation (5-50 μg) of PD-L1-Ig antigen and 5 -10x10^6 cells were used to immunize the HuMab mice intraperitonealy (IP), subcutaneously (Sc) or via footpad injection.

Transgenic mice were immunized twice with antigen in complete Freund's adjuvant or Ribi adjuvant IP, followed by 3-21 days IP (up to a total of 11 immunizations) with the antigen in incomplete Freund's or Ribi adjuvant. The immune response was monitored by retroorbital bleeds. The plasma was screened by ELISA (as described below), and mice with sufficient titers of anti-PD-L1 human immunogolobulin

were used for fusions. Mice were boosted intravenously with antigen 3 days before sacrifice and removal of the spleen. Typically, 10-35 fusions for each antigen were performed. Several dozen mice were immunized for each antigen.

Selection of KM-Mouse® Producing Anti-PD-L1 Antibodies:

To select HuMab mice producing antibodies that bound PD-L1, sera from immunized mice were tested by ELISA as described by Fishwild, D. *et al.* (1996). Briefly, microtiter plates were coated with purified recombinant PD-L1 fusion protein from transfected CHO cells at 1-2 μg /ml in PBS, 100 μl/wells incubated 4 °C overnight then blocked with 200 μl/well of 5% fetal bovine serum in PBS/Tween (0.05%). Dilutions of sera from PD-L1-immunized mice were added to each well and incubated for 1-2 hours at ambient temperature. The plates were washed with PBS/Tween and then incubated with a goat-anti-human IgG polyclonal antibody conjugated with horseradish peroxidase (HRP) for 1 hour at room temperature. After washing, the plates were developed with ABTS substrate (Sigma, A-1888, 0.22 mg/ml) and analyzed by spectrophotometer at OD 415-495. Mice that developed the highest titers of anti-PD-L1 antibodies were used for fusions. Fusions were performed as described below and hybridoma supernatants were tested for anti-PD-L1 activity by ELISA.

Generation of Hybridomas Producing Human Monoclonal Antibodies to PD-L1:

The mouse splenocytes, isolated from a KM mouse, were fused with PEG to a mouse myeloma cell line based upon standard protocols. The resulting hybridomas were then screened for the production of antigen-specific antibodies. Single cell suspensions of splenocytes from immunized mice were fused to one-fourth the number of SP2/0 nonsecreting mouse myeloma cells (ATCC, CRL 1581) with 50% PEG (Sigma). Cells were plated at approximately $1x10^5$/well in flat bottom microtiter plate, followed by about two week incubation in selective medium containing 10% fetal bovine serum, 10% P388D1 (ATCC, CRL TIB-63) conditioned medium, 3-5% origen (IGEN) in DMEM (Mediatech, CRL 10013, with high glucose, L-glutamine and sodium pyruvate) plus 5 mM HEPES, 0.055 mM 2-mercaptoethanol, 50 mg/ml gentamycin and 1x HAT (Sigma, CRL P-7185). After 1-2 weeks, cells were cultured in medium in which the HAT was replaced with HT. Individual wells were then screened by ELISA (described above) for human anti-PD-L1 monoclonal IgG antibodies. Once extensive hybridoma growth occurred, medium was monitored usually after 10-14 days. The antibody-secreting hybridomas were re-plated, screened again and, if still positive for human IgG, anti-PD-

L1 monoclonal antibodies were subcloned at least twice by limiting dilution. The stable subclones were then cultured *in vitro* to generate small amounts of antibody in tissue culture medium for further characterization.

Hybridoma clones 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4 were selected for further analysis.

Example 2: Structural Characterization of Human Monoclonal Antibodies 3G10, 12A4, and 10A5

The cDNA sequences encoding the heavy and light chain variable regions of the 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4 monoclonal antibodies were obtained from the 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4 hybridomas, respectively, using standard PCR techniques and were sequenced using standard DNA sequencing techniques.

The nucleotide and amino acid sequences of the heavy chain variable region of 3G10 are shown in Figure 1A and in SEQ ID NO:81 and 1, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 3G10 are shown in Figure 1B and in SEQ ID NO:91 and 11, respectively.

Comparison of the 3G10 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 3G10 heavy chain utilizes a VH segment from human germline VH 1-18, an undetermined D segment, and a JH segment from human germline JH 6b. The alignment of the 3G10 VH sequence to the germline VH 1-18 sequence is shown in Figure 11. Further analysis of the 3G10 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 1A and 11, and in SEQ ID NOs:21, 31 and 41, respectively.

Comparison of the 3G10 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 3G10 light chain utilizes a VL segment from human germline VK L6 and a JK segment from human germline JK 1. The alignment of the 3G10 VL sequence to the germline VK L6 sequence is shown in Figure 21. Further analysis of the 3G10 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 1B and 21, and in SEQ ID NOs:51, 61 and 71, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 12A4 are shown in Figure 2A and in SEQ ID NO:82 and 2, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 12A4 are shown in Figure 2B and in SEQ ID NO:92 and 12, respectively.

Comparison of the 12A4 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 12A4 heavy chain utilizes a VH segment from human germline VH 1-69, a D segment from human germline 3-10, and a JH segment from human germline JH 6b. The alignment of the 12A4 VH sequence to the germline VH 1-69 sequence is shown in Figure 12. Further analysis of the 12A4 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 2A and 12, and in SEQ ID NOs:22, 32 and 42, respectively.

Comparison of the 12A4 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 12A4 light chain utilizes a VL segment from human germline VK L6 and a JK segment from human germline JK 1. The alignment of the 12A4 VL sequence to the germline VK L6 sequence is shown in Figure 22. Further analysis of the 12A4 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 2B and 22, and in SEQ ID NOs:52, 62 and 72, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 10A5 are shown in Figure 3A and in SEQ ID NO:83 and 3, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 10A5 are shown in Figure 3B and in SEQ ID NO:93 and 13, respectively.

Comparison of the 10A5 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 10A5 heavy chain utilizes a VH segment from human germline VH 1-3, a D segment from human germline 5-5, and a JH segment from human germline JH 4b. The alignment of the 10A5 VH sequence to the germline VH 1-3 sequence is shown in Figure 13. Further analysis of the 10A5 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 3A and 13, and in SEQ ID NOs:23, 33, and 43, respectively.

Comparison of the 10A5 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 10A5 light chain utilizes a VL segment from human germline VK L15 and a JK segment from human germline JK 2. The alignment of the 10A5 VL sequence to the germline VK L15 sequence is shown in Figure 23. Further analysis of the 10A5 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 3B and 23, and in SEQ ID NOs:53, 63, and 73, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 5F8 are shown in Figure 4A and in SEQ ID NO:84 and 4, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 5F8 are shown in Figure 4B and in SEQ ID NO:94 and 14, respectively.

Comparison of the 5F8 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 5F8 heavy chain utilizes a VH segment from human germline VH 1-69, a D segment from human germline 6-13, and a JH segment from human germline JH 4b. The alignment of the 5F8 VH sequence to the germline VH 1-69 sequence is shown in Figure 14. Further analysis of the 5F8 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 4A and 14, and in SEQ ID NOs:24, 34, and 44, respectively.

Comparison of the 5F8 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 5F8 light chain utilizes a VL segment from human germline VK A27 and a JK segment from human germline JK 1. The alignment of the 5F8 VL sequence to the germline VK A27 sequence is shown in Figure 24. Further analysis of the 5F8 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 4B and 24, and in SEQ ID NOs:54, 64, and 74, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 10H10 are shown in Figure 5A and in SEQ ID NO:85 and 5, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 10H10 are shown in Figure 5B and in SEQ ID NO:95 and 15, respectively.

Comparison of the 10H10 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 10H10 heavy chain utilizes a VH segment from human germline VH 3-9, a D segment from human germline 4-17, and a JH segment from human germline JH 4b. The alignment of the 10H10 VH sequence to the germline VH 3-9 sequence is shown in Figure 15. Further analysis of the 10H10 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 5A and 15, and in SEQ ID NOs:25, 35, and 45, respectively.

Comparison of the 10H10 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 10H10 light chain utilizes a VL segment from human germline VK L15 and a JK segment from human germline JK 2. The alignment of the 10H10 VL sequence to the germline VK L15 sequence is shown in Figure 25. Further analysis of the 10H10 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 5B and 25, and in SEQ ID NOs:55, 65, and 75, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 1B12 are shown in Figure 6A and in SEQ ID NO:86 and 6, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 1B12 are shown in Figure 6B and in SEQ ID NO:96 and 16, respectively.

Comparison of the 1B12 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 1B12 heavy chain utilizes a VH segment from human germline VH 1-69, a D segment from human germline 3-10, and a JH segment from human germline JH 6b. The alignment of the 1B12 VH sequence to the germline VH 1-69 sequence is shown in Figure 16. Further analysis of the 1B12 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 6A and 16, and in SEQ ID NOs:26, 36, and 46, respectively.

Comparison of the 1B12 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 1B12 light chain utilizes a VL segment from human germline VK L6 and a JK segment from human germline JK 1. The alignment of the 1B12 VL sequence to the germline VK L6 sequence is shown in Figure 26. Further analysis of the 1B12 VL sequence using the Kabat system

of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 6B and 26, and in SEQ ID NOs:56, 66, and 76, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 7H1 are shown in Figure 7A and in SEQ ID NO:87 and 7, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 7H1 are shown in Figure 7B and in SEQ ID NO:97 and 17, respectively.

Comparison of the 7H1 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 7H1 heavy chain utilizes a VH segment from human germline VH 1-69, a D segment from human germline 3-10, and a JH segment from human germline JH 6b. The alignment of the 7H1 VH sequence to the germline VH 1-69 sequence is shown in Figure 17. Further analysis of the 7H1 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 7A and 17, and in SEQ ID NOs:27, 37, and 47, respectively.

Comparison of the 7H1 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 7H1 light chain utilizes a VL segment from human germline VK L6 and a JK segment from human germline JK 1. The alignment of the 7H1 VL sequence to the germline VK L6 sequence is shown in Figure 27. Further analysis of the 7H1 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 7B and 27, and in SEQ ID NOs:57, 67, and 77, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 11E6 are shown in Figure 4A and in SEQ ID NO:84 and 4, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 11E6 are shown in Figure 4B and in SEQ ID NO:94 and 14, respectively.

Comparison of the 11E6 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 11E6 heavy chain utilizes a VH segment from human germline VH 1-69, a D segment from human germline 6-19, and a JH segment from human germline JH 6c. The alignment of the 11E6 VH sequence to the germline VH 1-69 sequence is shown in Figure 18. Further analysis of the 11E6 VH sequence using the Kabat system of CDR region determination

led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 8A and 18, and in SEQ ID NOs:28, 38, and 48, respectively.

Comparison of the 11E6 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 11E6 light chain utilizes a VL segment from human germline VK A27 and a JK segment from human germline JK 4. The alignment of the 11E6 VL sequence to the germline VK A27 sequence is shown in Figure 27. Further analysis of the 11E6 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 8B and 28, and in SEQ ID NOs:58, 68, and 78, respectively. In addition, a second related clone included the VK sequence as shown in SEQ ID NO:109. This antibody is denoted herein as 11E6a.

The nucleotide and amino acid sequences of the heavy chain variable region of 12B7 are shown in Figure 9A and in SEQ ID NO:89 and 9, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 12B7 are shown in Figure 9B and in SEQ ID NO:99 and 19, respectively.

Comparison of the 12B7 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 12B7 heavy chain utilizes a VH segment from human germline VH 1-69, a D segment from human germline 3-10, and a JH segment from human germline JH 6b. The alignment of the 12B7 VH sequence to the germline VH 1-69 sequence is shown in Figure 19. Further analysis of the 12B7 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 9A and 19, and in SEQ ID NOs:29, 39, and 49, respectively.

Comparison of the 12B7 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 12B7 light chain utilizes a VL segment from human germline VK L6 and a JK segment from human germline JK 5. The alignment of the 12B7 VL sequence to the germline VK L6 sequence is shown in Figure 29. Further analysis of the 12B7 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 9B and 29, and in SEQ ID NOs:59, 69, and 79, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 13G4 are shown in Figure 10A and in SEQ ID NO:90 and 10, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 13G4 are shown in Figure 10B and in SEQ ID NO:100 and 20, respectively.

Comparison of the 13G4 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 13G4 heavy chain utilizes a VH segment from human germline VH 3-9, a D segment from human germline 3-9, and a JH segment from human germline JH 4b. The alignment of the 13G4 VH sequence to the germline VH 3-9 sequence is shown in Figure 20. Further analysis of the 13G4 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 10A and 20, and in SEQ ID NOs:30, 40, and 50, respectively.

Comparison of the 13G4 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 13G4 light chain utilizes a VL segment from human germline VK L18 and a JK segment from human germline JK 3. The alignment of the 13G4 VL sequence to the germline VK L18 sequence is shown in Figure 30. Further analysis of the 13G4 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 10B and 30, and in SEQ ID NOs:60, 70, and 80, respectively.

Example 3: Characterization of Binding Specificity and Binding Kinetics of Anti-PD-L1 Human Monoclonal Antibodies

In this example, binding affinity and binding kinetics of anti-PD-L1 antibodies were examined by Biacore analysis. Binding specificity, and cross-competition were examined by flow cytometry.

Binding affinity and kinetics

Anti-PD-L1 antibodies were characterized for affinities and binding kinetics by Biacore analysis (Biacore AB, Uppsala, Sweden). Purified recombinant human PD-L1 fusion protein was covalently linked to a CM5 chip (carboxy methyl dextran coated chip) via primary amines, using standard amine coupling chemistry and kit provided by Biacore, to a density of 562 RUs. Binding was measured by flowing the antibodies in HBS EP buffer (provided by Biacore AB) at a concentration of 133 nM at a flow rate of 50 µl/min. The antigen-antibody association kinetics was followed for 1 minute and the dissociation kinetics was followed for 1 minute. The association and dissociation curves

were fit to a 1:1 Langmuir binding model using BIAevaluation software (Biacore AB). To minimize the effects of avidity in the estimation of the binding constants, only the initial segment of data corresponding to association and dissociation phases were used for fitting. The K_D, k_{on} and k_{off} values that were determined are shown in Table 2.

Table 2. Biacore binding data for PD-L1 human monoclonal antibodies.

Sample #	Sample ID	Affinity K_D x 10^{-9} (M)	On rate k_{on} x 10^5 (1/Ms)	Off rate k_{off} x 10^{-4} 1/s
1	3G10	3.39	5.25	17.8
3	10A5	1.45	2.58	3.72

Additional binding data obtained by equilibrium binding method and analyzed on GraphPad Prizm is shown in Table 3..

Table 3. Biacore equilibrium binding data for PD-L1 human monoclonal antibodies.

Clone ID	K_D (nM) 37 C	K_D (nM) 25 C
12A4	1.94	0.76
7H1	2.15	nd
1B12	1.38	0.61
12B7	0.83	0.53
10A5	2.41	0.57
10H10	5.93	5.48
13G4	1.87	3.3
11E6	0.53	2.9
5F8	2.17	0.75

Binding specificity by flow cytometry

Chinese hamster ovary (CHO) cell lines that express recombinant human PD-L1 at the cell surface were developed and used to determine the specificity of PD-L1 human monoclonal antibodies by flow cytometry. CHO cells were transfected with expression plasmids containing full length cDNA encoding transmembrane forms of PD-L1. Binding of the 3G10, 10A5, and 12A4 anti-PD-L1 human monoclonal antibodies was assessed by incubating the transfected cells with the anti-PD-L1 human monoclonal antibody. The cells were washed and binding was detected with a FITC-labeled anti-human IgG Ab. Flow cytometric analyses were performed using a FACScan flow cytometry (Becton Dickinson, San Jose, CA). The binding was compared to the parent CHO cell line. The results are shown in Figures 32A (HuMAb 3G10), 32B (HuMAb

10A5) and 32C (HuMAb 12A4). Binding was also tested using varying concentrations of an anti-PD-L1 antibody. The results are shown in Figure 33. The anti-PD-L1 human monoclonal antibodies 3G10, 10A5, and 12A4 bound to the CHO cells transfected with PD-L1 in a concentration dependent manner. These data demonstrate that the anti-PD-L1 human monoclonal antibodies specifically bind to cell surface PD-L1.

Binding specificity by ELISA

The specificity of the anti-PD-L1 monoclonal antibodies was determined using a standard ELISA assay for binding to a human PD-L1 fusion to an immunoglobulin Fc region.

An Fc-fusion protein of human PD-L1 was tested for binding against the anti-PD-L1 human monoclonal antibodies 3G10, 12A4, and 10A5. Standard ELISA procedures were performed. The anti-PD-L1 human monoclonal antibodies were added at different concentrations. Goat-anti-human IgG (kappa chain-specific) polyclonal antibody conjugated with horseradish peroxidase (HRP) was used as secondary antibody. The results are shown in Figure 34. Each of the anti-PD-L1 human monoclonal antibodies 3G10, 12A4, and 10A5 bound with high specificity to PD-L1.

Example 4: Characterization of anti-PD-L1 antibody binding to PD-L1 expressed on the cell surface of human and monkey T cells

Anti-PD-L1 antibodies were tested by flow cytometry for binding to activated human or cynomolgus monkey T cells expressing PD-L1 on their surface.

Human or monkey T cells were activated by anti-CD3 antibody to induce PD-L1 expression prior to binding with a human anti-PD-L1 monoclonal antibody. Binding of the 3G10, 1B12, 13G4, and 12A4 anti-PD-L1 human monoclonal antibodies was assessed by incubating the activated cells with serial dilutions of the anti-PD-L1 human monoclonal antibodies. An isotype control antibody was used as a negative control. The cells were washed and binding was detected with a FITC-labeled anti-human Ig-kappa light chain Ab. Flow cytometric analyses were performed using a FACScalibur flow cytometer (Becton Dickinson, San Jose, CA). The results are shown in Figure 35 and 36. The anti-PD-L1 monoclonal antibodies 3G10, 1B12, 13G4, and 12A4 bound to activated human and monkey T cells. These data demonstrate that the anti-PD-L1 human monoclonal antibodies bind to human and cynomolgus monkey cell surface PD-L1.

Example 5: Characterization of anti-PD-L1 antibody binding to PD-L1 expressed on the cell surface of human T cells

Anti-PD-L1 antibodies were tested for binding to activated human T cells expressing PD-L1 on their cell surface by flow cytometry.

Human T cells were activated by anti-CD3 antibody to induce PD-L1 expression on T cells prior to binding with a human anti-PD-L1 monoclonal antibody. Binding of the 3G10, 10A5 and 12A4 anti-PD-L1 human monoclonal antibodies was assessed by incubating the activated T cells with the anti-PD-L1 human monoclonal antibodies at a concentration of 20 μg/ml. An isotype control antibody was used as a negative control. The cells were washed and binding was detected with a FITC-labeled anti-human IgG Ab. Flow cytometric analyses were performed using a FACScalibur flow cytometry (Becton Dickinson, San Jose, CA). The results are shown in Figures 37A (HuMAb 3G10), 37B (HuMAb 10A5) and 37C (HuMAb 12A4). The anti-PD-L1 monoclonal antibodies 3G10, 10A5, and 12A4 bound to activated human T cells (bold line), as shown in histogram plots compared to control (light line). These data demonstrate that the anti-PD-L1 human monoclonal antibodies bind to human cell surface PD-L1.

Example 6: Binding specificity by flow cytometry

The ES-2 human ovarian carcinoma cell line that expresses human PD-L1 at the cell surface was used to determine the specificity of PD-L1 human monoclonal antibodies by flow cytometry. ES-2 cells were treated overnight with 500 IU/mL of recombinant hIFN-γ to increase PD-L1 expression over the basal level. Binding of the 12A4, 1B12, 3G10, 10A5, 12B7, 13G4, 11E6, and 5F8 anti-PD-L1 human monoclonal antibodies was assessed by incubating the induced cells with serial dilutions of the anti-PD-L1 human monoclonal antibody. The cells were washed and binding was detected with a PE-labeled anti-human IgG Ab. Flow cytometric analyses were performed using a FACScalibur flow cytometer (Becton Dickinson, San Jose, CA). The binding was compared to isotype control antibody. The results are shown in Figures 38. The anti-PD-L1 human monoclonal antibodies 12A4, 1B12, 3G10, 10A5, 12B7, 13G4, 11E6, and 5F8 bound to the hIFN-γ -induced ES-2 cells in a concentration dependent manner. These data demonstrate that the anti-PD-L1 human monoclonal antibodies specifically bind to cell surface PD-L1.

Example 7: Effect of human anti-PD-L1 antibodies on cell proliferation and cytokine production in a Mixed Lymphocyte Reaction

A mixed lymphocyte reaction was employed to demonstrate the effect of blocking the PD-L1/PD-1 pathway to lymphocyte effector cells. T cells in the assay were tested for proliferation, IFN-γ secretion and IL-2 secretion in the presence or absence of an anti-PD-L1 human monoclonal antibody.

Human CD4+ T-cells were purified from PBMC using a CD4+ positive selection kit (Dynal Biotech). Dendritic cells were derived from purified monocytes cultured with 1000 U/ml of IL-4 and 500 U/ml of GM-CSF (R&D Biosystems) for seven days. Monocytes were prepared using a monocyte negative selection kig (Mitenyi Biotech). Each culture contained 10^5 purified T-cells and 10^4 allogeneic dendritic cells in a total volume of 200 μl. Anti-PD-L1 monoclonal antibody 10A5, 12A4, or 3G10 was added to each culture at different antibody concentrations. Either no antibody or an isotype control antibody was used as a negative control. The cells were cultured for 5 days at 37°C. After day 5, 100 μl of medium was taken from each culture for cytokine measurement. The levels of IFN-γ and IL-2 were measured using OptEIA ELISA kits (BD Biosciences). The cells were labeled with ^{3}H-thymidine, cultured for another 18 hours, and analyzed for cell proliferation. The results are shown in Figures 39A (T cell proliferation), 39B (IFN-γ secretion using HuMAb 10A5), 39C (IFN-γ secretion using HuMAb 12A4 or 3G10) and 39D (IL-2 secretion). The anti-PD-L1 human monoclonal antibody 10A5 promotes T-cell proliferation, IFN-γ secretion and IL-2 secretion in a concentration dependent manner. The anti-PD-L1 human monoclonal antibodies 12A4 and 3G10 also showed an increase in IFN-γ secretion. In contrast, cultures containing the control antibody did not show an increase in T cell proliferation, IFN-γ or IL-2 secretion.

In a separate experiment, an allogeneic mixed lymphocyte reaction (MLR) was employed to demonstrate the effect of blocking the PD-L1/PD-1 pathway in lymphocyte effector cells. T cells in the assay were tested for proliferation and IFN-γ secretion in the presence or absence of an anti-PD-L1 human monoclonal antibody or isotype control antibody.

Human CD4+ T-cells were purified from PBMC using a CD4+ negative selection kit (Miltenyi). Monocytes were prepared using a monocyte negative selection kit (Mitenyi Biotech). Dendritic cells were derived from purified monocytes cultured with 1000 U/ml of IL-4 and 500 U/ml of GM-CSF (R&D Biosystems) for seven days. Each MLR culture

contained 10^5 purified T-cells and 10^4 allogeneic dendritic cells in a total volume of 200 µl. Anti-PD-L1 monoclonal antibody12A4, 11E6, 3G10, 13G4, 1B12, 10A5, and 12B7 were added to each culture at different antibody concentrations. Either no antibody or an isotype control antibody was used as a negative control. The cells were cultured for 5 days at 37°C. On day 5, 50 µl of medium was taken from each culture for cytokine measurement and replaced with an equal volume of culture medium containing 1 µCi of ^{3}H-thymidine. The cells were cultured for another 18 hours, harvested, and analyzed for cell proliferation. The levels of IFN-γ in the culture fluid were measured using an OptEIA hIFN-γ ELISA kit (BD Biosciences). The results are shown in Figure 40. The anti-PD-L1 human monoclonal antibodies promote T-cell proliferation and IFN-γ secretion in a concentration-dependent manner. In contrast, cultures containing the control antibody did not show an increase in T cell proliferation or IFN-γ secretion.

Example 8: Effect of human anti-PD-L1 antibody on function of T regulatory cells

T regulatory cells (CD4+, CD25+) are lymphocytes that suppress the immune response. The effect of the addition of T regulatory cells on proliferation and IFN-γ secretion in the allogeneic dendritic cell and T cell MLR in the presence or absence of an anti-PD-L1 human monoclonal antibody was tested.

T regulatory cells were purified from PBMC using a CD4+CD25+ regulatory T cell isolation kit (Miltenyi Biotec). T regulatory cells were added into a mixed lymphocyte reaction (see above) containing purified CD4+CD25- T cells and allogeneic dendritic cells in a 2:1 ratio of CD4+CD25- to T regulatory cells. Anti-PD-L1 monoclonal antibody 10A5 was added to each culture at a concentration of 10 µg/ml. Either no antibody or an isotype control antibody was used as a negative control. The cells were cultured for 5 days at 37°C at which time the supernatants were analyzed for IFN-γ secretion using a Beadlyte cytokine detection system (Upstate). The cells were labeled with ^{3}H-thymidine, cultured for another 18 hours, and analyzed for cell proliferation. The results are shown in Figures 41A (T cell proliferation) and 41B (IFN-γ secretion). The addition of anti-PD-L1 human monoclonal antibody 10A5 promotes both T cell proliferation and IFN-γ secretion in cell cultures of allogeneic dendritic cells, T cells and T regulatory cells, indicating that anti-PD-L1 antibodies can reverse the effect of T regulatory cells in the allogeneic DC – T cell -MLR

In a separate experiment, human anti-PD-L1 antibodies 12A4 and 13G4, and a control antibody 1D12, were tested in the MLR assay with T regulatory cells. The results are shown in Figures 42 (T cell proliferation) and 43 (IFN-γ secretion). The addition of anti-PD-L1 human monoclonal antibodies 12A4 or 13G4 partially reverses the suppression of both T cell proliferation and IFN-γ secretion in cell cultures of allogeneic dendritic cells and T cells containing T-regulatory cells, indicating that anti-PD-L1 antibodies may have an effect on T-regulatory cells.

Example 9: Effect of anti-PD-1 antibodies on cytokine secretion by viral antigen-stimulated PBMC cells from a positiveCMV responsive donor

CMV antigen-responsive human PBMC (Astarte Biologics, Redmond, WA) were cultured at 2e5 cells/well in flat bottom TC-treated 96 well plates, in the presence of 0.5 ug/ml CMV lysate (Astarte Biologics) +/- titrated anti-PD-L1 antibodies. AIM-V medium (Invitrogen) supplemented with heat-inactivated FBS (10% final) was used at a total volume of 200 ul/well. The cells were cultured for 4 days at 37°C, 5%CO_2 at which time culture supernatant was harvested for determination of secreted interferon-γ by ELISA (OptEIA hIFN-γ ELISA kit - BD Biosciences). The results are shown in Figure 44. The anti-PD-L1 human monoclonal antibodies promote IFN-γ secretion by CMV-specific T-cells in a dose-dependent manner. The most robust response was generated by antibodies 13G4, 1B12, and 12A4 compared to isotype control. These results shows that anti-PD-L1 HuMAbs can stimulate IFN-γ release in a memory T cell response from PBMC cells previously stimulated against an antigen.

Example 10: Blocking of PD-L1 ligand binding to PD-1 by human anti-PD-L1 antibodies

Anti-PD-L1 human monoclonal antibodies were tested for the ability to block binding of the ligand PD-L1 to PD-1 expressed on transfected CHO cells by using a cell cytometry assay.

PD-1 expressing CHO cells were suspended in FACS buffer (PBS with 4% fetal calf serum). Various concentrations of the anti-PD-L1 HuMAbs 3G10, 10A5 or 12A4 was added to the cell suspension tubes at 4°C for 30 minutes, followed by addition FITC-labeled PD-L1 fused to an immunoglobulin Fc-region. Flow cytometric analyses were performed using a FACScalibur flow cytometer (Becton Dickinson, San Jose, CA). The

results are depicted in Figure 45. The anti-PD-L1 monoclonal antibodies 3G10, 10A5, and 12A4 blocked binding of PD-L1 to CHO cells transfected with human PD-1, as measured by the mean fluorescent intensity (MFI) of staining. These data demonstrate that the anti-PD-L1 HuMAbs block binding of PD-L1 ligand to cell surface PD-1.

Example 11: Inhibition of the binding of soluble PD-1 to cell-surface PD-L1 by human anti-PD-L1 antibodies.

Anti-PD-L1 human monoclonal antibodies were tested for the ability to block binding of a soluble dimeric version of the PD-1 receptor (PD-1-hFc) to PD-L1 expressed on hIFN-γ -induced ES-2 human ovarian carcinoma cells using a flow cytometry assay. The blocking was compared to isotype control antibody.

ES-2 cells were induced overnight with 500 IU/mL of hIFN-γ to upregulate hPD-L1 cell surface expression. Induced cells were suspended in FACS buffer. Serial dilutions of the anti-PD-L1 HuMAbs 12A4, 1B12, 3G10, 10A5, 12B7, 13G4, 11E6, and 5F8 were added to the cell suspension tubes at 4°C for 30 minutes, followed by two washes to remove unbound antibody. Next PD-1-hFc protein was added at a constant 2 ug/mL to all wells at 4°C for 30 minutes, followed by two washes to remove unbound PD-1-hFc. Next bound PD-1-Fc was detected on the ES-2 cells by addition of biotinylated-non-blocking anti-PD-1 HuMab 26D5, which binds to PD-1 when bound to PD-L1, at 4°C for 30 minutes, followed by two washes to remove unbound antibody. Finally, bound 26D5 antibody was detected by addition of streptavidin-PE conjugate at 4°C for 30 minutes, followed by two washes to remove unbound conjugate. Flow cytometric analysis was performed using a FACScalibur flow cytometer (Becton Dickinson, San Jose, CA). The results are depicted in Figure 46. The anti-PD-L1 monoclonal antibodies 12A4, 1B12, 3G10, 10A5, 12B7, 13G4, 11E6, and 5F8 blocked binding of PD-1 to ES-2 cells that express human PD-L1, as measured by the geometric mean fluorescent intensity (GMFI) of staining. These data demonstrate that the anti-PD-L1 HuMAbs block binding of soluble PD-1 receptor to cell surface PD-L1.

Example 12: Treatment of *in vivo* tumor model using anti-PD-L1 antibodies

Mice implanted with a cancerous tumor are treated *in vivo* with anti-PD-L1 antibodies to examine the *in vivo* effect of the antibodies on tumor growth. For the tumor studies, female AJ mice between 6-8 weeks of age (Harlan Laboratories) are randomized

by weight into 6 groups. The mice are implanted subcutaneously in the right flank with 2 x 10^6 SA1/N fibrosarcoma cells dissolved in 200 µl of DMEM media on day 0. The mice are treated with PBS vehicle, or anti-PD-L1 antibodies at 10 mg/kg. The animals are dosed by intraperitoneal injection with approximately 200 µl of PBS containing antibody or vehicle on days 1, 4, 8 and 11. Each group contains 10 animals and the groups consist of: (i) a vehicle group, (ii) control mouse IgG, and (iii) an anti-PD-L1 antibody. The mice are monitored twice weekly for tumor growth for approximately 6 weeks. Using an electronic caliper, the tumors are measured three dimensionally (height x width x length) and tumor volume is calculated. Mice are euthanized when the tumors reached tumor end point (1500 mm^3) or show greater than 15% weight loss.

Example 13: ***In Vivo*** **Efficacy of Combination Therapy (anti-CTLA-4 and anti-PD-L1 Antibodies) on Tumor Establishment and Growth**

MC38 colorectal cancer cells (available from Dr. N. Restifo, National Cancer Institute, Bethesda, MD; or Jeffrey Schlom, National Institutes of Health, Bethesda, MD) were implanted in C57BL/6 mice (2 x 10^6 cells/mouse) and selected for treatment when tumors reached a size of 100-200 mm^3). On day 0 (*i.e.*, the first day of treatment), each of four groups of 10 mice each was injected intraperitoneally (IP) with one of the following: (1) 10 mg/kg mouse IgG and 10 mg/kg of rat IgG (control), (2) 10 mg/kg anti-CTLA-4 monoclonal antibody 9D9 (mouse anti-mouse CTLA-4, obtained from J. Allison, Memorial Sloan–Kettering Cancer Center, New York, NY) and 10 mg/kg rat IgG, (3) anti-PD-L1 monoclonal antibody MIH5 (rat anti-mouse PD-L1, eBioscience) and 10 mg/kg mouse IgG, or (4) 10 mg/kg anti-CTLA-4 antibody 9D9 and 10 mg/kg anti-PD-L1 antibody MIH5. Antibody injections were then further administered on days 3 and, 6. Using an electronic caliper, the tumors were measured three dimensionally (height x width x length) and tumor volume was calculated. Mice were euthanized when the tumors reached a designated tumor end-point. The results are shown in Figure 47.

This study indicates that, in the MC38 murine tumor model, anti-PD-L1 antibody treatment alone has a modest effect on tumor growth resulting in a delay of tumor growth while anti-CTLA-4 has little effect in this model. However, the combination treatment of CTLA-4 antibody and PD-L1 antibody has a significantly greater effect on tumor growth and results in tumor free mice.

Example 14: Immunohistochemistry Using Anti-PD-L1 Antibodies

To assess the tissue binding profiles of HuMab anti-PD-L1, unmodified 12A4, 13G4, 3G10 and 12B7 were examined in a panel of normal (non-neoplastic) human tissues, including spleen, tonsil, cerebrum, cerebellum, heart, liver, lung, kidney, pancreas, pituitary, skin, and small intestine, as well as lung carcinoma tissues (1 sample/each). ES-2 cells were used as positive control. Hu-IgG_1 and Hu-IgG_4 were used as isotype control antibodies.

Snap frozen and OCT embedded normal and tumor tissues were purchased from Cooperative Human Tissue Network (Philadelphia, PA) or National Disease Research Institute (Philadelphia, PA). Cryostat sections at 5 μm were fixed with acetone for 10 min at room temperature, and stored at –80 °C until use. A Medarex developed immunohistochemistry protocol was performed using unmodified HuMab anti-PD-L1 by pre-complex of the primary antibodies (12A4, 13G4, 3G10 and 12B7) and secondary antibody (FITC conjugated Fab fragment of goat anti-Hu-IgG. Jackson ImmunoResearch Laboratories. West Grove, PA) before applying onto the sections. Briefly, 1 μg/ml or 5 μg/ml of the un-conjugated primary antibodies were mixed with 3 fold excess of secondary antibody respectively and incubated for 30 min at room temperature, and then excess human gamma globulin was added for another 30 min to block the unbound secondary antibody. In parallel, isotype control antibodies Hu-IgG_1 or Hu-IgG_4 were pre-complexed in the same manner. Slides were washed with PBS (Sigma, St. Louis, MO) twice, and then incubated with peroxidase block supplied in Dako EnVision+System (Dako. Carpinteria, CA) for 10 minutes. After two washes with PBS, slides were incubated with Dako protein block to block the non-specific binding sites. Subsequently, the pre-complex of primary antibodies or isotype controls were applied onto sections and incubated for 1 hr. Following three washes with PBS, slides were incubated with mouse anti-FITC antibody (20 μg/ml. Sigma) for 30 min. After another three washes with PBS, the slides were incubated with the peroxidase-conjugated anti-mouse IgG polymer supplied in the Dako EnVision+System for 30 min. Finally, slides were washed as above and reacted with DAB substrate-chromogen solution supplied in the Dako EnVision+System for 6 min. Slides were then washed with deionized water, counterstained with Mayer's hematoxylin (Dako), dehydrated, cleared and coverslipped with Permount (Fischer Scientific, Fair Lawn, NJ) following routine histological procedure.

Weak to moderate staining was observed in ES-2 cells, as well as in tumor cells of lung carcinoma tissues. In tonsil sections, strong staining was seen in crypt epithelium that is heavily infiltrated by lymphoid cells, but not in the mucous stratified squamous epithelial cells. Moderate staining was seen in some cells in the inter-follicular region, and very weak staining was seen in scattered large cells (dendritic reticulum-like cells) in the germinal center. In lung, weak staining was found in alveoli macrophages. The staining patterns in tonsil and lung tissues were similarly seen in immunohistochemistry sections using commercial anti-PD-L1 mAb (eBiosciences. San Diego, CA). There was overall less intense staining by HuMabs, especially for the staining in the germinal centers. In spleen, diffuse weak immunoreactivity in red pulp was slightly above the background staining. In addition, weak to moderate staining was displayed in Kupffer-like cells in liver and scattered cells in Peyer's patch, as well as in scattered macrophage-like cells and fibroblasts mainly in focal region of the muscularis externa of small intestine.

In cerebellum, cerebrum, heart, kidney, pancreas, pituitary and skin tissues, no meaningful staining was observed when stained with all four anti-PD-L1 HuMabs. No evident difference in staining was noted among these four antibodies except 12B7 and/or 3G10 displayed slightly stronger staining in liver and ES-2 cells.

PD-L1 Antibody Summary

SEQ ID NO:	SEQUENCE	SEQ ID NO:	SEQUENCE
1	VH a.a. 3G10	26	VH CDR1 a.a. 1B12
2	VH a.a. 12A4	27	VH CDR1 a.a. 7H1
3	VH a.a. 10A5	28	VH CDR1 a.a. 11E6
4	VH a.a. 5F8	29	VH CDR1 a.a. 12B7
5	VH a.a. 10H10	30	VH CDR1 a.a. 13G4
6	VH a.a. 1B12		
7	VH a.a. 7H1	31	VH CDR2 a.a. 3G10
8	VH a.a. 11E6	32	VH CDR2 a.a. 12A4
9	VH a.a. 12B7	33	VH CDR2 a.a. 10A5
10	VH a.a. 13G4	34	VH CDR2 a.a. 5F8
		35	VH CDR2 a.a. 10H10
11	VK a.a. 3G10	36	VH CDR2 a.a. 1B12
12	VK a.a. 12A4	37	VH CDR2 a.a. 7H1
13	VK a.a. 10A5	38	VH CDR2 a.a. 11E6
14	VK a.a. 5F8	39	VH CDR2 a.a. 12B7
15	VK a.a. 10H10	40	VH CDR2 a.a. 13G4
16	VK a.a. 1B12		
17	VK a.a. 7H1	41	VH CDR3 a.a. 3G10
18	VK a.a. 11E6	42	VH CDR3 a.a. 12A4
19	VK a.a. 12B7	43	VH CDR3 a.a. 10A5
20	VK a.a. 13G4	44	VH CDR3 a.a. 5F8
		45	VH CDR3 a.a. 10H10
21	VH CDR1 a.a. 3G10	46	VH CDR3 a.a. 1B12
22	VH CDR1 a.a. 12A4	47	VH CDR3 a.a. 7H1
23	VH CDR1 a.a. 10A5	48	VH CDR3 a.a. 11E6
24	VH CDR1 a.a. 5F8	49	VH CDR3 a.a. 12B7
25	VH CDR1 a.a. 10H10	50	VH CDR3 a.a. 13G4

51	VK CDR1 a.a. 3G10	79	VK CDR3 a.a. 12B7
52	VK CDR1 a.a. 12A4	80	VK CDR3 a.a. 13G4
53	VK CDR1 a.a. 10A5		
54	VK CDR1 a.a. 5F8	81	VH n.t. 3G10
55	VK CDR1 a.a. 10H10	82	VH n.t. 12A4
56	VK CDR1 a.a. 1B12	83	VH n.t. 10A5
57	VK CDR1 a.a. 7H1	84	VH n.t. 5F8
58	VK CDR1 a.a. 11E6	85	VH n.t. 10H10
59	VK CDR1 a.a. 12B7	86	VH n.t. 1B12
60	VK CDR1 a.a. 13G4	87	VH n.t. 7H1
		88	VH n.t. 11E6
61	VK CDR2 a.a. 3G10	89	VH n.t. 12B7
62	VK CDR2 a.a. 12A4	90	VH n.t. 13G4
63	VK CDR2 a.a. 10A5		
64	VK CDR2 a.a. 5F8	91	VK n.t. 3G10
65	VK CDR2 a.a. 10H10	92	VK n.t. 12A4
66	VK CDR2 a.a. 1B12	93	VK n.t. 10A5
67	VK CDR2 a.a. 7H1	94	VK n.t. 5F8
68	VK CDR2 a.a. 11E6	95	VK n.t. 10H10
69	VK CDR2 a.a. 12B7	96	VK n.t. 1B12
70	VK CDR2 a.a. 13G4	97	VK n.t. 7H1
		98	VK n.t. 11E6
71	VK CDR3 a.a. 3G10	99	VK n.t. 12B7
72	VK CDR3 a.a. 12A4	100	VK n.t. 13G4
73	VK CDR3 a.a. 10A5		
74	VK CDR3 a.a. 5F8	101	VH 1-18 germline a.a.
75	VK CDR3 a.a. 10H10	102	VH 1-69 germline a.a.
76	VK CDR3 a.a. 1B12	103	VH 1-3 germline a.a.
77	VK CDR3 a.a. 7H1	104	VH 3-9 germline a.a.
78	VK CDR3 a.a.11E6		

105	VK L6 germline a.a.		
106	VK L15 germline a.a.		
107	VK A27 germline a.a.		
108	VK L18 germline a.a.		
109	VK a.a. 11E6a		

We claim:

1. An isolated human monoclonal antibody, or an antigen-binding portion thereof, wherein the antibody specifically binds to human PD-L1 and wherein the antibody exhibits at least one of the following properties:

(a) binds to human PD-L1 with a K_D of $1x10^{-7}$M or less;

(b) increases T-cell proliferation in a mixed lymphocyte reaction (MLR) assay;

(c) increases interferon-γ production in an MLR assay; or

(d) increases interleukin-2 (IL-2) secretion in an MLR assay.

2. The antibody of claim 1 which is a full-length antibody of an IgG1, IgG2 or IgG4 isotype.

3. The antibody of claim 1 which is an antibody fragment or a single chain antibody.

4. The antibody of claim 1, wherein the antibody binds to human PD-L1 with a K_D of $5x10^{-9}$M or less.

5. The antibody of claim 1, wherein the antibody binds to human PD-L1 with a K_D of $2x10^{-9}$M or less.

6. An isolated human monoclonal antibody, or an antigen-binding portion thereof, wherein the antibody cross-competes for binding to PD-L1 with a reference antibody which comprises:

(a) the human heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:1, 2, 3, 4, 5, 6, 7, 8, 9, and 10; and

(b) the human light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:11, 12, 13, 14, 15, 16, 17, 18, 19, and 20.

7. The antibody of claim 6, wherein the human heavy chain variable region comprises the amino acid sequence of SEQ ID NO:1 and the human light chain variable region comprises the amino acid sequence of SEQ ID NO:11.

8. The antibody of claim 6, wherein the human heavy chain variable region comprises the amino acid sequence of SEQ ID NO:2 and the human light chain variable region comprises the amino acid sequence of SEQ ID NO:12.

9. The antibody of claim 6, wherein the human heavy chain variable region comprises the amino acid sequence of SEQ ID NO:3 and the human light chain variable region comprises the amino acid sequence of SEQ ID NO:13.

10. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 1-18 gene, wherein the antibody specifically binds PD-L1.

11. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 1-69 gene, wherein the antibody specifically binds PD-L1.

12. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 1-3 gene, wherein the antibody specifically binds PD-L1.

13. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L6 gene, wherein the antibody specifically binds PD-L1.

14. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L15 gene, wherein the antibody specifically binds PD-L1.

15. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising:

(a) a heavy chain variable region of a human V_H 1-18 gene; and

(b) a light chain variable region of a human Vk L6 gene;

wherein the antibody specifically binds to PD-L1.

16. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising:

(a) a heavy chain variable region of a human V_H 1-69 gene; and

(b) a light chain variable region of a human Vk L6 gene;

wherein the antibody specifically binds to PD-L1.

17. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising:

(a) a heavy chain variable region of a human V_H 1-3 gene; and

(b) a light chain variable region of a human Vk L15 gene;

wherein the antibody specifically binds to PD-L1.

18. An isolated monoclonal antibody, or antigen binding portion thereof, comprising a heavy chain variable region that comprises CDR1, CDR2, and CDR3 sequences; and a light chain variable region that comprises CDR1, CDR2, and CDR3 sequences, wherein:

(a) the heavy chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:41, 42, 43, 44, 45, 46, 47, 48, 49, and 50, and conservative modifications thereof;

(b) the light chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:71, 72, 73, 74, 75, 76, 77, 78, 79, and 80, and conservative modifications thereof; and

(c) the antibody specifically binds to human PD-L1.

19. The antibody of claim 18, wherein the heavy chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs:31, 32, 33, 34, 35, 36, 37, 38, 39, and 40, and conservative modifications thereof; and the light chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs:61, 62, 63, 64, 65, 66, 67, 68, 69, and 70, and conservative modifications thereof.

20. The antibody of claim 19, wherein the heavy chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs:21, 22, 23, 24, 25, 26, 27, 28, 29, and 30, and conservative modifications thereof; and the light chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs:51, 52, 53, 54, 55, 56, 57, 58, 59, and 60, and conservative modifications thereof.

21. An isolated monoclonal antibody, or antigen binding portion thereof, comprising a heavy chain variable region and a light chain variable region, wherein:

(a) the heavy chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs:1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

(b) the light chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs:11, 12, 13, 14, 15, 16, 17, 18, 19, and 20; and
the antibody binds to human PD-L1 with a K_D of $1x10^{-7}$M or less.

22. The antibody of claim 21, wherein the antibody further comprises one or more properties selected from:

(a) the antibody increases T-cell proliferation in a mixed lymphocyte reaction (MLR) assay;

(b) the antibody increases interferon-γ production in an MLR assay; and

(c) the antibody increases IL-2 secretion in an MLR assay.

23. An isolated monoclonal antibody, or antigen binding portion thereof, comprising:

(a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:21, 22, 23, 24, 25, 26, 27, 28, 29, and 30;

(b) a heavy chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:31, 32, 33, 34, 35, 36, 37, 38, 39, and 40;

(c) a heavy chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:41, 42, 43, 44, 45, 46, 47, 48, 49, and 50;

(d) a light chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:51, 52, 53, 54, 55, 56, 57, 58, 59, and 60;

(e) a light chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:61, 62, 63, 64, 65, 66, 67, 68, 69, and 70; and

(f) a light chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:71, 72, 73, 74, 75, 76, 77, 78, 79, and 80;

wherein the antibody specifically binds PD-L1.

24. The antibody of claim 23, which comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:21;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:31;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:41;

(d) a light chain variable region CDR1 comprising SEQ ID NO:51;

(e) a light chain variable region CDR2 comprising SEQ ID NO:61; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:71.

25. The antibody of claim 23, which comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:22;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:32;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:42;

(d) a light chain variable region CDR1 comprising SEQ ID NO:52;

(e) a light chain variable region CDR2 comprising SEQ ID NO:62; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:72.

26. The antibody of claim 23, which comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO:23;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO:33;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO:43;

(d) a light chain variable region CDR1 comprising SEQ ID NO:53;

(e) a light chain variable region CDR2 comprising SEQ ID NO:63; and

(f) a light chain variable region CDR3 comprising SEQ ID NO:73.

27. An isolated monoclonal antibody, or antigen binding portion thereof comprising:

(a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:1, 2, 3, 4, 5, 6, 7, 8, 9, and 10; and

(b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:11, 12, 13, 14, 15, 16, 17, 18, 19, and 20; wherein the antibody specifically binds PD-L1.

28. The antibody of claim 27, which comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:1; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:11.

29. The antibody of claim 27, which comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:2; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:12.

30. The antibody of claim 27, which comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:3; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:13.

31. A composition comprising the antibody, or antigen-binding portion thereof, of any one of claims 1-30, and a pharmaceutically acceptable carrier.

32. An immunoconjugate comprising the antibody, or antigen-binding portion thereof, of any one of claims 1-30, linked to a therapeutic agent.

33. A composition comprising the immunoconjugate of claim 32 and a pharmaceutically acceptable carrier.

34. The immunoconjugate of claim 32, wherein the therapeutic agent is a cytotoxin.

35. A composition comprising the immunoconjugate of claim 34 and a pharmaceutically acceptable carrier.

36. The immunoconjugate of claim 32, wherein the therapeutic agent is a radioactive isotope.

37. A composition comprising the immunoconjugate of claim 34 and a pharmaceutically acceptable carrier.

38. A bispecific molecule comprising the antibody, or antigen-binding portion thereof, of any one of claims 1-30, linked to a second functional moiety having a different binding specificity than said antibody, or antigen binding portion thereof.

39. A composition comprising the bispecific molecule of claim 38, and a pharmaceutically acceptable carrier.

40. An isolated nucleic acid molecule encoding the antibody, or antigen-binding portion thereof, of any one of claims 1-30.

41. An expression vector comprising the nucleic acid molecule of claim 40.

42. A host cell comprising the expression vector of claim 41.

43. A transgenic mouse comprising human immunoglobulin heavy and light chain transgenes, wherein the mouse expresses the antibody of any one of claims 1-30.

44. A hybridoma prepared from the mouse of claim 43, wherein the hybridoma produces said antibody.

45. A method of modulating an immune response in a subject comprising administering to the subject the antibody, or antigen-binding portion thereof, of any one of claims 1-30 such that the immune response in the subject is modulated.

46. A method of inhibiting growth of tumor cells in a subject, comprising administering to the subject a therapeutically effective amount of an anti-PD-L1 antibody, or antigen-binding portion thereof.

47. The method of claim 46, wherein the antibody is a chimeric antibody.

48. The method of claim 46, wherein the antibody is a humanized antibody.

49. The method of claim 46, wherein the antibody is a fully human antibody.

50. The method of claim 46, wherein the tumor cells are of a cancer selected from the group consisting of melanoma, renal cancer, prostate cancer, breast cancer, colon cancer and lung cancer.

51. The method of claim 46, wherein the tumor cells are of a cancer selected from the list consisting of bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood,

lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers.

52. A method of inhibiting growth of tumor cells in a subject, comprising administering to a subject the antibody, or antigen-binding portion thereof, of any one of claims 1-30 in an amount effective to inhibit growth of the tumor cells.

53. A method of treating an infectious disease in a subject comprising administering to the subject the antibody, or antigen-binding portion thereof, of any one of claims 1-30 such that the subject is treated for the infectious disease.

54. The method of claim 53, wherein the infectious disease is selected from the list consisting of: HIV, Influenza, Herpes, Giardia, Malaria, Leishmania, the pathogenic infection by the virus Hepatitis (A, B, & C), herpes virus (e.g., VZV, HSV-1, HAV-6, HSV-II, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus, pathogenic infection by the bacteria chlamydia, rickettsial bacteria, mycobacteria, staphylococci, streptococci, pneumonococci, meningococci and conococci, klebsiella, proteus, serratia, pseudomonas, legionella, diphtheria, salmonella, bacilli, cholera, tetanus, botulism, anthrax, plague, leptospirosis, and Lyme's disease bacteria, pathogenic infection by the fungi Candida (albicans, krusei, glabrata, tropicalis, etc.), Cryptococcus neoformans, Aspergillus (fumigatus, niger, etc.), Genus Mucorales (mucor, absidia, rhizophus), Sporothrix schenkii, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Coccidioides immitis and Histoplasma capsulatum, and pathogenic infection by the parasites Entamoeba histolytica, Balantidium coli, Naegleriafowleri, Acanthamoeba sp., Giardia lambia, Cryptosporidium sp., Pneumocystis

carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondi, Nippostrongylus brasiliensis.

55. A method of enhancing an immune response to an antigen in a subject, comprising administering to the subject: (i) the antigen; and (ii) the antibody, or antigen-binding portion thereof, of any one of claims 1-30 such that an immune response to the antigen in the subject is enhanced.

56. The method of claim 55, wherein the antigen is a tumor antigen, a viral antigen, a bacterial antigen or an antigen from a pathogen.

57. A method of treating or preventing an inflammatory disease in a subject comprising administering to the subject the antibody, or antigen-binding portion thereof, of any one of claims 1-30 such that the subject is treated for the inflammatory disease.

58. The method of claim 57, wherein the inflammatory disease is lichen planus (LP).

59. A method for preparing an anti-PD-L1 antibody comprising:

(a) providing: (i) a heavy chain variable region antibody sequence comprising a CDR1 sequence that is selected from the group consisting of SEQ ID NOs:21, 22, 23, 24, 25, 26, 27, 28, 29, and 30, a CDR2 sequence that is selected from the group consisting of SEQ ID NOs:31, 32, 33, 34, 35, 36, 37, 38, 39, and 40; and a CDR3 sequence that is selected from the group consisting of SEQ ID NOs:41, 42, 43, 44, 45, 46, 47, 48, 49, and 50; or (ii) a light chain variable region antibody sequence comprising a CDR1 sequence that is selected from the group consisting of SEQ ID NOs:51, 52, 53, 54, 55, 56, 57, 58, 59, and 60, a CDR2 sequence that is selected from the group consisting of SEQ ID NOs:61, 62, 63, 64, 65, 66, 67, 68, 69, and 70, and a CDR3 sequence that is selected from the group consisting of SEQ ID NOs:71, 72, 73, 74, 75, 76, 77, 78, 79, and 80;

(b) altering at least one amino acid residue within at least one variable region antibody sequence, said sequence being selected from the heavy chain variable region antibody sequence and the light chain variable region antibody sequence, to create at least one altered antibody sequence; and

(c) expressing the altered antibody sequence as a protein.

60. An anti-PD-L1 antibody, or antigen-binding portion thereof, for use in a method of inhibiting growth of tumor cells.

61. Use of an anti-PD-L1 antibody, or antigen-binding portion thereof, for the preparation of a medicament for inhibiting growth of tumor cells.

62. An antibody, or antigen-binding portion thereof, of any one of claims 1-30 for use in a method of inhibiting growth of tumor cells.

63. Use of an antibody, or antigen-binding portion thereof, of any one of claims 1-30 for the preparation of a medicament for inhibiting growth of tumor cells.

64. An antibody, or antigen-binding portion thereof, of any one of claims 1-30 for use in a method of treating an infectious disease.

65. Use of an antibody, or antigen-binding portion thereof, of any one of claims 1-30 for the preparation of a medicament for treating an infectious disease.

66. An antibody, or antigen-binding portion thereof, of any one of claims 1-30 for use in a method of treating an inflammatory disease.

67. Use of an antibody, or antigen-binding portion thereof, of any one of claims 1-30 for the preparation of a medicament for treating an inflammatory disease.

Anti-PD-L1 3G10 VH

V segment: 1-18
D segment: undetermined
J segment: JH6b

```
     Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   A   S   V
1    CAG GTT CAG CTG GTG CAG TCT GGA GCT GAG GTG AAG AAG CCT GGG GCC TCA GTG

                                                        CDR1
                                                        ~~~~~~~~~~~~~~~~~~~
     K   V   S   C   K   A   S   G   Y   T   F   T   D   Y   G   F   S   W
55   AAG GTC TCC TGC AAG GCT TCT GGT TAC ACC TTT ACC GAC TAT GGT TTC AGC TGG

                                                            CDR2
                                                            ~~~~~~~~~~~~~~~~~~~
     V   R   Q   A   P   G   Q   G   L   E   W   M   G   W   I   T   A   Y
109  GTG CGA CAG GCC CCT GGA CAA GGG CTT GAG TGG ATG GGA TGG ATC ACC GCT TAC

                    CDR2
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     N   G   N   T   N   Y   A   Q   K   L   Q   G   R   V   T   M   T   T
163  AAT GGT AAC ACA AAC TAT GCA CAG AAG CTC CAG GGC AGA GTC ACC ATG ACC ACA

     D   T   S   T   S   T   V   Y   M   E   L   R   S   L   R   S   D   D
217  GAC ACA TCC ACG AGC ACA GTC TAC ATG GAG CTG AGG AGC CTG AGA TCT GAC GAC

                                                CDR3
                                                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     T   A   V   Y   Y   C   A   R   D   Y   F   Y   G   M   D   V   W   G
271  ACG GCC GTG TAT TAC TGT GCG AGA GAC TAC TTC TAC GGT ATG GAC GTC TGG GGC

     Q   G   T   T   V   T   V   S   S
325  CAA GGG ACC ACG GTC ACC GTC TCC TCA
```

Figure 1a

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Anti-PD-L1 3G10 VK

V segment: L6
J segment: JK1

```
      E   I   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R
1     GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                CDR1
                    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      A   T   L   S   C   R   A   S   Q   S   V   S   S   Y   L   V   W   Y
55    GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC TAC TTA GTC TGG TAC

                                                            CDR2
                                                    ~~~~~~~~~~~~~~~~~~~~
      Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   D   A   S   N   R
109   CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GAT GCA TCC AAC AGG

      CDR2
      ~~~~~~~
      A   T   G   I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
163   GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                                    CDR3
                                                                    ~~~~~~~
      L   T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q
217   CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAG

                CDR3
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      R   S   N   W   P   R   T   F   G   Q   G   T   K   V   E   I   K
271   CGT AGC AAC TGG CCT CGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA
```

Figure 1b

Anti-PD-L1 12A4 VH

V segment: 1-69
D segment: 3-10
J segment: JH6b

```
          Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   S   S   V
  1      CAG GTC CAG CTG GTG CAG TCT GGG GCT GAG GTG AAG AAG CCT GGG TCC TCG GTG

                                                              CDR1
                                                  ~~~~~~~~~~~~~~~~~~~~~~~
          K   V   S   C   K   T   S   G   D   T   F   S   T   Y   A   I   S   W
 55      AAG GTC TCC TGC AAG ACT TCT GGA GAC ACC TTC AGC ACC TAT GCT ATC AGC TGG

                                                                  CDR2
                                                      ~~~~~~~~~~~~~~~~~~~
          V   R   Q   A   P   G   Q   G   L   E   W   M   G   G   I   I   P   I
109      GTG CGA CAG GCC CCT GGA CAA GGG CTT GAG TGG ATG GGA GGG ATC ATC CCT ATA

                      CDR2
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          F   G   K   A   H   Y   A   Q   K   F   Q   G   R   V   T   I   T   A
163      TTT GGT AAA GCA CAC TAC GCA CAG AAG TTC CAG GGC AGA GTC ACG ATT ACC GCG

          D   E   S   T   S   T   A   Y   M   E   L   S   S   L   R   S   E   D
217      GAC GAA TCC ACG AGC ACA GCC TAC ATG GAG CTG AGC AGC CTG AGA TCT GAG GAC

                                                      CDR3
                                      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          T   A   V   Y   F   C   A   R   K   F   H   F   V   S   G   S   P   F
271      ACG GCC GTG TAT TTT TGT GCG AGA AAG TTT CAC TTT GTT TCG GGG AGC CCC TTC

           CDR3
         ~~~~~~~~~~~
          G   M   D   V   W   G   Q   G   T   T   V   T   V   S   S
325      GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA
```

Figure 2a

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Anti-PD-L1 12A4 VK

V segment: L6
J segment: JK1

```
                E   I   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R
1       GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                    CDR1
                                    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                A   T   L   S   C   R   A   S   Q   S   V   S   S   Y   L   A   W   Y
55      GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC TAC TTA GCC TGG TAC

                                                                    CDR2
                                                                    ~~~~~~~~~~~~~~~~~~~~
                Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   D   A   S   N   R
109     CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GAT GCA TCC AAC AGG

                CDR2
                ~~~~~~~
                A   T   G   I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
163     GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                                                CDR3
                                                                                ~~~~~~~
                L   T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q
217     CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAG

                        CDR3
                        ~~~~~~~~~~~~~~~~~~~~~~~~
                R   S   N   W   P   T   F   G   Q   G   T   K   V   E   I   K
271     CGT AGC AAC TGG CCG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA
```

Figure 2b

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Anti-PD-L1 10A5 VH

V segment: 1-3
D segment: 5-5
J segment: JH4b

```
   Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   A   S   V
1  CAG GTC CAA CTT GTG CAG TCT GGG GCT GAG GTG AAG AAG CCT GGG GCC TCA GTG

                                                   CDR1
                                                   ~~~~~~~~~~~~~~~~~~~
   K   V   S   C   K   A   S   G   Y   T   F   T   S   Y   D   V   H   W
55 AAG GTT TCC TGC AAG GCT TCT GGA TAC ACC TTC ACT AGC TAT GAT GTA CAT TGG

                                                           CDR2
                                                           ~~~~~~~~~~~~~~~~~~~
   V   R   Q   A   P   G   Q   R   L   E   W   M   G   W   L   H   A   D
109 GTG CGC CAG GCC CCC GGA CAA AGG CTT GAG TGG ATG GGA TGG CTC CAC GCT GAC

                    CDR2
   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   T   G   I   T   K   F   S   Q   K   F   Q   G   R   V   T   I   T   R
163 ACT GGT ATC ACA AAA TTT TCA CAG AAG TTC CAG GGC AGA GTC ACC ATT ACC AGG

   D   T   S   A   S   T   A   Y   M   E   L   S   S   L   R   S   E   D
217 GAC ACA TCC GCG AGC ACA GCC TAC ATG GAG CTG AGC AGC CTG AGA TCT GAA GAC

                                       CDR3
                                       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   T   A   V   Y   Y   C   A   R   E   R   I   Q   L   W   F   D   Y   W
271 ACG GCT GTG TAT TAC TGT GCG AGG GAG AGG ATA CAG CTA TGG TTT GAC TAC TGG

   G   Q   G   T   L   V   T   V   S   S
325 GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA
```

Figure 3a

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Anti-PD-L1 10A5 VK

V segment: L15
J segment: JK2

```
   D   I   Q   M   T   Q   S   P   S   S   L   S   A   S   V   G   D   R
1 GAC ATC CAG ATG ACC CAG TCT CCA TCC TCA CTG TCT GCA TCT GTA GGA GAC AGA

                          CDR1
                          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   V   T   I   T   C   R   A   S   Q   G   I   S   S   W   L   A   W   Y
55 GTC ACC ATC ACT TGT CGG GCG AGT CAG GGT ATT AGC AGC TGG TTA GCC TGG TAT

                                                          CDR2
                                                          ~~~~~~~~~~~~~~~~~~~~
   Q   Q   K   P   E   K   A   P   K   S   L   I   Y   A   A   S   S   L
109 CAG CAG AAA CCA GAG AAA GCC CCT AAG TCC CTG ATC TAT GCT GCA TCC AGT TTG

    CDR2
    ~~~~~~~
   Q   S   G   V   P   S   R   F   S   G   S   G   S   G   T   D   F   T
163 CAA AGT GGG GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA GAT TTC ACT

                                                                  CDR3
                                                                  ~~~~~~~
   L   T   I   S   S   L   Q   P   E   D   F   A   T   Y   Y   C   Q   Q
217 CTC ACC ATC AGC AGC CTG CAG CCT GAA GAT TTT GCA ACT TAT TAC TGC CAA CAG

            CDR3
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~
   Y   N   S   Y   P   Y   T   F   G   Q   G   T   K   L   E   I   K
271 TAT AAT AGT TAC CCG TAC ACT TTT GGC CAG GGG ACC AAG CTG GAG ATC AAA
```

Figure 3b

Anti-PD-L1 5F8 VH

```
V segment:     1-69
D segment:     6-13
J segment:     JH4b

      Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   S   S   V
1     CAG GTC CAG CTG GTG CAG TCT GGG GCT GAG GTG AAG AAG CCT GGG TCC TCG GTG

                                                                CDR1
                                                    ~~~~~~~~~~~~~~~~~~~
      K   V   S   C   K   V   S   G   G   I   F   S   T   Y   A   I   N   W
55    AAG GTC TCC TGC AAG GTT TCT GGA GGC ATC TTC AGC ACC TAT GCT ATC AAC TGG

                                                                    CDR2
                                                        ~~~~~~~~~~~~~~~~~~~
      V   R   Q   A   P   G   Q   G   L   E   W   M   G   G   I   I   P   I
109   GTG CGA CAG GCC CCT GGA CAA GGG CTT GAG TGG ATG GGA GGG ATC ATC CCT ATC

                     CDR2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      F   G   T   A   N   H   A   Q   K   F   Q   G   R   V   T   I   T   A
163   TTT GGT ACA GCA AAC CAC GCA CAG AAG TTC CAG GGC AGA GTC ACG ATT ACC GCG

      D   E   S   T   S   T   A   Y   M   E   L   S   S   L   R   S   E   D
217   GAC GAA TCC ACG AGC ACA GCC TAC ATG GAG CTG AGC AGC CTG AGA TCT GAG GAC

                                                    CDR3
                                  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      T   A   V   Y   Y   C   A   R   D   Q   G   I   A   A   A   L   F   D
271   ACG GCC GTG TAT TAC TGT GCG AGA GAT CAG GGT ATA GCA GCA GCC CTT TTT GAC

      CDR3
      ~~~
      Y   W   G   Q   G   T   L   V   T   V   S   S
325   TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA
```

Figure 4a

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Anti-PD-L1 5F8 VK1

V segment: A27
J segment: JK1

```
                E   I   V   L   T   Q   S   P   G   T   L   S   L   S   P   G   E   R
1       GAA ATT GTG TTG ACG CAG TCT CCA GGC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                        CDR1
                        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                A   T   L   S   C   R   A   S   Q   S   V   S   S   S   Y   L   A   W
55      GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC AGC TAC TTA GCC TGG

                                                                    CDR2
                                                                    ~~~~~~~~~~~~~~~~
                Y   Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   G   A   S   S
109     TAC CAG CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GGT GCA TCC AGC

                CDR2
                ~~~~~~~~~~~~
                R   A   T   G   I   P   D   R   F   S   G   S   G   S   G   T   D   F
163     AGG GCC ACT GGC ATC CCA GAC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC

                                        CDR3
                                                                            ~~~
                T   L   T   I   S   R   L   E   P   E   D   F   A   V   Y   Y   C   Q
217     ACT CTC ACC ATC AGC AGA CTG GAG CCT GAA GAT TTT GCA GTG TAT TAC TGT CAG

                        CDR3
                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                Q   Y   G   S   S   P   W   T   F   G   Q   G   T   K   V   E   I   K
271     CAG TAT GGT AGC TCA CCG TGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA
```

Figure 4b

Anti-PD-L1 10H10 VH

V segment: 3-9
D segment: 4-17
J segment: JH4b

```
                E   V   Q   L   V   E   S   G   G   G   L   V   Q   P   G   R   S   L
1       GAA GTG CAG CTG GTG GAG TCT GGG GGA GGC TTG GTA CAG CCT GGC AGG TCC CTG

                                                                CDR1
                                                            ~~~~~~~~~~~~~~~~~~~
                R   L   S   C   A   V   S   G   F   T   F   D   D   Y   V   V   H   W
55      AGA CTC TCC TGT GCA GTC TCT GGA TTC ACC TTT GAT GAT TAT GTC GTG CAC TGG

                                                                        CDR2
                                                                    ~~~~~~~~~~~~~~~~~~~
                V   R   Q   A   P   G   K   G   L   E   W   V   S   G   I   S   G   N
109     GTC CGG CAA GCT CCA GGG AAG GGC CTG GAG TGG GTC TCA GGT ATT AGT GGG AAT

                        CDR2
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                S   G   N   I   G   Y   A   D   S   V   K   G   R   F   T   I   S   R
163     AGT GGT AAC ATA GGC TAT GCG GAC TCT GTG AAG GGC CGA TTC ACC ATC TCC AGA

                D   N   A   K   N   S   L   Y   L   Q   M   N   S   L   R   A   E   D
217     GAC AAC GCC AAG AAC TCC CTG TAT CTG CAA ATG AAC AGT CTG AGA GCT GAG GAC

                                                CDR3
                                            ~~~~~~~~~~~~~~~~
                T   A   L   Y   Y   C   A   V   P   F   D   Y   W   G   Q   G   T   L
271     ACG GCC TTG TAT TAC TGT GCG GTC CCC TTT GAC TAC TGG GGC CAG GGA ACC CTG

                V   T   V   S   S
325     GTC ACC GTC TCC TCA
```

Figure 5a

Anti-PD-L1 10H10 VK

V segment: L15
J segment: JK2

```
          D   I   Q   M   T   Q   S   P   S   S   L   S   A   S   V   G   D   R
1     GAC ATC CAG ATG ACC CAG TCT CCA TCC TCA CTG TCT GCA TCT GTA GGA GAC AGA

                                  CDR1
                          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          V   T   I   T   C   R   A   S   Q   G   I   S   S   W   L   A   W   Y
55    GTC ACC ATC ACT TGT CGG GCG AGT CAG GGT ATT AGC AGC TGG TTA GCC TGG TAT

                                                                CDR2
                                                        ~~~~~~~~~~~~~~~~~~~~
          Q   Q   K   P   E   K   A   P   K   S   L   I   Y   A   A   S   S   L
109   CAG CAG AAA CCA GAG AAA GCC CCT AAG TCC CTG ATC TAT GCT GCA TCC AGT TTG

          CDR2
          ~~~~~~~~
          Q   S   G   V   P   S   R   F   S   G   S   G   S   G   T   D   F   T
163   CAA AGT GGG GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA GAT TTC ACT

                                                                        CDR3
                                                                        ~~~~~~~~
          L   T   I   S   S   L   Q   P   E   D   F   A   T   Y   Y   C   Q   Q
217   CTC ACC ATC AGC AGC CTG CAG CCT GAA GAT TTT GCA ACT TAT TAC TGC CAA CAG

                  CDR3
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          Y   N   S   Y   P   Y   T   F   G   Q   G   T   K   L   E   I   K
271   TAT AAT AGT TAC CCG TAC ACT TTT GGC CAG GGG ACC AAG CTG GAG ATC AAA
```

Figure 5b

Anti-PD-L1 1B12 VH

V segment: 1-69
D segment: 3-10
J segment: JH6b

```
                Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   S   S   V
1       CAG GTC CAG CTG GTG CAG TCT GGG GCT GAG GTG AAG AAG CCT GGG TCC TCG GTG

                                                                CDR1
                                                    ~~~~~~~~~~~~~~~~~~~~
                K   V   S   C   K   T   S   G   D   T   F   S   S   Y   A   I   S   W
55      AAG GTC TCC TGC AAG ACT TCT GGA GAC ACC TTC AGC AGC TAT GCT ATC AGC TGG

                                                                CDR2
                                                    ~~~~~~~~~~~~~~~~~~~~
                V   R   Q   A   P   G   Q   G   L   E   W   M   G   G   I   I   P   I
109     GTG CGA CAG GCC CCT GGA CAA GGG CTT GAG TGG ATG GGA GGG ATC ATC CCT ATC

                        CDR2
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                F   G   R   A   H   Y   A   Q   K   F   Q   G   R   V   T   I   T   A
163     TTT GGT AGA GCA CAC TAC GCA CAG AAG TTC CAG GGC AGA GTC ACG ATT ACC GCG

                D   E   S   T   S   T   A   Y   M   E   L   S   S   L   R   S   E   D
217     GAC GAA TCC ACG AGC ACA GCC TAC ATG GAG CTG AGC AGC CTG AGA TCT GAG GAC

                                                        CDR3
                                        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                T   A   V   Y   F   C   A   R   K   F   H   F   V   S   G   S   P   F
271     ACG GCC GTG TAT TTT TGT GCG AGA AAG TTT CAC TTT GTT TCG GGG AGC CCC TTC

                    CDR3
        ~~~~~~~~~~~~~~~~
                G   M   D   V   W   G   Q   G   T   T   V   T   V   S   S
325     GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA
```

Figure 6a

Anti-PD-L1 1B12 VK

V segment: L6
J segment: JK1

```
          E   I   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R
1         GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                  CDR1
                          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          A   T   L   S   C   R   A   S   Q   S   V   S   S   Y   L   A   W   Y
55        GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC TAC TTA GCC TGG TAC

                                                                  CDR2
                                                          ~~~~~~~~~~~~~~~~~~~~
          Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   D   A   S   N   R
109       CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GAT GCA TCC AAC AGG

          CDR2
          ~~~~~~~
          A   T   G   I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
163       GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                                          CDR3
                                                                          ~~~~~~~~
          L   T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q
217       CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAG

                  CDR3
          ~~~~~~~~~~~~~~~~~~~~~~~~
          R   S   N   W   P   T   F   G   Q   G   T   K   V   E   I   K
271       CGT AGC AAC TGG CCG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA
```

Figure 6b

Anti-PD-L1 7H1 VH

V segment: 1-69
D segment: 3-10
J segment: JH6b

```
          Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   S   S   V
1         CAG GTC CAG CTG GTG CAG TCT GGG GCT GAG GTG AAG AAG CCT GGG TCC TCG GTG

                                                                CDR1
                                                            ~~~~~~~~~~~~~~~~~~~
          K   V   S   C   K   T   S   G   G   T   F   S   S   Y   A   I   S   W
55        AAG GTC TCC TGC AAG ACT TCT GGA GGC ACC TTC AGC AGC TAT GCT ATC AGC TGG

                                                                    CDR2
                                                                ~~~~~~~~~~~~~~~~~~~
          V   R   Q   A   P   G   Q   G   L   E   W   M   G   G   I   I   P   I
109       GTG CGA CAG GCC CCT GGA CAA GGG CTT GAG TGG ATG GGA GGG ATC ATC CCT ATC

                        CDR2
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          F   G   K   A   H   Y   A   Q   K   F   Q   G   R   V   T   I   T   A
163       TTT GGT AAA GCA CAC TAC GCA CAG AAG TTC CAG GGC AGA GTC ACG ATT ACC GCG

          D   E   S   T   T   T   A   Y   M   E   L   S   S   L   R   S   E   D
217       GAC GAA TCC ACG ACC ACA GCC TAC ATG GAG CTG AGC AGC CTG AGA TCT GAG GAC

                                                    CDR3
                                                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          T   A   V   Y   Y   C   A   R   K   Y   D   Y   V   S   G   S   P   F
271       ACG GCC GTG TAT TAC TGT GCG AGA AAG TAT GAC TAT GTT TCG GGG AGC CCC TTC

              CDR3
          ~~~~~~~~~~~~~~~~
          G   M   D   V   W   G   Q   G   T   T   V   T   V   S   S
325       GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA
```

Figure 7a

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Anti-PD-L1 7H1 VK

V segment: L6
J segment: JK1

```
                E   I   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R
1       GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                    CDR1
                                    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                A   T   L   S   C   R   A   S   Q   S   V   S   S   Y   L   A   W   Y
55      GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC TAC TTA GCC TGG TAC

                                                                    CDR2
                                                                    ~~~~~~~~~~~~~~~~~~~~
                Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   D   A   S   N   R
109     CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GAT GCA TCC AAC AGG

                CDR2
                ~~~~~~~~
                A   T   G   I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
163     GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                                                CDR3
                                                                                ~~~~~~~~
                L   T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q
217     CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAG

                        CDR3
                        ~~~~~~~~~~~~~~~~~~~~~~~~
                R   S   N   W   P   T   F   G   Q   G   T   K   V   E   I   K
271     CGT AGC AAC TGG CCG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA
```

Figure 7b

Anti-PD-L1 11E6 VH

```
     V segment:      1-69
     D segment:           6-19
     J segment:      JH6c

          Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   S   S   V
1        CAG GTC CAG CTG GTG CAG TCT GGG GCT GAG GTG AAG AAG CCT GGG TCC TCG GTG

                                                           CDR1
                                                      ~~~~~~~~~~~~~~~~~~~
          K   V   S   C   K   A   S   G   G   T   F   S   S   Y   A   I   N   W
55       AAG GTC TCC TGC AAG GCT TCT GGA GGC ACC TTC AGC AGC TAT GCT ATC AAC TGG

                                                               CDR2
                                                          ~~~~~~~~~~~~~~~~~~~
          V   R   Q   A   P   G   Q   G   L   E   W   M   G   G   I   I   P   I
109      GTG CGA CAG GCC CCT GGA CAA GGG CTT GAG TGG ATG GGA GGG ATC ATC CCT ATC

                      CDR2
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          F   G   S   A   N   Y   A   Q   K   F   Q   D   R   V   T   I   T   A
163      TTT GGT TCA GCA AAC TAC GCA CAG AAG TTC CAG GAC AGA GTC ACG ATT ACC GCG

          D   E   S   T   S   A   A   Y   M   E   L   S   S   L   R   S   E   D
217      GAC GAA TCC ACG AGC GCA GCC TAC ATG GAG CTG AGC AGC CTG AGA TCT GAG GAC

                                                       CDR3
                                         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          T   A   V   Y   Y   C   A   R   D   S   S   G   W   S   R   Y   Y   M
271      ACG GCC GTA TAT TAC TGT GCG AGA GAC AGC AGT GGC TGG TCT CGG TAC TAT ATG

          CDR3
         ~~~~~~~
          D   V   W   G   Q   G   T   T   V   T   V   S   S
325      GAC GTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA
```

Figure 8a

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Anti-PD-L1 11E6 VK1

V segment: A27
J segment: JK4

```
          E   I   V   L   T   Q   S   P   G   T   L   S   L   S   P   G   E   R
1     GAA ATT GTG TTG ACG CAG TCT CCA GGC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                  CDR1
                          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          A   T   L   S   C   R   A   S   Q   S   V   S   S   S   Y   L   A   W
55    GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC AGC TAC TTA GCC TGG

                                                                  CDR2
                                                          ~~~~~~~~~~~~~~~~
          Y   Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   G   A   S   S
109   TAC CAG CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GGT GCA TCC AGC

            CDR2
          ~~~~~~~~~~~
          R   A   T   G   I   P   D   R   F   S   G   S   G   S   G   T   D   F
163   AGG GCC ACT GGC ATC CCA GAC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC

                                                                          CDR3
                                                                          ~~~
          T   L   T   I   S   R   L   E   P   E   D   F   A   V   Y   Y   C   Q
217   ACT CTC ACC ATC AGC AGA CTG GAG CCT GAA GAT TTT GCA GTG TAT TAC TGT CAG

                  CDR3
          ~~~~~~~~~~~~~~~~~~~~~~~
          Q   Y   G   S   S   P   F   G   G   G   T   K   V   E   I   K
271   CAG TAT GGT AGC TCA CCT TTC GGC GGA GGG ACC AAG GTG GAG ATC AAA
```

Figure 8b

Anti-PD-L1 12B7 VH

V segment: 1-69
D segment: 3-10
J segment: JH6b

```
          Q   V   Q   L   V   Q   S   G   A   E   V   K   E   P   G   S   S   V
1       CAG GTC CAG CTG GTG CAG TCT GGG GCT GAG GTG AAG GAG CCT GGG TCC TCG GTG

                                                        CDR1
                                                    ~~~~~~~~~~~~~~~~~~~
          K   V   S   C   K   A   S   G   G   T   F   N   S   Y   A   I   S   W
55      AAG GTC TCC TGC AAG GCT TCT GGA GGC ACC TTC AAC AGC TAT GCT ATC AGC TGG

                                                            CDR2
                                                        ~~~~~~~~~~~~~~~~~~~
          V   R   Q   A   P   G   Q   G   L   E   W   M   G   G   I   I   P   L
109     GTG CGA CAG GCC CCT GGA CAA GGG CTT GAG TGG ATG GGA GGG ATC ATC CCT CTT

                        CDR2
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          F   G   I   A   H   Y   A   Q   K   F   Q   G   R   V   T   I   T   A
163     TTC GGT ATA GCA CAC TAC GCA CAG AAG TTC CAG GGC AGA GTC ACG ATT ACC GCG

          D   E   S   T   N   T   A   Y   M   D   L   S   S   L   R   S   E   D
217     GAC GAA TCC ACG AAC ACA GCC TAT ATG GAC CTG AGC AGC CTG AGA TCT GAG GAC

                                                CDR3
                                        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          T   A   V   Y   Y   C   A   R   K   Y   S   Y   V   S   G   S   P   F
271     ACG GCC GTA TAT TAT TGT GCG AGA AAG TAT TCC TAT GTT TCG GGG AGC CCC TTC

              CDR3
        ~~~~~~~~~~~~~~~~~
          G   M   D   V   W   G   Q   G   T   T   V   T   V   S   S
325     GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA
```

Figure 9a

Anti-PD-L1 12B7 VK

V segment: L6
J segment: JK5

```
                E   I   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R
1       GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                    CDR1
                                    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                A   T   L   S   C   R   A   S   Q   S   V   S   S   Y   L   A   W   Y
55      GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC TAC TTA GCC TGG TAC

                                                                    CDR2
                                                                    ~~~~~~~~~~~~~~~~~~~~
                Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   D   A   S   N   R
109     CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GAT GCA TCC AAC AGG

            CDR2
            ~~~~~~~~
                A   T   G   I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
163     GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                                                CDR3
                                                                                ~~~~~~~~
                L   T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q
217     CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAG

                    CDR3
                    ~~~~~~~~~~~~~~~~~~~~~~~~
                R   S   N   W   P   T   F   G   Q   G   T   R   L   E   I   K
271     CGT AGC AAC TGG CCC ACC TTC GGC CAA GGG ACA CGA CTG GAG ATT AAA
```

Figure 9b

Anti-PD-L1 13G4 VH

V segment: 3-9
D segment: 3-9
J segment: JH4b

```
          E   V   Q   L   V   E   S   G   G   G   L   V   Q   P   G   R   S   L
1       GAA GTG CAG TTG GTG GAG TCT GGG GGA GGC TTG GTA CAG CCT GGC AGG TCC CTG

                                                        CDR1
                                                    ~~~~~~~~~~~~~~~~~~~
          R   L   S   C   A   A   S   G   I   T   F   D   D   Y   G   M   H   W
55      AGA CTC TCC TGT GCA GCC TCT GGA ATC ACC TTT GAT GAT TAT GGC ATG CAC TGG

                                                            CDR2
                                                        ~~~~~~~~~~~~~~~~~~~
          V   R   Q   A   P   G   K   G   L   E   W   V   S   G   I   S   W   N
109     GTC CGG CAA GCT CCA GGG AAG GGC CTG GAG TGG GTC TCA GGT ATT AGC TGG AAT

                        CDR2
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          R   G   R   I   E   Y   A   D   S   V   K   G   R   F   T   I   S   R
163     AGA GGT AGA ATA GAG TAT GCG GAC TCT GTG AAG GGC CGA TTC ACC ATC TCC AGA

          D   N   A   K   N   S   L   Y   L   Q   M   N   S   L   R   A   E   D
217     GAC AAC GCC AAG AAC TCC CTG TAT CTG CAA ATG AAC AGT CTG AGA GCT GAG GAC

                                                    CDR3
                                        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          T   A   L   Y   Y   C   A   K   G   R   F   R   Y   F   D   W   F   L
271     ACG GCC TTG TAT TAC TGT GCA AAA GGG CGG TTC CGA TAT TTT GAC TGG TTT CTT

          CDR3
          ~~~~~~~~
          D   Y   W   G   Q   G   T   L   V   T   V   S   S
325     GAC TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA
```

Figure 10a

Anti-PD-L1 13G4 VK

V segment: L18
J segment: JK3

```
          A   I   Q   L   T   Q   S   P   S   S   L   S   A   S   V   G   D   R
1         GCC ATC CAG TTG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGA GAC AGA

                                  CDR1
                              ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          V   T   I   T   C   R   A   S   Q   G   I   S   S   A   L   A   W   Y
55        GTC ACC ATC ACT TGC CGG GCA AGT CAG GGC ATT AGC AGT GCT TTA GCC TGG TAT

                                                                  CDR2
                                                              ~~~~~~~~~~~~~~~~~~~~
          Q   Q   K   P   G   K   A   P   K   L   L   I   Y   D   A   S   S   L
109       CAG CAG AAA CCA GGG AAA GCT CCT AAG CTC CTG ATC TAT GAT GCC TCC AGT TTG

          CDR2
          ~~~~~~~~
          E   S   G   V   P   S   R   F   S   G   S   G   S   G   T   D   F   T
163       GAA AGT GGG GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA GAT TTC ACT

                                                                          CDR3
                                                                      ~~~~~~~~
          L   T   I   S   S   L   Q   P   E   D   F   A   T   Y   Y   C   Q   Q
217       CTC ACC ATC AGC AGC CTG CAG CCT GAA GAT TTT GCA ACT TAT TAC TGT CAA CAG

                  CDR3
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          F   N   S   Y   P   F   T   F   G   P   G   T   K   V   D   I   K
271       TTT AAT AGT TAC CCA TTC ACT TTC GGC CCT GGG ACC AAA GTG GAT ATC AAA
```

Figure 10b

Anti-PD-L1 3G10 VH region

```
                                                                                CDR1
1-18 germline:  Q V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y G I S W V R Q
3G10 VH:        - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - D - - F - - - - -

                                                CDR2
1-18 germline:  A P G Q G L E W M G W I S A Y N G N T N Y A Q K L Q G R V T M T T D T S T S T
3G10 VH:        - - - - - - - - - - - - T - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                        CDR3
1-18 germline:  A Y M E L R S L R S D D T A V Y Y C A R
3G10 VH:        V - - - - - - - - - - - - - - - - - - - D Y F Y G M D V W G Q G T T V T V S S
(JH6b)
```

Figure 11

Anti-PD-L1 12A4 VH Region

1-69 germline:	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	S	S	V	K	V	S	C	K	A
12A4 VH:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T

							CDR1																	
1-69 germline:	S	G	G	T	F	S	S	Y	A	I	S	W	V	R	Q	A	P	G	Q	G	L	E	W	M
12A4 VH:	-	-	D	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

									CDR2															
1-69 germline:	G	G	I	I	P	I	F	G	T	A	N	Y	A	Q	K	F	Q	G	R	V	T	I	T	A
12A4 VH:	-	-	-	-	-	-	-	-	K	-	H	-	-	-	-	-	-	-	-	-	-	-	-	-

1-69 germline:	D	E	S	T	S	T	A	Y	M	E	L	S	S	L	R	S	E	D	T	A	V	Y	Y	C
12A4 VH:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	F	-

										CDR3														
1-69 germline:	A	R																						
JH6b germline:												Y	G	M	D	V	W	G	Q	G	T	T	V	T
12A4 VH:	-	-	K	F	H	F	V	S	G	S	P	F	-	-	-	-	-	-	-	-	-	-	-	-

JH6b germline:	V	S	S
12A4 VH:	-	-	-

Figure 12

Anti-PD-L1 10A5 VH region

```
                                                                          __CDR1__
1-3 germline    Q V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y A M H W
10A5 VH         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - D V - -

                                             ________CDR2________________
1-3 germline    V R Q A P G Q R L E W M G W I N A G N G N T K Y S Q K F Q G R V T I T R
10A5 VH         - - - - - - - - - - - - - - L H - D T - I - - F - - - - - - - - - - - -

                                                               ____CDR3____
1-3 germline    D T S A S T A Y M E L S S L R S E D T A V Y Y C A R
JH4b germline                                                                   F D Y W
10A5 VH         - - - - - - - - - - - - - - - - - - - - - - - - - - E R I Q L W - - - -

JH4b germline   G Q G T L V T V S S
10A5 VH         - - - - - - - - - - (JH4b)
```

Figure 13

Anti-PD-L1 5F8 VH region

```
                                                                                CDR1
1-69 germline   Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F S S Y A I S W
5F8 VH          - - - - - - - - - - - - - - - - - - - - - - V - - - I - - T - - - N -

                                    CDR2
1-69 germline   V R Q A P G Q G L E W M G G I I P I F G T A N Y A Q K F Q G R V T I T A
5F8 VH          - - - - - - - - - - - - - - - - - - - - H - - - - - - - - - - - - - -

                                                                    CDR3
1-69 germline   D E S T S T A Y M E L S S L R S E D T A V Y Y C A R
JH4b germline                                                                   F D
5F8 VH          - - - - - - - - - - - - - - - - - - - - - - - - - D Q G I A A A L - -

JH4b germline   Y W G Q G T L V T V S S
5F8 VH          - - - - - - - - - - - (JH4b)
```

Figure 14

Anti-PD-L1 10H10 VH region

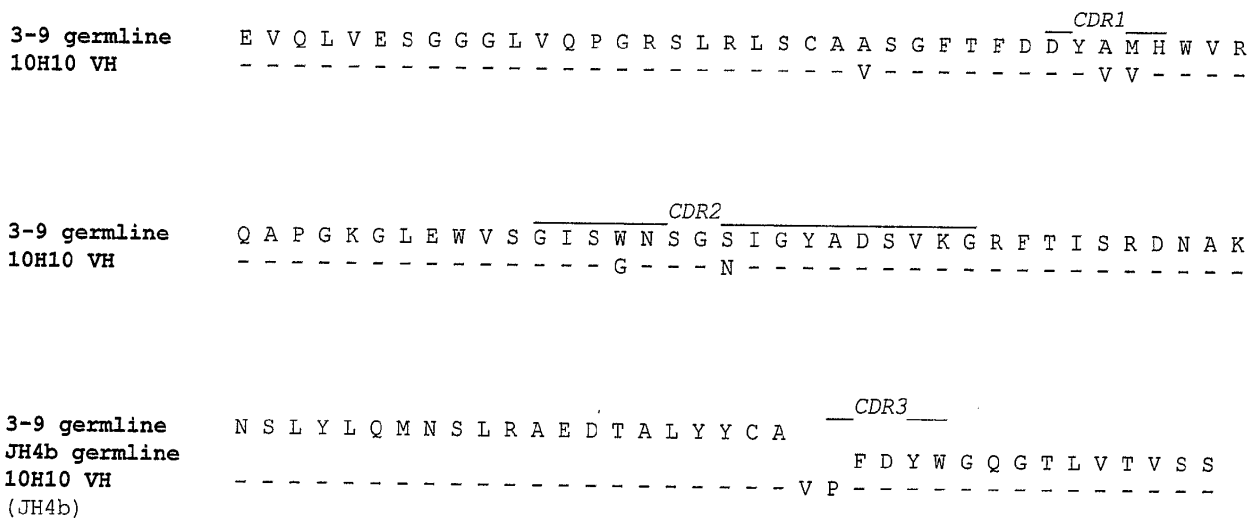


Figure 15

Anti-PD-L1 1B12 VH region

```
                                                                                                  CDR1
1-69 germline     Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F S S Y A I S W
1B12 VH           - - - - - - - - - - - - - - - - - - - - - T - - D - - - - - - - - - -

                                                  CDR2
1-69 germline     V R Q A P G Q G L E W M G G I I P I F G T A N Y A Q K F Q G R V T I T A
1B12 VH           - - - - - - - - - - - - - - - - - - - - R - H - - - - - - - - - - - - -

                                                                                  CDR3
1-69 germline     D E S T S T A Y M E L S S L R S E D T A V Y Y C A R
JH6b germline                                                                           Y
1B12 VH           - - - - - - - - - - - - - - - - - - - - - F - - - K F H F V S G S P F

                  ------
JH6b germline     G M D V W G Q G T T V T V S S
1B12 VH           - - - - - - - - - - - - - - -  (JH6b)
```

Figure 16

Anti-PD-L1 7H1 VH region

```
                                                                                CDR1
1-69 germline   Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F S S Y A I S W
7H1 VH          - - - - - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - -

                                        CDR2
1-69 germline   V R Q A P G Q G L E W M G G I I P I F G T A N Y A Q K F Q G R V T I T A
7H1 VH          - - - - - - - - - - - - - - - - - - - - K - H - - - - - - - - - - - -

                                                                        CDR3
1-69 germline   D E S T S T A Y M E L S S L R S E D T A V Y Y C A R
JH6b germline                                                                   Y
7H1 VH          - - - - T - - - - - - - - - - - - - - - - - - - - K Y D Y V S G S P F

JH6b germline   G M D V W G Q G T T V T V S S
7H1 VH          - - - - - - - - - - - - - - -  (JH6b)
```

Figure 17

Anti-PD-L1 11E6 VH region

```
                                                                                                  CDR1
1-69 germline   Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F S S Y A I S W
11E6 VH         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - N -

                                                  CDR2
1-69 germline   V R Q A P G Q G L E W M G G I I P I F G T A N Y A Q K F Q G R V T I T A
11E6 VH         - - - - - - - - - - - - - - - - - - - S - - - - - - - - D - - - - - -

                                                                                  CDR3
1-69 germline   D E S T S T A Y M E L S S L R S E D T A V Y Y C A R
JH6c germline                                                                       Y Y M
11E6 VH         - - - - - A - - - - - - - - - - - - - - - - - - - D S S G W S R - - -

JH6c germline   D V W G Q G T T V T V S S
11E6 VH         - - - - - - - - - - - - - (JH6c)
```

Figure 18

Anti-PD-L1 12B7 VH region

```
                                                                                    CDR1
1-69 germline    Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F S S Y A I S W
12B7 VH          - - - - - - - - - - - - E - - - - - - - - - - - - - - - - N - - - - - -

                                                  CDR2
1-69 germline    V R Q A P G Q G L E W M G G I I P I F G T A N Y A Q K F Q G R V T I T A
12B7 VH          - - - - - - - - - - - - - - - - - - L - - I - H - - - - - - - - - - - -

                                                                                CDR3
1-69 germline    D E S T S T A Y M E L S S L R S E D T A V Y Y C A R
JH6b germline                                                                       Y
12B7 VH          - - - - N - - - - D - - - - - - - - - - - - - - - - K Y S Y V S G S P F

JH6b germline    G M D V W G Q G T T V T V S S
12B7 VH          - - - - - - - - - - - - - - - (JH6b)
```

Figure 19

Anti-PD-L1 13G4 VH region

```
                                                                                                                CDR1
3-9 germline      E V Q L V E S G G G L V Q P G R S L R L S C A A S G F T F D D Y A M H W
13G4 VH           - - - - - - - - - - - - - - - - - - - - - - - - - - - I - - - - - G - - -

                                                  CDR2
3-9 germline      V R Q A P G K G L E W V S G I S W N S G S I G Y A D S V K G R F T I S R
13G4 VH           - - - - - - - - - - - - - - - - - - R - R - E - - - - - - - - - - - - - -

                                                                                                  CDR3
3-9 germline      D N A K N S L Y L Q M N S L R A E D T A L Y Y C A K
13G4 VH           - - - - - - - - - - - - - - - - - - - - - - - - - G R F R Y F D W F L

JH4b germline     D Y W G Q G T L V T V S S
13G4 VH           - - - - - - - - - - - - - (JH4b)
```

Figure 20

Anti-PD-L1 3G10 VK Region

```
                                                                CDR1
                                                          ___________
L6 germline:    E I V L T Q S P A T L S L S P G E R A T L S C R A S Q S V S S Y L A
3G10 VK#1:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - V

                                    CDR2
                                ___________
L6 germline:    W Y Q Q K P G Q A P R L L I Y D A S N R A T G I P A R F S G S G S G
3G10 VK#1:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                CDR3
                                            ___________
L6 germline:    T D F T L T I S S L E P E D F A V Y Y C Q Q R S N W P
JK1 germline:                                             W T F G Q G T
3G10 VK#1:      - - - - - - - - - - - - - - - - - - - - - - R - - - - - -

JK1 germline:   K V E I K
3G10 VK#1:      - - - - -  (JK1)
```

Figure 21

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PD-L1 12A4 VK Region

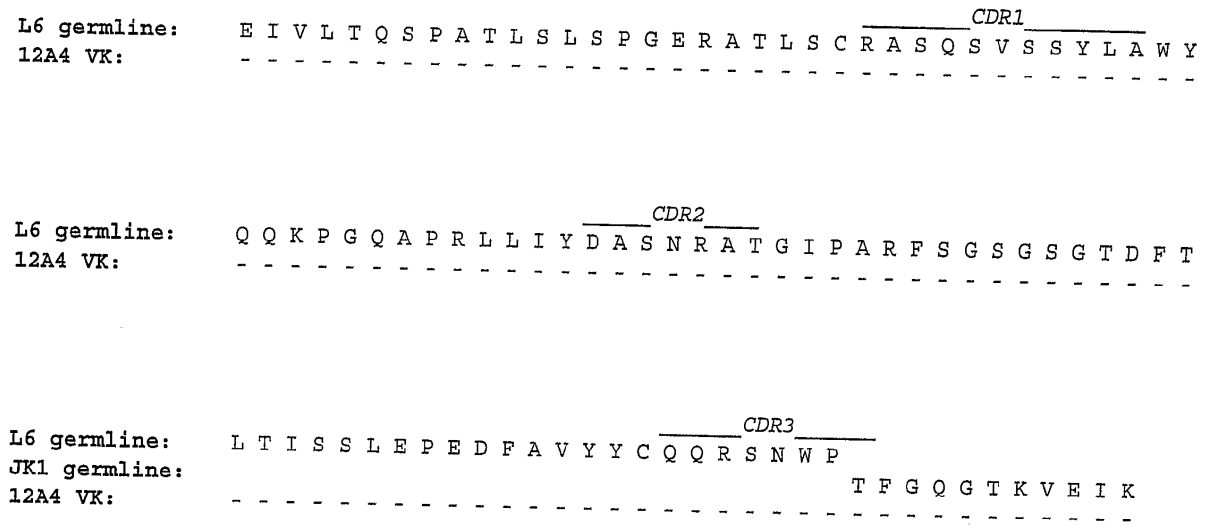


Figure 22

Anti-PD-L1 10A5 VK region

```
                                                                                                  CDR1
L15 germline    D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q G I S S
10A5 VK         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                  CDR2
L15 germline    W L A W Y Q Q K P E K A P K S L I Y A A S S L Q S G V P S R F
10A5 VK         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                                          CDR3
L15 germline    S G S G S G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y N S
10A5 VK         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

L15 germline    Y P
JK2 germline        Y T F G Q G T K L E I K
10A5 VK         - - - - - - - - - - - - - - (JK2)
```

Figure 23

Anti-PD-L1 5F8 VK1 region

```
                                                        _______CDR1___________
A27 germline      E I V L T Q S P G T L S L S P G E R A T L S C R A S Q S V S S S Y L A W
5F8 VK1           - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                  ____CDR2____
A27 germline      Y Q Q K P G Q A P R L L I Y G A S S R A T G I P D R F S G S G S G T D F
5F8 VK1           - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                              _____CDR3_______
A27 germline      T L T I S R L E P E D F A V Y Y C Q Q Y G S S P
JK1 germline                                                    W T F G Q G T K V E I K
5F8 VK1           - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
(JK1)
```

Figure 24

Anti-PD-L1 10H10 VK region

```
                                                                  CDR1
L15 germline    D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q G I S S
10H10 VK        - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                  CDR2
L15 germline    W L A W Y Q Q K P E K A P K S L I Y A A S S L Q S G V P S R F
10H10 VK        - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                  CDR3
L15 germline    S G S G S G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y N S
10H10 VK        - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

L15 germline    Y P
JK2 germline        Y T F G Q G T K L E I K
10H10 VK        - - - - - - - - - - - - - - - - (JK2)
```

Figure 25

Anti-PD-L1 1B12 VK region

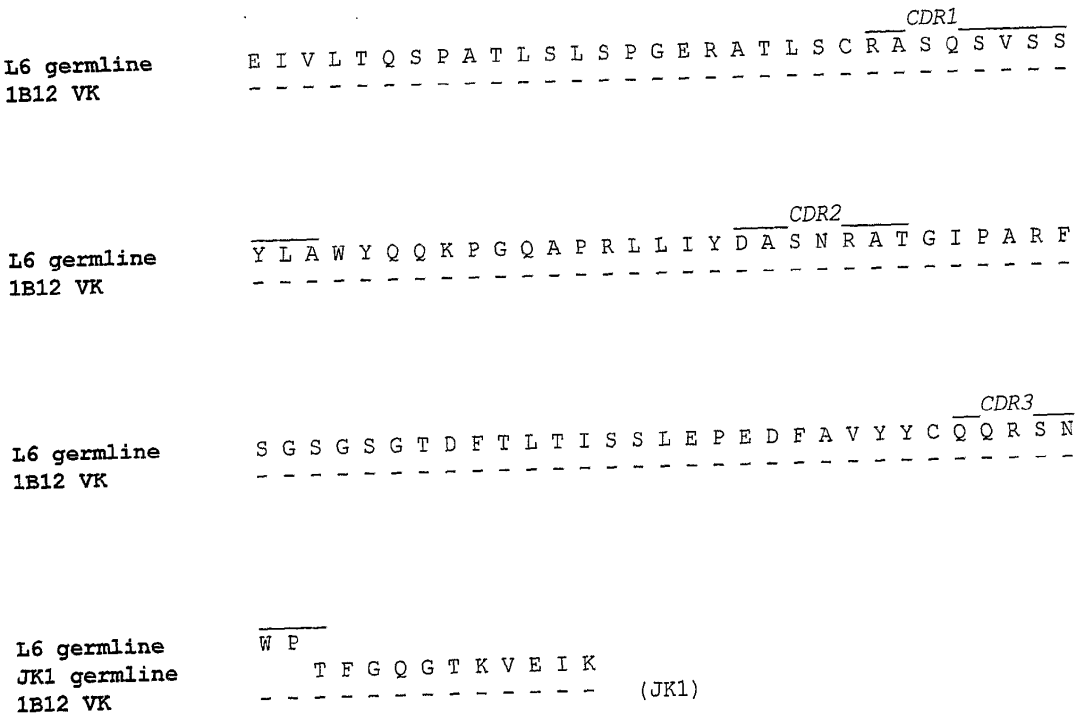


Figure 26

Anti-PD-L1 7H1 VK region

```
                                                                  ____CDR1_____
L6 germline     E I V L T Q S P A T L S L S P G E R A T L S C R A S Q S V S S
7H1 VK          - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                _____                                 ____CDR2_____
L6 germline     Y L A W Y Q Q K P G Q A P R L L I Y D A S N R A T G I P A R F
7H1 VK          - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                  __CDR3___
L6 germline     S G S G S G T D F T L T I S S L E P E D F A V Y Y C Q Q R S N
7H1 VK          - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                _____
L6 germline     W P
JK1 germline        T F G Q G T K V E I K
7H1 VK          - - - - - - - - - - -    (JK1)
```

Figure 27

Anti-PD-L1 11E6 VK1 region

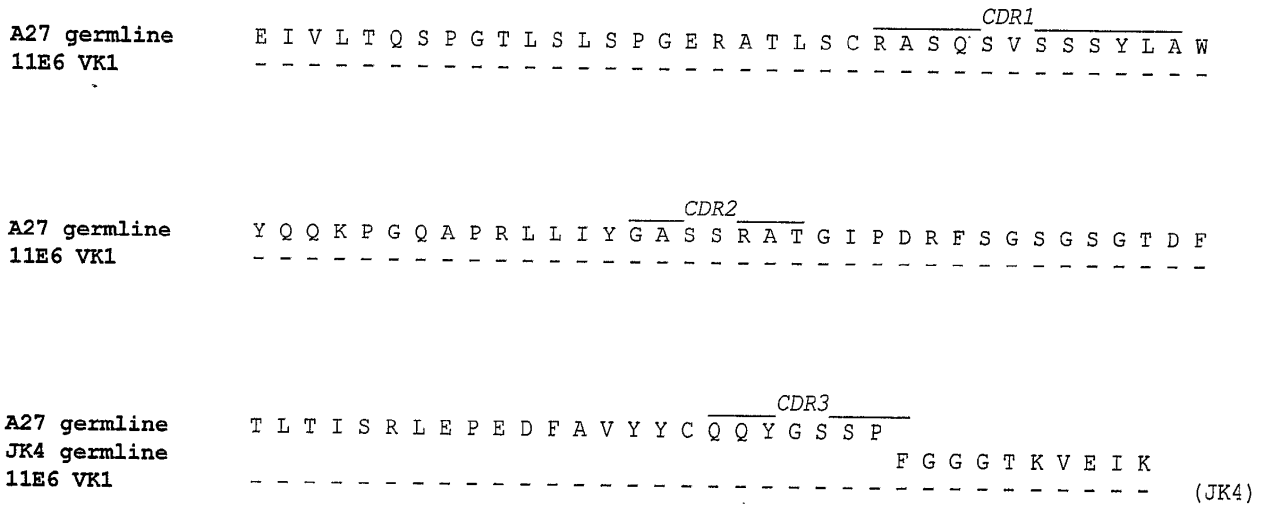


Figure 28

Anti-PD-L1 11E6a VK2 region

```
                                                        CDR1
A27 germline    E I V L T Q S P G T L S L S P G E R A T L S C R A S Q S V S S S Y L A W
11E6 VK2        - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                        CDR2
A27 germline    Y Q Q K P G Q A P R L L I Y G A S S R A T G I P D R F S G S G S G T D F
11E6 VK2        - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                CDR3
A27 germline    T L T I S R L E P E D F A V Y Y C Q Q Y G S S P
JK4 germline                                                  T F G G G T K V E I K
11E6 VK2        - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - (JK4)
```

Figure 29

Anti-PD-L1 12B7 VK region

```
                                                                  CDR1
L6 germline     E I V L T Q S P A T L S L S P G E R A T L S C R A S Q S V S S
12B7 VK         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                  CDR2
L6 germline     Y L A W Y Q Q K P G Q A P R L L I Y D A S N R A T G I P A R F
12B7 VK         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                  CDR3
L6 germline     S G S G S G T D F T L T I S S L E P E D F A V Y Y C Q Q R S N
12B7 VK         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

L6 germline     W P
JK5 germline      T F G Q G T R L E I K
12B7 VK         - - - - - - - - - - -   (JK5)
```

Figure 30

Anti-PD-L1 13G4 VK region

```
                                                                  CDR1
L18 germline    A I Q L T Q S P S S L S A S V G D R V T I T C R A S Q G I S S
13G4 VK         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                        CDR2
L18 germline    A L A W Y Q Q K P G K A P K L L I Y D A S S L E S G V P S R F
13G4 VK         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                  CDR3
L18 germline    S G S G S G T D F T L T I S S L Q P E D F A T Y Y C Q Q F N S
13G4 VK         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

L18 germline    Y P
JK3 germline        F T F G P G T K V D I K
13G4 VK         - - - - - - - - - - - - - - - (JK3)
```

Figure 31

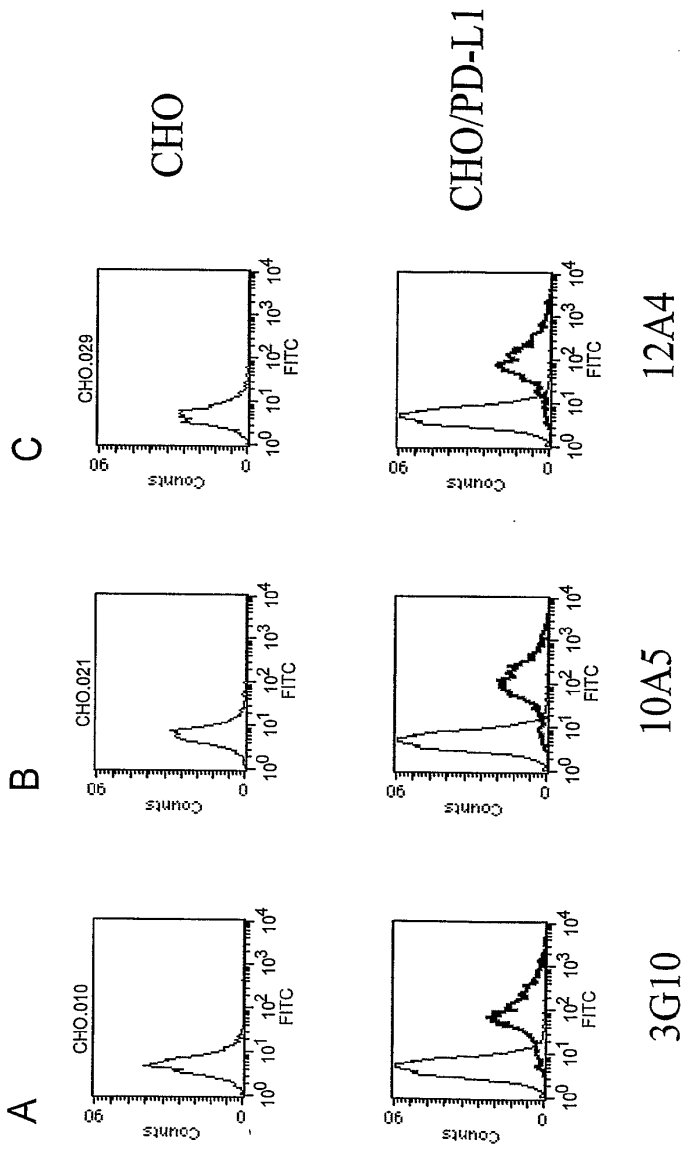


Figure 32

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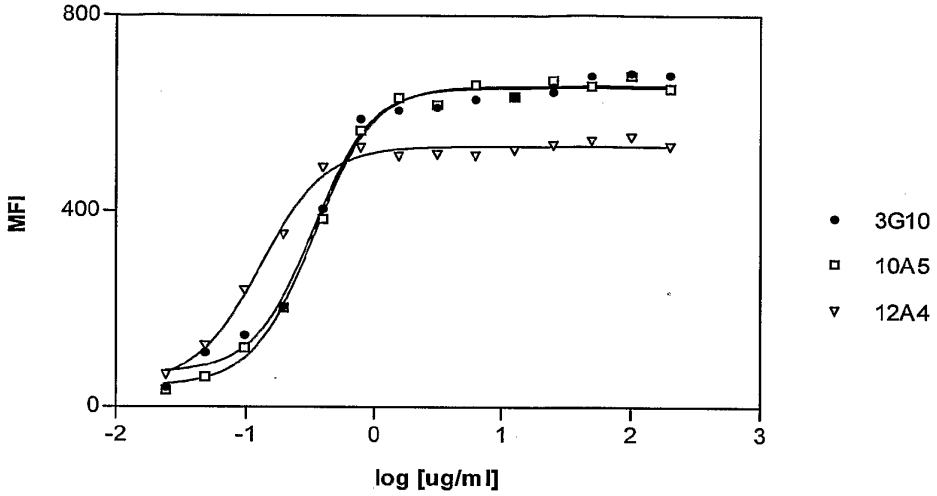


Figure 33

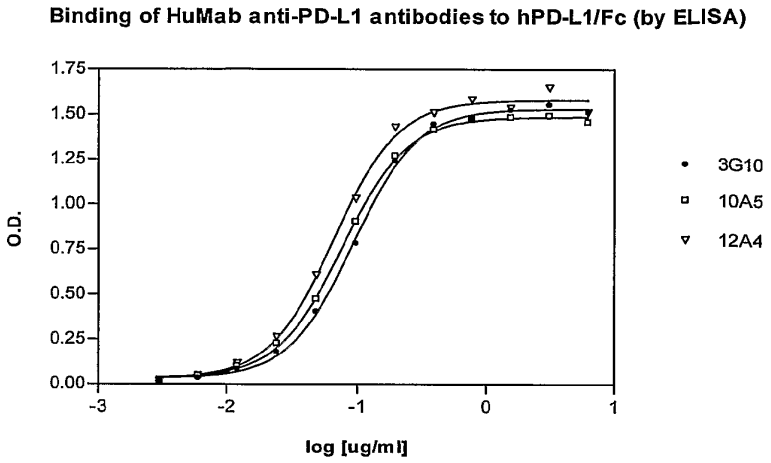

Binding of HuMab anti-PD-L1 antibodies to hPD-L1/Fc (by ELISA)
O.D.
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-3
-2
-1
0
1
log [ug/ml]
3G10
10A5
12A4

Figure 34

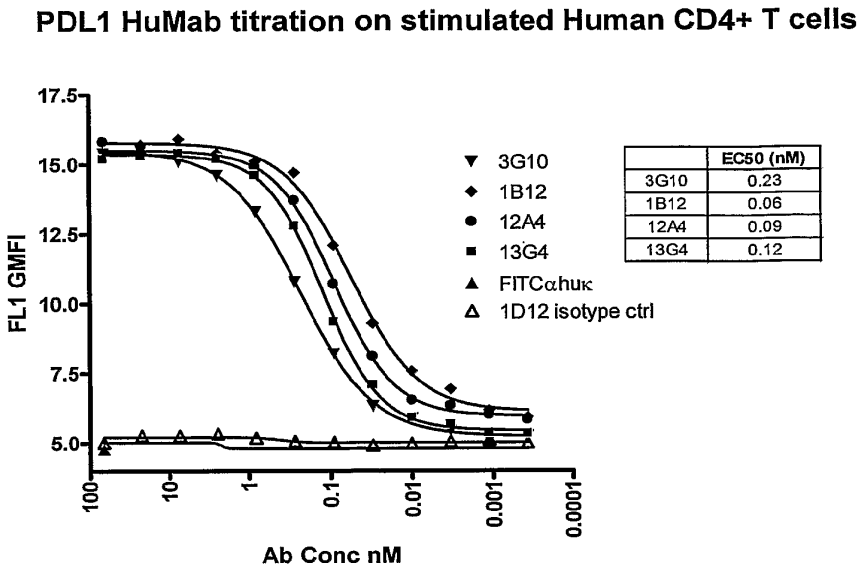

PDL1 HuMab titration on stimulated Human CD4+ T cells
FL1 GMFI
17.5
15.0
12.5
10.0
7.5
5.0
100
10
1
0.1
0.01
0.001
0.0001
Ab Conc nM
3G10
1B12
12A4
13G4
FITCαhuκ
1D12 isotype ctrl
EC50 (nM)
3G10 0.23
1B12 0.06
12A4 0.09
13G4 0.12

Figure 35

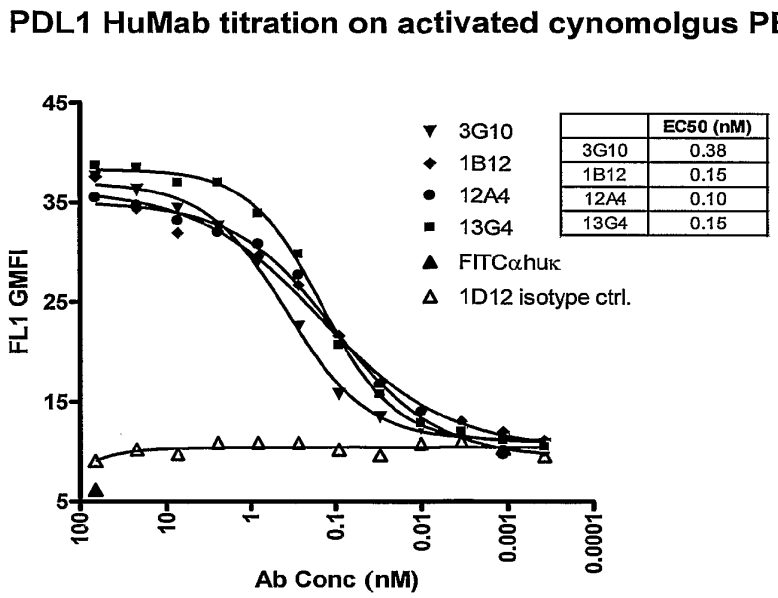

PDL1 HuMab titration on activated cynomolgus PBMC
FL1 GMFI
45
35
25
15
5
100
10
1
0.1
0.01
0.001
0.0001
Ab Conc (nM)
3G10
1B12
12A4
13G4
FITCαhuκ
1D12 isotype ctrl.
EC50 (nM)
3G10 0.38
1B12 0.15
12A4 0.10
13G4 0.15

Figure 36

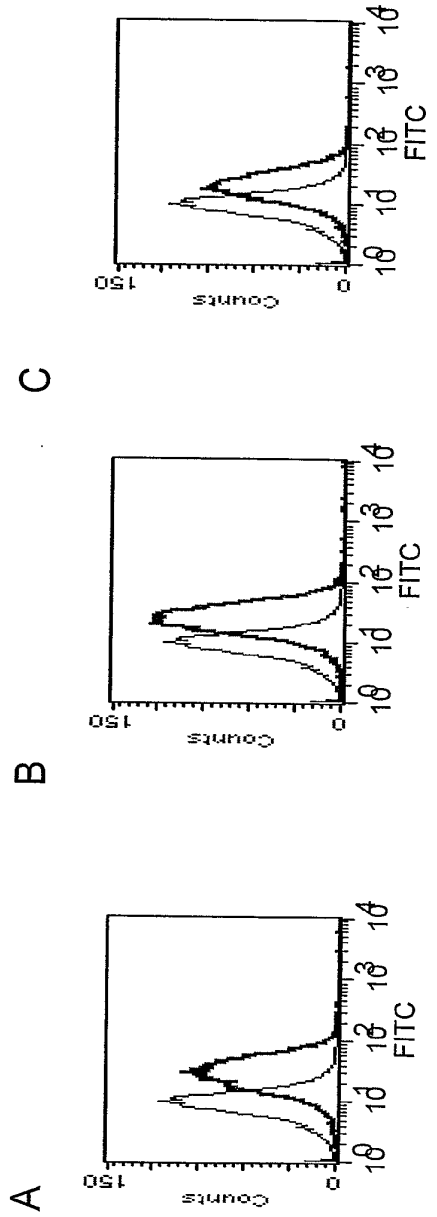


Figure 37

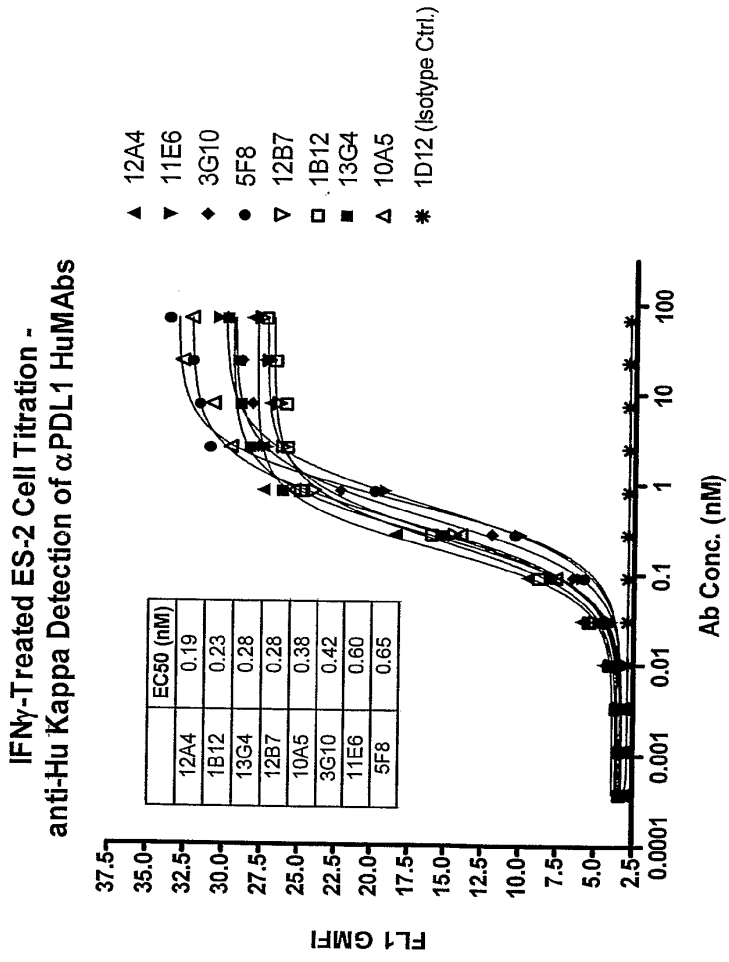


Figure 38

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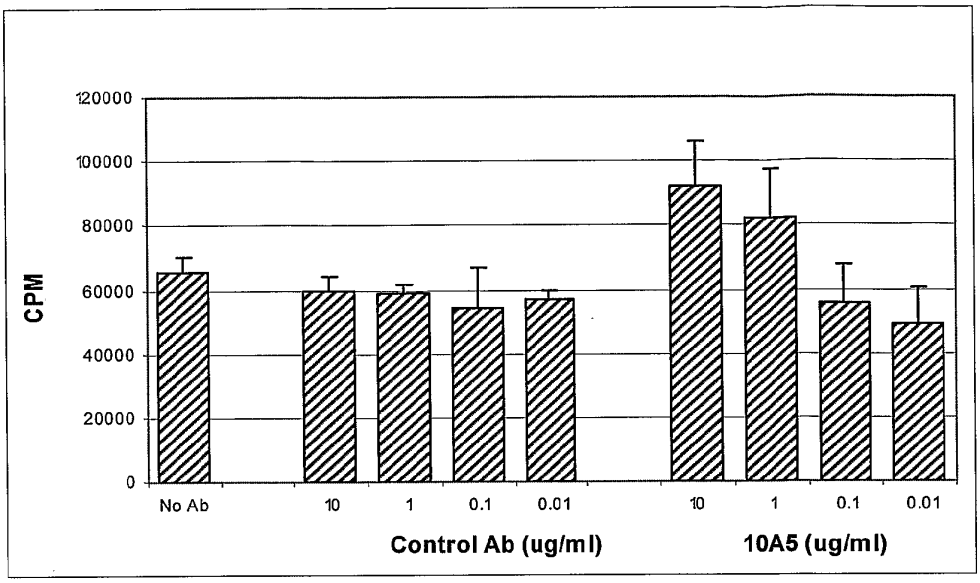

120000
100000
80000
60000
40000
20000
0
CPM
No Ab
10
1
0.1
0.01
10
1
0.1
0.01
Control Ab (ug/ml)
10A5 (ug/ml)

Figure 39A

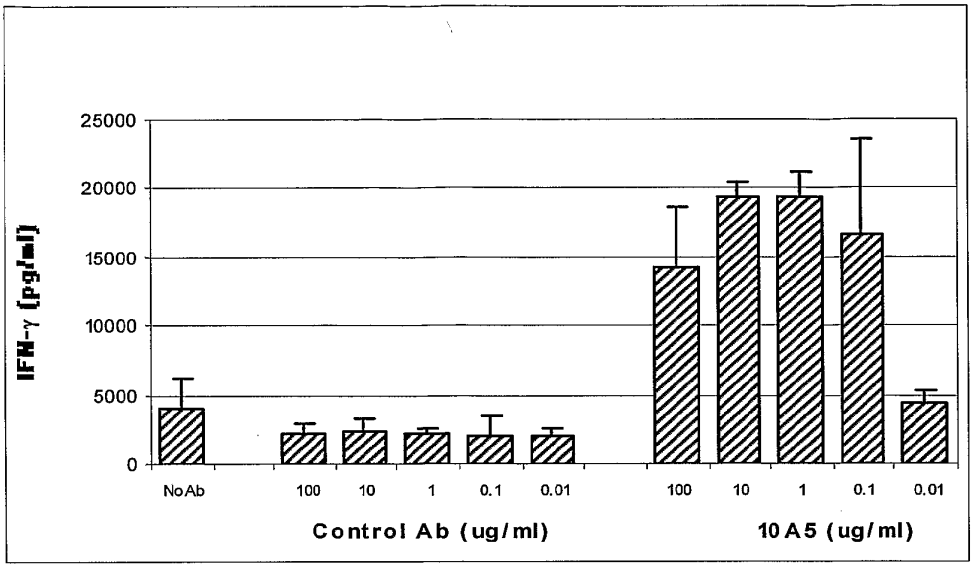

IFN-γ (pg/ml)
25000
20000
15000
10000
5000
0
NoAb
100
10
1
0.1
0.01
100
10
1
0.1
0.01
Control Ab (ug/ml)
10A5 (ug/ml)

Figure 39B

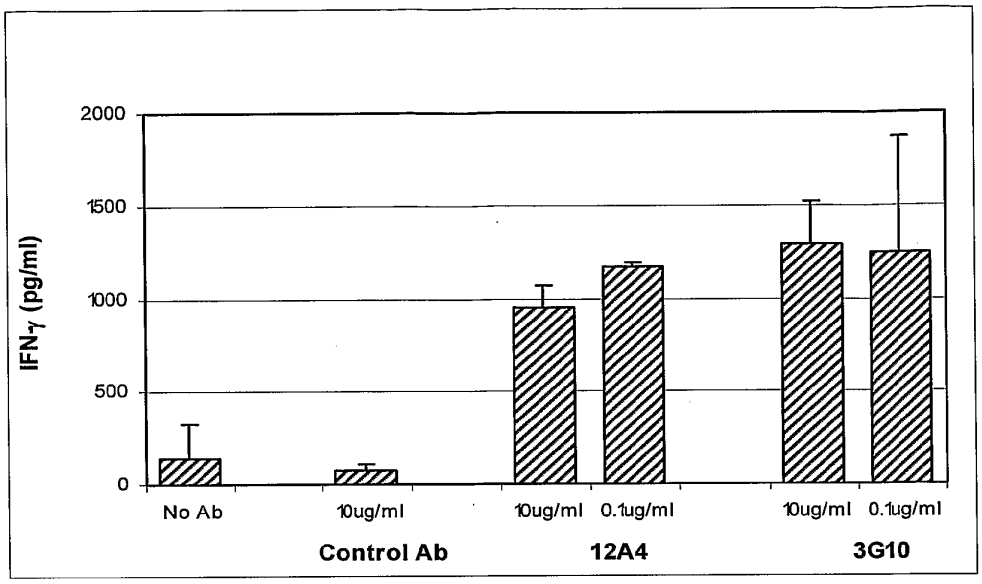

2000
1500
1000
500
0
IFN-γ (pg/ml)
No Ab
10ug/ml
10ug/ml
0.1ug/ml
10ug/ml
0.1ug/ml
Control Ab
12A4
3G10

Figure 39C

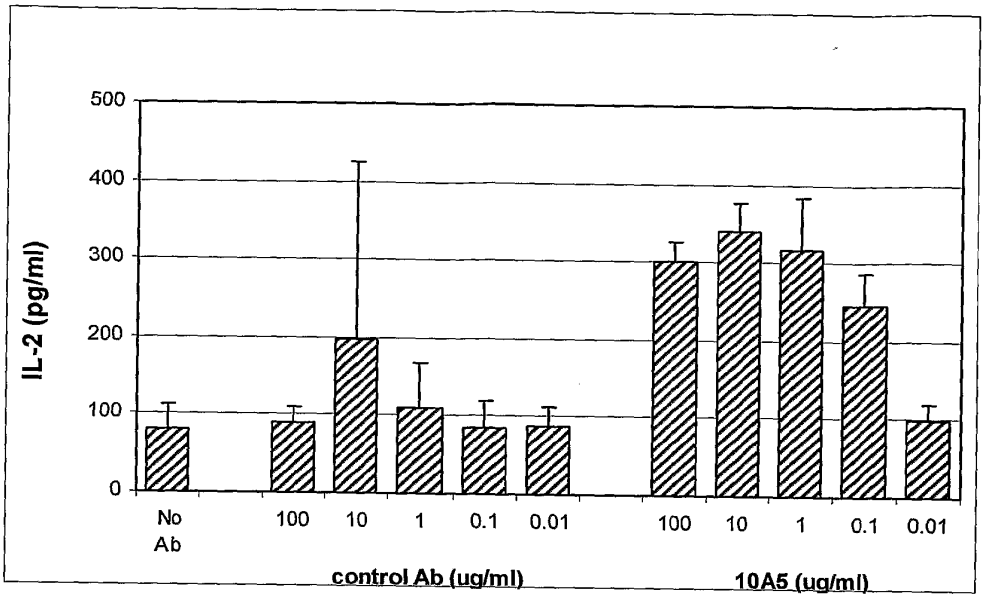
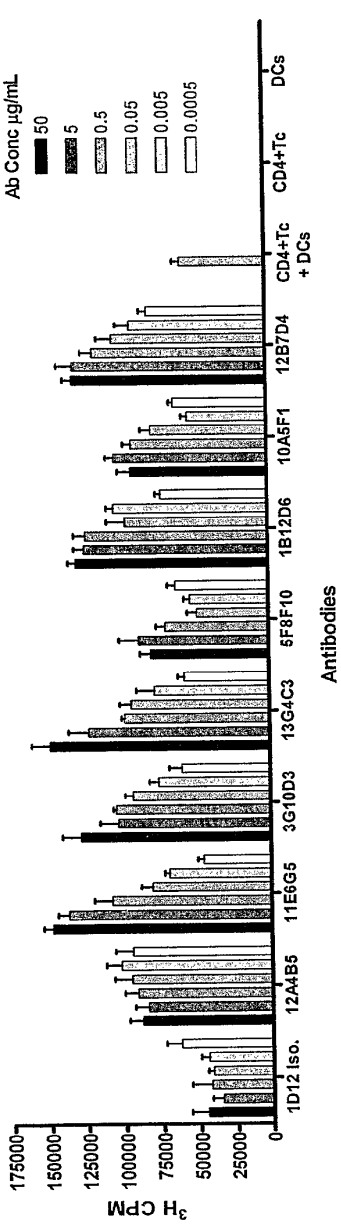

IL-2 (pg/ml)
500
400
300
200
100
0
No Ab
100
10
1
0.1
0.01
100
10
1
0.1
0.01
control Ab (ug/ml)
10A5 (ug/ml)

Figure 39D



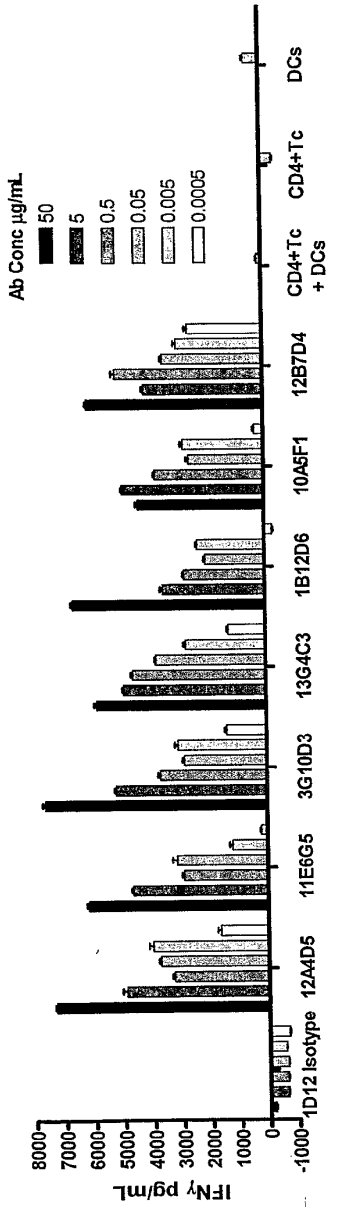


Figure 40

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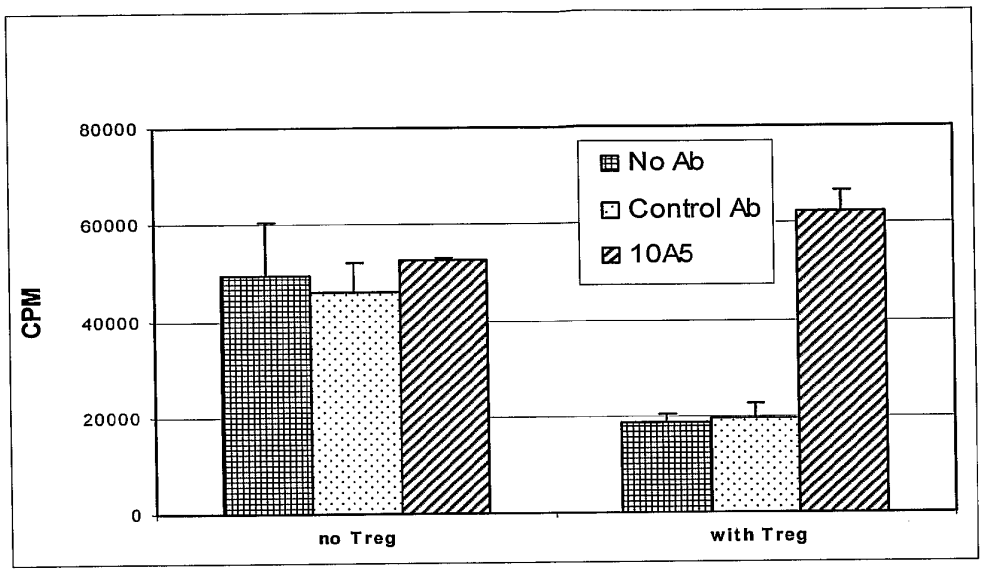

80000
60000
40000
20000
0
CPM
No Ab
Control Ab
10A5
no Treg
with Treg

Figure 41A

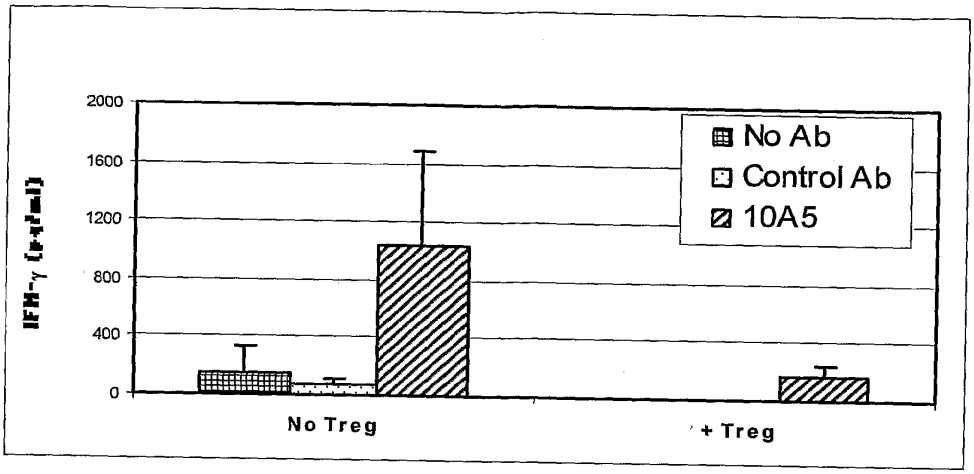


Figure 41B

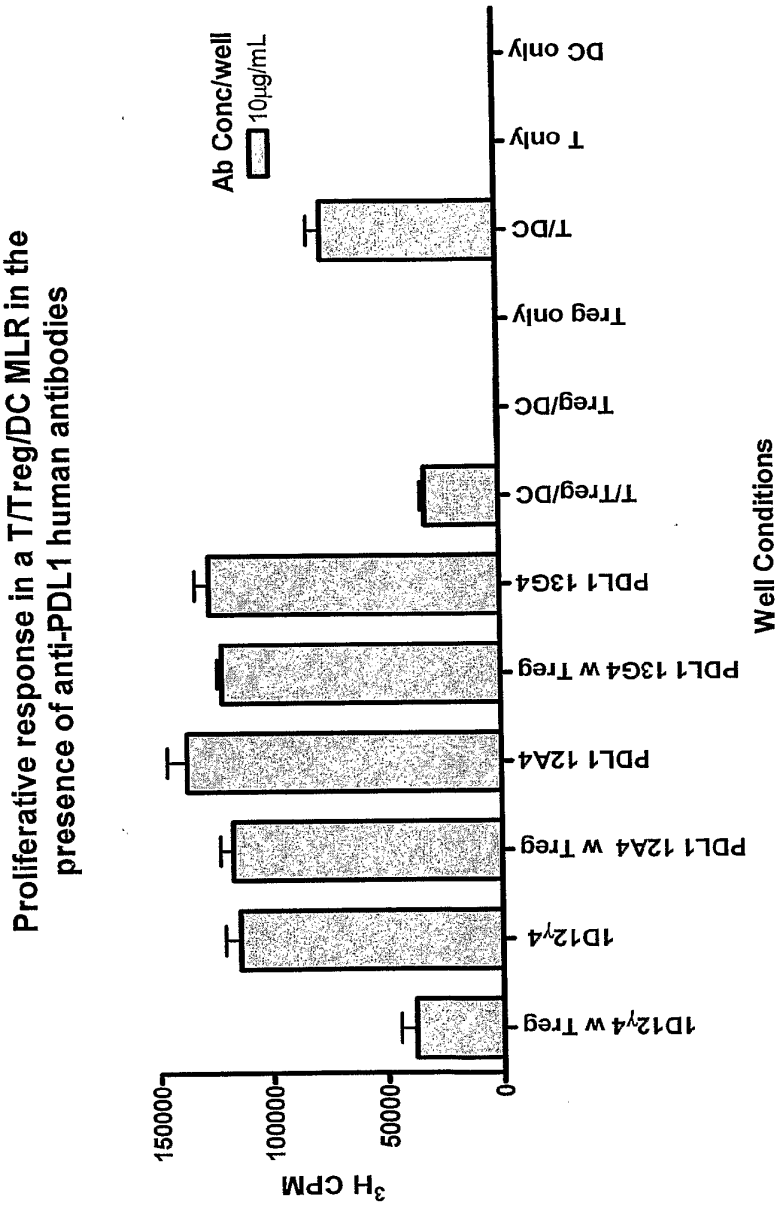

Proliferative response in a T/Treg/DC MLR in the presence of anti-PDL1 human antibodies
Ab Conc/well
10µg/mL
3H CPM
150000
100000
50000
0
1D12γ4 w Treg
1D12γ4
PDL1 12A4 w Treg
PDL1 12A4
PDL1 13G4 w Treg
PDL1 13G4
T/Treg/DC
Treg/DC
Treg only
T/DC
T only
DC only
Well Conditions

Figure 42

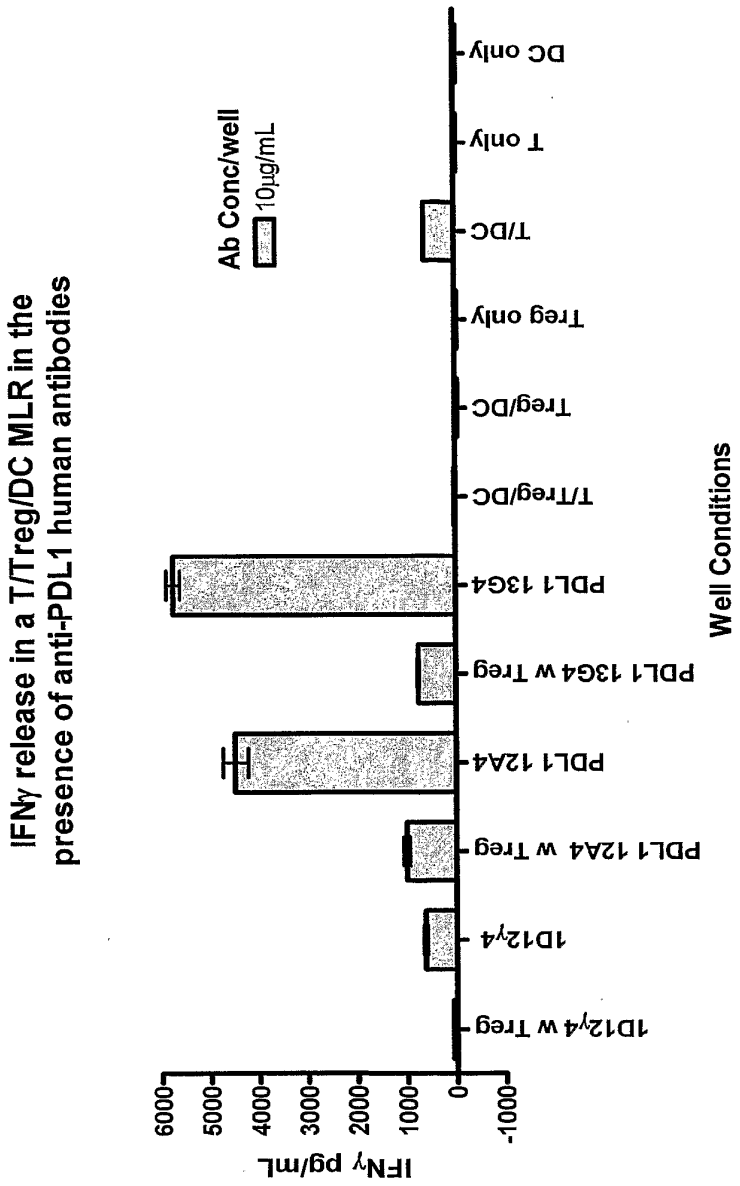

IFNγ release in a T/Treg/DC MLR in the presence of anti-PDL1 human antibodies
Ab Conc/well
10µg/mL
IFNγ pg/mL
6000
5000
4000
3000
2000
1000
0
-1000
1D12γ4 w Treg
1D12γ4
PDL1 12A4 w Treg
PDL1 12A4
PDL1 13G4 w Treg
PDL1 13G4
T/Treg/DC
Treg/DC
Treg only
T/DC
T only
DC only
Well Conditions

Figure 43

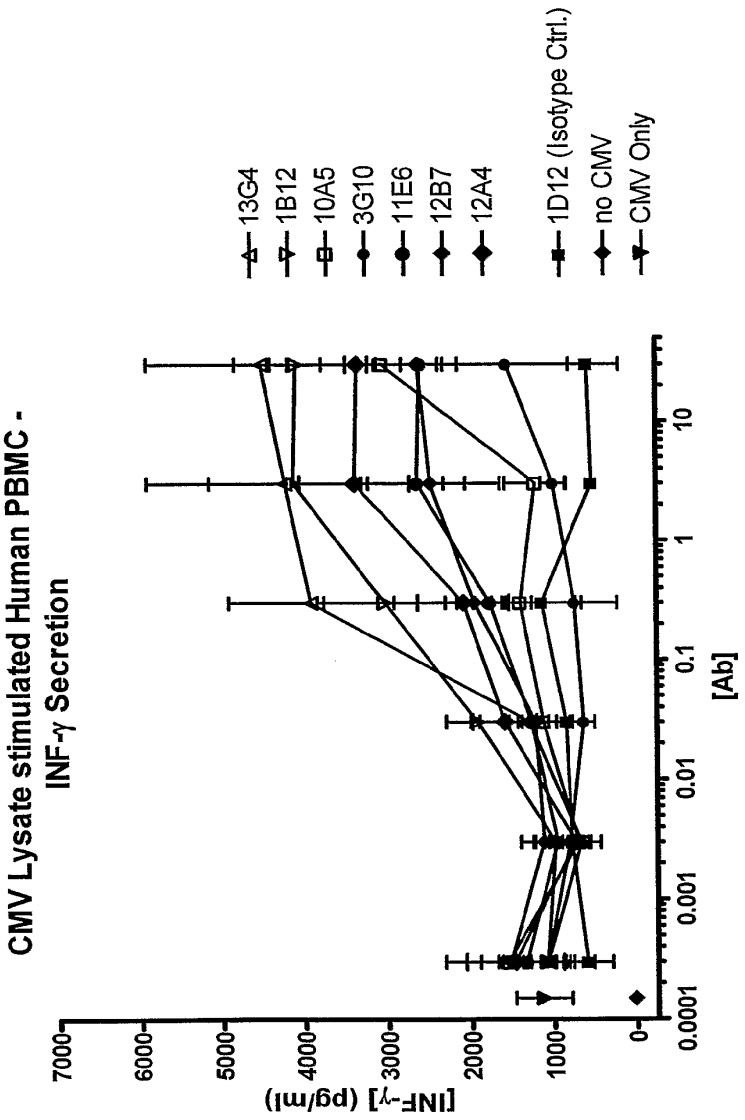


Figure 44

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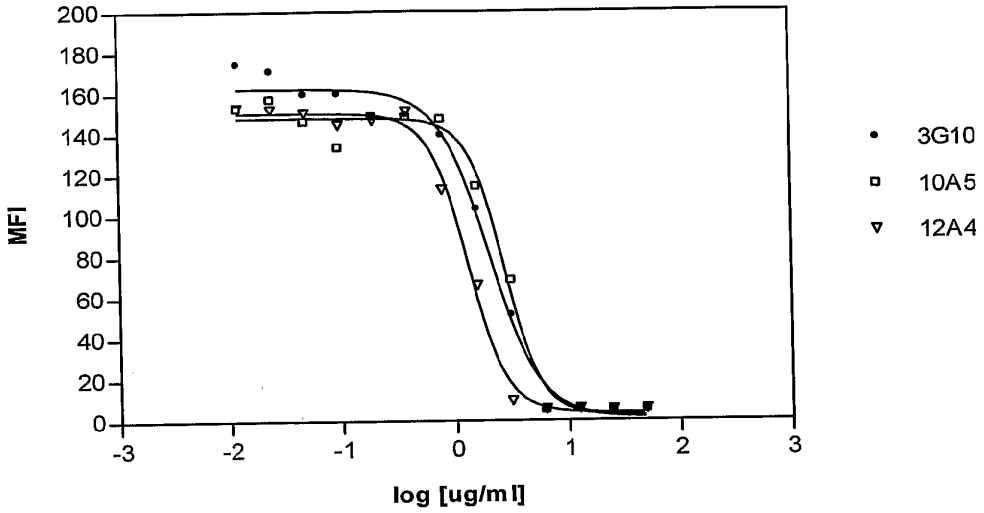
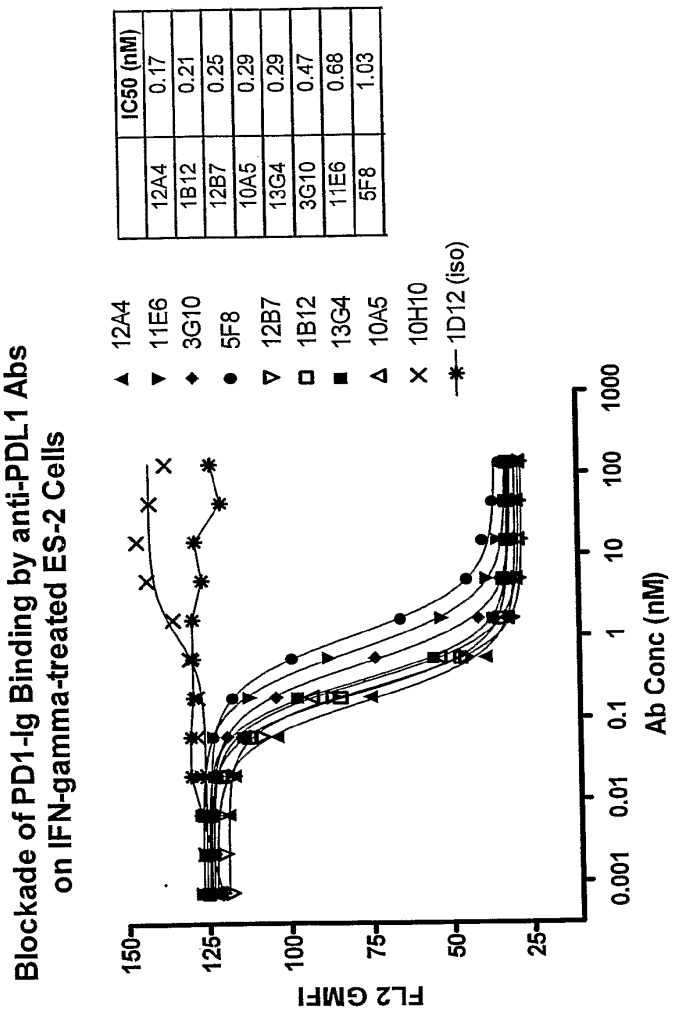

200
180
160
140
120
100
80
60
40
20
0
MFI
-3
-2
-1
0
1
2
3
log [ug/ml]
3G10
10A5
12A4

Figure 45



	IC50 (nM)
12A4	0.17
1B12	0.21
12B7	0.25
10A5	0.29
13G4	0.29
3G10	0.47
11E6	0.68
5F8	1.03

Figure 46

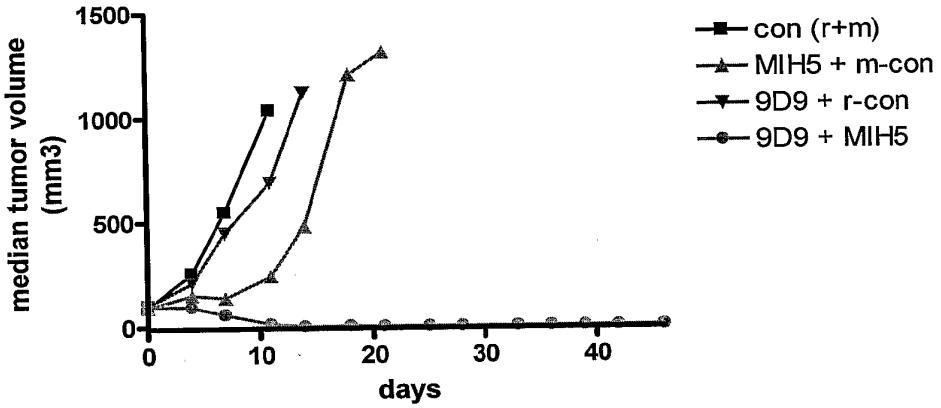


Figure 47

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(71) Applicant *(for all designated States except US)*: **PFIZER PRODUCTS INC.** [US/US]; Eastern Point Road, Groton, CT 06340 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **GOMEZ-NAVARRO, Jesus** [ES/US]; Pfizer Global Research & Development, 50 Pequot Avenue, New London, CT 06320 (US).

(74) Agents: **FULLER, Grover, F., Jr.** et al.; Pfizer Inc., 150 East 42nd Street, New York, NY 10017 (US).

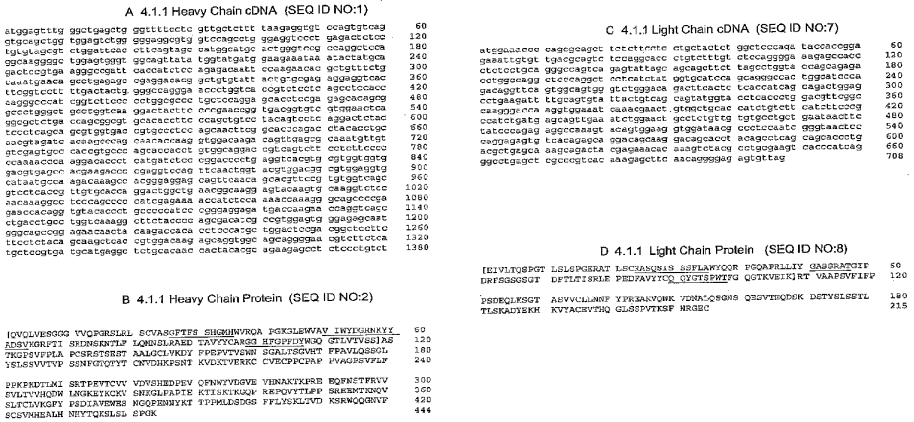
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(54) **Title:** CTLA4 ANTIBODY COMBINATION THERAPY



(57) **Abstract:** The invention relates to administration of an anti-CTLA4 antibody, particularly human antibodies to human CTLA4, such as those having amino acid sequences of antibodies 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, CP-675,206 (11.2.1), and ipilimumab, in combination with a therapeutic agent for treatment of cancer. An exemplary method of the invention comprises administering an anti-CTLA4 antibody, preferably, CP-675,206, and a chemotherapeutic agent, preferably, gemcitabine, for treatment of pancreatic cancer, among other treatment modalities.

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CTLA4 ANTIBODY COMBINATION THERAPY

Background of the Invention

The present invention relates to uses and compositions comprising an anti-CTLA4 antibody and at least one therapeutic agent for treatment of cancer. The invention further relates to administering, in addition to the antibody-therapeutic agent inhibitor combination, at least one additional therapy such as, among others, additional therapeutic agents, radiation and stem cell transplant.

Current antitumor agents act by a variety of mechanisms that inhibit cancer cell growth and division, ultimately destroying the malignant cell. However, because these cytotoxic agents are generally not selective for neoplastic cells, they destroy normal cells, disrupt physiologic functions, and are often associated with adverse effects. An alternative approach to cancer therapy is to target the immune system ("immunotherapy") rather than the tumor itself so that the patient's own immune system attacks tumors while sparing non-tumor cells.

One cancer immunotherapy approach targets cytotoxic T lymphocyte-associated antigen 4 (CTLA4; CD152), which is a cell surface receptor expressed on activated T cells. Binding of CTLA4 to its natural ligands, B7.1 (CD80) and B7.2 (CD86), delivers a negative regulatory signal to T cells, and blocking this negative signal results in enhanced T cell immune function and antitumor activity in animal models (Thompson and Allison *Immunity* **7**:445-450 (1997); McCoy and LeGros *Immunol.& Cell Biol.* **77**:1-10 (1999)). Several studies have demonstrated that CTLA4 blockade using antibodies markedly enhances T cell-mediated killing of tumors and can induce antitumor immunity (*see, e.g.*, Leach et al., *Science* 271:1734-1736 (1996); Kwon et al. *Proc. Natl. Acad. Sci. USA* **94**:8099-8103 (1997); Kwon et al., *Natl. Acad. Sci. USA* **96**:15074-15079 (1999); Yang et al. *Cancer Res* **57**:4036-41 (1997); Hurwitz et al. *Proc. Natl. Acad. Sci. USA* **95**:10067-71 (1998)). CTLA4 antibodies and their uses are described in, *e.g.*, the following applications and patents: U.S. Patent Application No. 09/472,087, now issued as U.S. Patent No. 6,682,736; Int. Appl. No. PCT/US99/30895 (published June 29, 2000, as WO 00/37504); U.S. Pat. Appl. No. 10/612,497 (published November 18, 2004, as US 2004/0228858); U.S. Pat. Appl. No. 10/776,649 (published November 18, 2004, as US 2004/0228861); Int. Appl. No. Int. Appl. No. PCT/US00/23356 (published March 1, 2001, as WO 01/14424) (*e.g.*, antibody 10D1, also known as MDX-010, and ipilimumab, Medarex, Princeton, NJ); Int. Appl. No. PCT/US99/28739 (published June 8, 2000, as WO 00/32231); U.S. Pat. Nos. 5,811,097, 5,855,887, 6,051,227, and 6,207,156; U.S. Pat. No. 5,844,095, to Linsley et al.; Int. Appl. No. PCT/US92/05202 (published Jan. 7, 1993, as WO 93/00431); U.S. Patent Appl. No. 10/153,382 (published May 8, 2003, as US 2003/0086930); U.S. Pat. Appl. No. 10/673,738 (published February 24, 2005 as US

2005/0042223); U.S. Pat. Appl. No. 11/085,368 (published October 13, 2005, as US 2005/0226875); U.S. Pat. Appl. No. 60/624,856 (filed Nov. 4, 2004), now published as WO2006/048749, May 11, 2006; U.S. Pat. Appl. No. 60/664,364 (filed March 23, 2005) and U.S. Pat. Appl. No. 60/711,707 (filed Aug. 26, 2005) now published as WO 2006/101691 Sept. 28, 2006; U.S. Pat. Appl. No. 60/664,653 (filed March 23, 2005) now published as WO 2006/101692, Sept. 28, 2006; U.S. Pat. Appl. No. 60/697,082 (filed July 7, 2005) now WO 2007/008463 published Jan. 18, 2007.

Despite the successes of currently available anti-cancer treatments, complete responses to these treatments or prolonged survival are infrequently observed, and the patient population refractory to these treatments is still large. Thus, there is an unmet need for the development of new therapeutic regimens, particularly those capable of augmenting or potentiating the anti-tumor activity of other anti-neoplastic agents while reducing the cytotoxic side effects of current chemotherapeutics, and the present invention meets this need.

Summary of the Invention

The present invention includes a method for the treatment of cancer in a patient in need of such treatment. The method comprises administering to the patient a therapeutically effective amount of an anti-CTLA4 antibody, *e.g.*, ipilimumab (also referred to as MDX-010) and CP-675,206 (also referred to as 11.2.1. and ticilimumab), in combination with a therapeutically effective amount of at least one therapeutic agent, wherein the cancer and the agent are selected from the group consisting of:

(a) the cancer is non-Hodgkin's lymphoma (NHL) and wherein the agent is rituximab;

(b) the cancer is NHL and the agent is cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP);

(c) the cancer is NHL and the agent is cyclophosphamide, doxorubicin, vincristine, prednisone and rituximab (CHOP-R);

(d) the cancer is lung cancer and the agent is bevacizumab;

(e) the cancer is non-small cell lung cancer (NSCLC) and the agent is gefitinib;

(f) the cancer is NSCLC and the agent is bevacizumab;

(g) the cancer is NSCLC and the agent is a taxane and gemcitabine, and further wherein the taxane is selected from the group consisting of docetaxel and paclitaxel;

(h) the cancer is NSCLC and the agent is a taxane and a platinum compound;

(i) the cancer is NSCLC and the agent is docetaxel;

(j) the cancer is NSCLC and the agent is erlotinib;

(k) the cancer is NSCLC and the agent is pemetrexed;

(l) the cancer is NSCLC and the agent is a platinum compound;

(m) the cancer is gastric cancer and the agent is irinotecan;

(n) the cancer is gastric cancer and the agent is fluorouracil and leucovorin;

(o) the cancer is liver cancer and the agent is doxorubicin, ifosfamide and vincristine;

(p) the cancer is liver cancer and the agent is doxorubicin and vincristine;

(q) the cancer is colorectal carcinoma (CRC) and the agent is fluorouracil;

(r) the cancer is CRC and the agent is capecitabine;

(s) the cancer is CRC and the agent is fluorouracil, leucovorin, and oxaliplatin (FOLFOX);

(t) the cancer is CRC and the agent is fluorouracil, leucovorin, and irinotecan (FOLFIRI);

(u) the cancer is CRC and the agent is cetuximab;

(v) the cancer is chronic myeloid leukemia (CML) and the agent is imatinib mesylate;

(w) the cancer is chronic lymphocytic leukemia (CLL) and the agent is imatinib mesylate;

(x) the cancer is pancreatic cancer and the agent is gemcitabine;

(y) the cancer is breast cancer and the agent is a taxane;

(z) the cancer is breast cancer and the agent is cyclophosphamide, doxorubicin and a taxane;

(aa) the cancer is breast cancer and the agent is selected from the group consisting of tamoxifen, anastrazole, letrozole, and fulvestrant;

(bb) the cancer is breast cancer and the agent is trastuzumab;

(cc) the cancer is breast cancer and the agent is bevacizumab;

(dd) the cancer is breast cancer and the agent is cetuximab;

(ee) the cancer is breast cancer and the agent is axitinib;

(ff) the cancer is bladder cancer and the agent is Bacillus Calmette-Guerin (BCG);

(gg) the cancer is bladder cancer and the agent is gemcitabine and cisplatin;

(hh) the cancer is melanoma and the agent is interferon alpha;

(ii) the cancer is multiple myeloma and the agent is bortezomib;

(jj) the cancer is multiple myeloma and the agent is dexamethasone and thalidomide; and

(kk) the cancer is ovarian cancer and the agent is carboplatin and paclitaxel.

In one aspect, the treatment is selected from the group consisting of neoadjuvant therapy, adjuvant therapy, first-line therapy, second-line therapy, and third-line therapy.

In another aspect, the agent is administered sequentially or contemporaneously with the antibody.

In yet another aspect, the taxane is paclitaxel and the platinum compound is carboplatin.

In one aspect, the method further comprises administering at least one agent selected from the group consisting of bevacizumab, PF03512676, and sunitinib.

In another aspect, the method further comprises administering at least one agent selected from the group consisting of erlotinib and pemetrexed, wherein the treatment comprises second line therapy.

In one aspect, the therapeutically effective amount of the antibody ranges from about 1 mg/kg to 40 mg/kg.

In another aspect, the therapeutically effective amount of the antibody ranges from about 3 mg/kg to 15 mg/kg.

In one aspect, the NHL is indolent NHL and the treatment comprises first line therapy.

In another aspect, the NHL is aggressive NHL and the treatment comprises second line therapy.

In a further aspect, the treatment comprises first line therapy.

In one aspect, the cancer is CRC and the agent is capecitabine and the treatment comprises first line therapy.

In another aspect, the cancer is CRC and the agent is FOLFOX and the treatment is selected from the group consisting of first line therapy and adjuvant therapy following surgical resection of a primary colon tumor.

In one aspect, the cancer is CML and the agent is imatinib mesylate and the treatment comprises first line therapy.

In another aspect, the cancer is CLL and the agent is imatinib mesylate and the treatment comprises first line therapy.

In a further aspect, the cancer is pancreatic cancer and the agent is gemcitabine and the pancreatic cancer is selected from the group consisting of non-resectable Stage II, locally advanced Stage III, and metastatic Stage IV and wherein the treatment comprises first line therapy.

In another aspect, the cancer is breast cancer and the agent is a taxane and the treatment comprises first line therapy.

In one aspect, the cancer is ovarian cancer and the agent is carboplatin and paclitaxel and the treatment comprises first line therapy.

The invention includes a kit for the treatment of NSCLC comprising a therapeutically effective amount of CP-675,206; a therapeutically effective amount of a carboplatin; a therapeutically effective amount of paclitaxel; an applicator; and an instructional material for the use of the kit.

In one aspect, the kit further comprises a therapeutically effective amount of at least one agent selected from the group consisting of bevacizumab, sunitinib, and PF03512676.

The invention includes a kit for the treatment of NSCLC comprising a therapeutically effective amount of CP-675,206; a therapeutically effective amount of an agent selected from the group consisting of docetaxel, erlotinib and pemetrexed; an applicator; and an instructional material for the use of the kit.

The invention includes a kit for the treatment of CRC comprising a therapeutically effective amount of CP-675,206; a therapeutically effective amount of carboplatin; a therapeutically effective amount of paclitaxel; an applicator; and an instructional material for the use of the kit.

The invention includes a kit for the treatment of CRC comprising a therapeutically effective amount of CP-675,206; a therapeutically effective amount of a fluorouracil; a therapeutically effective amount of leucovorin; a therapeutically effective amount of oxaliplatin; an applicator; and an instructional material for the use of the kit.

The invention includes a kit for the treatment of pancreatic cancer comprising a therapeutically effective amount of CP-675,206; a therapeutically effective amount of gemcitabine; an applicator; and an instructional material for the use of the kit.

The invention includes a kit for the treatment of ovarian cancer comprising a therapeutically effective amount of CP-675,206; a therapeutically effective amount of carboplatin; a therapeutically effective amount of paclitaxel; an applicator; and an instructional material for the use of the kit.

The invention includes a method for preventing or treating infection by HIV or for preventing, treating or delaying the onset of AIDS in a patient in need thereof. The method comprises administering to the patient a therapeutically effective amount of anti-CTLA4 antibody CP-675,206 and further comprises administering a therapeutically effective amount of at least one antiviral agent selected from the group consisting of an HIV protease inhibitor, a non-nucleoside reverse transcriptase inhibitor, a nucleoside/nucleotide reverse transcriptase inhibitor, a CCR5 antagonist, an inhibitor of gp120 interaction with CD4, an HIV fusion inhibitor, a HIV integrase inhibitor, an RNaseH inhibitor, a prenylation inhibitor, and a maturation inhibitor.

In one aspect, the CCR5 antagonist is maraviroc.

In another aspect, the method further comprises assessing the co-receptor tropism of the HIV.

The invention includes a method for preventing or treating infection by HIV or for preventing, treating or delaying the onset of AIDS in a patient in need thereof, where the

method comprises administering to the patient a therapeutically effective amount of an anti-CTLA4 antibody and maraviroc.

In one aspect, the anti-CTLA4 antibody is selected from the group consisting of CP-675,206 and ipilimumab.

Alternate embodiments of the invention are described below, such as those employing alternate anti-CTLA4 antibodies and involving different methods.

Brief Description of the Drawings

The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention there are shown in the drawings embodiment(s) which are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities shown.

In the drawings:

Figure 1, comprising Figures 1A-1D, shows the nucleotide and amino acid sequences of anti-CTLA4 antibody 4.1.1. Figure 1A shows the full length nucleotide sequence for the 4.1.1 heavy chain (SEQ ID NO:1). Figure 1B shows the full length amino acid sequence for the 4.1.1 heavy chain (SEQ ID NO:2), and the amino acid sequence for the 4.1.1 heavy chain variable region (SEQ ID NO:3) designated between brackets "[ ]". The amino acid sequence of each 4.1.1 heavy chain CDR is underlined. The CDR sequences are as follows: CDR1: GFTFSSHGMH (SEQ ID NO:4); CDR2: VIWYDGRNKYYADSV (SEQ ID NO:5); and CDR3: GGHFGPFDY (SEQ ID NO:6). Figure 1C shows the nucleotide sequence for the 4.1.1 light chain (SEQ ID NO:7). Figure 1D shows the amino acid sequence of the full length 4.1.1 light chain (SEQ ID NO:8), and the variable region as indicated between brackets "[ ]" (SEQ ID NO:9). The amino acid sequence of each CDR is indicated as follows: CDR1: RASQSISSSFLA (SEQ ID NO:10); CDR2: GASSRAT (SEQ ID NO:11); and CDR3: QQYGTSPWT (SEQ ID NO:12).

Figure 2, comprising Figures 2A-2D, shows the nucleotide and amino acid sequences of anti-CTLA4 antibody 4.13.1. Figure 2A shows the full length nucleotide sequence for the 4.13.1 heavy chain (SEQ ID NO:13). Figure 2B shows the full length amino acid sequence for the 4.13.1 heavy chain (SEQ ID NO:14), and the amino acid sequence for the 4.13.1 heavy chain variable region (SEQ ID NO:15) designated between brackets "[ ]". The amino acid sequence of each 4.13.1 heavy chain CDR is underlined. The CDR sequences are as follows: CDR1: GFTFSSHGIH (SEQ ID NO:16); CDR2: VIWYDGRNKDYADSV (SEQ ID NO:12); and CDR3: VAPLGPLDY (SEQ ID NO:18). Figure 2C shows the nucleotide

sequence for the 4.13.1 light chain (SEQ ID NO:19). Figure 2D shows the amino acid sequence of the full length 4.13.1 light chain (SEQ ID NO:20), and the variable region as indicated between brackets "[ ]" (SEQ ID NO:21). The amino acid sequence of each CDR is indicated as follows: CDR1: RASQSVSSYLA (SEQ ID NO:22); CDR2: GASSRAT (SEQ ID NO:23); and CDR3: QQYGRSPFT (SEQ ID NO:24).

Figure 3, comprising Figures 3A-3D, shows the nucleotide and amino acid sequences of anti-CTLA4 antibody CP-675,206. Figure 3A shows the full length nucleotide sequence for the CP-675,206 heavy chain (SEQ ID NO:25). Figure 3B shows the full length amino acid sequence for the CP-675,206 heavy chain (SEQ ID NO:26), and the amino acid sequence for the CP-675,206 heavy chain variable region (SEQ ID NO:27) designated between brackets "[ ]". The amino acid sequence of each CP-675,206 heavy chain CDR is underlined. The CDR sequences are as follows: CDR1: GFTFSSYGMH (SEQ ID NO:28); CDR2: VIWYDGSNKYYADSV (SEQ ID NO:29); and CDR3: DPRGATLYYYYYGMDV (SEQ ID NO:30). Figure 3C shows the nucleotide sequence for the CP-675,206 light chain (SEQ ID NO:31). Figure 3D shows the amino acid sequence of the full length CP-675,206 light chain (SEQ ID NO:32), and the variable region as indicated between brackets "[ ]" (SEQ ID NO:33). The amino acid sequence of each CDR is indicated as follows: CDR1: RASQSINSYLD (SEQ ID NO:34); CDR2: AASSLQS (SEQ ID NO:35); and CDR3: QQYYSTPFT (SEQ ID NO:36).

Figure 4 is a graph depicting the plasma serum levels in Rhesus monkeys following exposure to an anti-CTLA4 antibody. All Rhesus monkeys were administered influenza vaccine FLUZONE intramuscularly (IM) on week 0 and again on week 4. In addition to FLUZONE, animals in Group 1 (animal number 1 (closed circle), 2 (closed square), and 3 (closed triangle)) received a single dose of anti-CTL4 antibody 4.1.1 (also referred to as CP-642,570) at 5 mg/kg intravenously (IV) on week 0. Animals in Group 3 (animal number 4 (open circle), 5 (open square), 6 (open triangle), and 7 (marked by "x")) received a single dose of an irrelevant human antibody (anti-KLH antibody) at 5 mg/kg IV on week 0.

Figure 5 is a graph depicting the serum level of a neopterin, a marker which is suggestive of immune activity, in Rhesus monkeys immunized with FLUZONE IM on week 0 and week 4 and administered anti-CTLA4 antibody 4.1.1 at 5 mg/kg IV on week 0. Animal number AG40 (closed diamond), number AK80 (closed square) and number AM90 (closed triangle) were in group 1 and received anti-CTLA4 antibody and FLUZONE on day 0 followed by FLUZONE on week 0. Animal number AK76 (x), number AM84 (*), and number BC13 (open circle) were in control group 2 and were administered FLUZONE on week 0 and week 4 and also administered an irrelevant human antibody (anti-KLH antibody) at 5 mg/ml IV on week 0 of the protocol.

Figure 6 is a graph depicting the level of a 2-5 adenylate synthetase in white blood cell pellets isolated from the blood of Rhesus monkeys immunized with FLUZONE IM on weeks 0 and 4 and further administered anti-CTLA4 antibody 4.1.1 (5mg/kg IV) on week 0. Peripheral blood cells were obtained from each animal in each group 1 (FLUZONE IM weeks 0 and 4 and anti-CTLA4 antibody week 0) and group 2 (FLUZONE IM on weeks 0 and 4 and an irrelevant antibody (anti-KLH 5 mg/kg IV) administered on week 0) at week 2 (light gray shading), week 4 (darker gray shading) and week 6 (unshaded bars). Where the values exceeded 4000 pmol/dl, the values are shown in each bar. The cells were pelleted and the level of 2-5 adenylate synthetase in the pellets obtained from each animal was assessed.

Figure 7 is a graph depicting the IgG anti-FLUZONE titer in Rhesus monkeys administered anti-CTLA4 antibody. Serum anti-FLUZONE IgG titers were assessed for animals in Group 1 (open triangle; administered FLUZONE on weeks 0 and 4 and administered anti-CTLA4 antibody on week 0), Group 2 (open square; administered FLUZONE on weeks 0 and 4 and administered an irrelevant human anti-KLH antibody on week 0), Group 3 (closed circle; administered FLUZONE on weeks 0 and 4 and administered anti-CTLA4 antibody on week 4), Group 4 (open circle; administered FLUZONE on weeks 0 and 4 and administered an irrelevant human anti-KLH antibody on week 4) and Group 5 (closed triangle; administered FLUZONE on weeks 0 and 4 but no antibody was administered). FLUZONE was administered IM and antibodies were administered IV at 5 mg/kg. Anti-FLUZONE IgG titers were assessed one week before immunization with FLUZONE (week -1, also referred to as "prebleed") and then at week 0, week 2, week 4, week 6 and week 8. Animals in Group 3 (immunized with FLUZONE on weeks 0 and 4 and administered anti-CTLA4 antibody on week 4) demonstrated increased anti-FLUZONE IgG serum titers compared with animals in the other groups.

Figure 8 is a graph depicting the anti-FLUZONE IgG serum titer in Rhesus monkeys immunized with FLUZONE and on weeks 0 and 4 and administered anti-CLTA4 antibody on week 0 (Group 1), immunized with FLUZONE on weeks 0 and 4 and administered an irrelevant antibody on week 0 (Group 2), immunized with FLUZONE on weeks 0 and 4 and administered anti-CTLA4 antibody on week 4 (Group 3), immunized with FLUZONE on weeks 0 and 4 and administered an irrelevant antibody on week 0 (Group 4), and immunized with FLUZONE on weeks 0 and 4 (Group 5). The data points are separated by animal group and the titers for each individual animal within each group for the following data points: prebleed (week -1; +), week 0 (open triangle), week 2 (closed triangle), week 4 (open squares), week 6 (closed circle) and week 8 (open circle).

Figure 9 is a bar graph depicting the anti-FLUZONE IgG antibody titers at week 6 in Rhesus monkeys immunized with FLUZONE on weeks 0 and 4 (Groups 1-5). Group 1

animals received anti-CTLA4 (5 mg/kg IV) on week 0. Group 2 animals received irrelevant antibody anti-KLH (5 mg/kg IV) on week 0. Group 3 animals received anti-CTLA4 (5 mg/kg IV) on week 4. Group 4 animals received irrelevant antibody anti-KLH (5 mg/kg IV) on week 4. Group 5 animals received only FLUZONE immunizations on weeks 0 and 4.

Detailed Description Of The Invention

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art.

The methods and techniques of the present invention are generally performed according to methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. Such references include, *e.g.*, Sambrook and Russell, *Molecular Cloning, A Laboratory Approach*, Cold Spring Harbor Press, Cold Spring Harbor, NY (2001), Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (2002), and Harlow and Lane *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1990), which are incorporated herein by reference. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

As used herein, each of the following terms has the meaning associated with it in this section.

The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See *Immunology--A Synthesis* (2nd Edition, E. S. Golub and D. R. Gren,

Eds., Sinauer Associates, Sunderland, Mass. (1991)), which is incorporated herein by reference.

A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain R group with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See, e.g., Pearson, *Methods Mol. Biol.* **243**:307-31 (1994).

Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine, and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartic acid and glutamic acid; and 7) sulfur-containing side chains: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine.

Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet et al., *Science* **256**:1443-45 (1992), herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, and (4) confer or modify other physicochemical or functional properties of such analogs. Analogs comprising substitutions, deletions, and/or insertions can include various muteins of a sequence other than the specified peptide sequence. For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the specified sequence (preferably in the portion of the polypeptide outside the domain(s) forming intermolecular contacts, *e.g.*, outside of the CDRs). A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman and Company, New York (1984)); *Introduction to Protein Structure* (C. Branden and J. Tooze,

eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al., *Nature* **354**:105 (1991), which are each incorporated herein by reference.

Sequence similarity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, Genetics Computer Group (GCG available from Genetics Computer Group, Inc.), also referred to as the Wisconsin Package, is an integrated software package of over 130 programs for accessing, analyzing and manipulating nucleotide and protein sequences. GCG contains programs such as "Gap" and "Bestfit" which can be used with default parameters to determine sequence similarity, homology and/or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, e.g., GCG version 6.1, version 7.0, version 9.1, and version 10.0.

Polypeptide sequences also can be compared using FASTA, a program in GCG, using default or recommended parameters. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, *Methods Enzymol.* **183**:63-98 (1990); Pearson, *Methods Mol. Biol.* **132**:185-219 (2000)). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially blastp or tblastn, using default parameters. *See, e.g.*, Altschul et al., *J. Mol. Biol.* **215**:403-410 (1990); Altschul et al., *Nucleic Acids Res.* **25**:3389-402 (1997); herein incorporated by reference.

An intact "antibody" comprises at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. See generally, *Fundamental Immunology*, Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). Each heavy chain is comprised of a heavy chain variable region (HCVR or V_H) and a heavy chain constant region (C_H). The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (LCVR or V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxyl-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat, *Sequences of Proteins of*

Immunological Interest (National Institutes of Health, Bethesda, MD (1987 and 1991)), or Chothia & Lesk, *J. Mol. Biol.* **196**:901-917 (1987); Chothia et al., *Nature* **342**:878-883 (1989).

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., CTLA4). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_H1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_H1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) *Nature* **341**:544-546), which consists of a V_H domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv)); see e.g., Bird et al. *Science* **242**:423-426 (1988) and Huston et al. *Proc. Natl. Acad. Sci. USA* **85**:5879-5883 (1988)). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which V_H and V_L domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see e.g., Holliger et al. *Proc. Natl. Acad. Sci. USA* **90**:6444-6448 (1993); Poljak et al. *Structure* **2**:1121-1123 (1994)).

Still further, an antibody or antigen-binding portion thereof may be part of larger immunoadhesion molecules, formed by covalent or noncovalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov et al. *Human Antibodies and Hybridomas* **6**:93-101 (1995)) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov et al. *Mol. Immunol.* **31**:1047-1058 (1994)). Other examples include where one or more CDRs from an antibody are incorporated into a molecule either covalently or noncovalently to make it an immunoadhesin that specifically binds to an antigen of interest, such as CTLA4. In such embodiments, the CDR(s) may be incorporated as part of a larger polypeptide chain, may be covalently linked to another

polypeptide chain, or may be incorporated noncovalently. Antibody portions, such as Fab and $F(ab')_2$ fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein.

Where an "antibody" is referred to herein with respect to the present invention, it should be understood that an antigen-binding portion thereof may also be used. An antigen-binding portion competes with the intact antibody for specific binding. *See generally, Fundamental Immunology*, Ch. 7 (Paul, W., ed., 2nd ed., Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). Antigen-binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. In some embodiments, antigen-binding portions include Fab, Fab', $F(ab')_2$, Fd, Fv, dAb, and complementarity determining region (CDR) fragments, single-chain antibodies (scFv), chimeric antibodies, diabodies and polypeptides that contain at least a portion of an antibody that is sufficient to confer specific antigen binding to the polypeptide. In embodiments having one or more binding sites, the binding sites may be identical to one another or may be different.

The terms "human antibody" or "human sequence antibody", as used interchangeably herein, include antibodies having variable and constant regions (if present) derived from human germline immunoglobulin sequences. The human sequence antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo). However, the term "human antibody", as used herein, is not intended to include "chimeric" antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences (*i.e.*, "humanized" or PRIMATIZED™ antibodies).

The term "chimeric antibody" as used herein means an antibody that comprises regions from two or more different antibodies. In one embodiment, one or more of the CDRs are derived from a human anti-CTLA4 antibody. In another embodiment, all of the CDRs are derived from a human anti-CTLA4 antibody. In another embodiment, the CDRs from more than one human anti-CTLA4 antibodies are combined in a chimeric human antibody. For instance, a chimeric antibody may comprise a CDR1 from the light chain of a first human anti-CD40 antibody, a CDR2 from the light chain of a second human anti-CTLA4 antibody and a CDR3 and CDR3 from the light chain of a third human anti-CTLA4 antibody, and the CDRs from the heavy chain may be derived from one or more other anti-CD40 antibodies. Further,

the framework regions may be derived from one of the same anti-CTLA4 antibodies or from one or more different human(s).

Moreover, as discussed previously herein, chimeric antibody includes an antibody comprising a portion derived from the germline sequences of more than one species.

"Glycoform" refers to a complex oligosaccharide structure comprising linkages of various carbohydrate units. Such structures are described in, *e.g.*, *Essentials of Glycobiology* Varki et al., eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1999), which also provides a review of standard glycobiology nomenclature. Such glycoforms include, but are not limited to, G2, G1, G0, G-1, and G-2 (*see*, *e.g.*, International Patent Publication No. WO 99/22764).

"Glycosylation pattern" is defined as the pattern of carbohydrate units that are covalently attached to a protein (*e.g.*, the glycoform) as well as to the site(s) to which the glycoform(s) are covalently attached to the peptide backbone of a protein, more specifically to an immunoglobulin protein.

It is likely that antibodies expressed by different cell lines or in transgenic animals will have different glycoforms and/or glycosylation patterns compared with each other. However, all antibodies encoded by the nucleic acid molecules provided herein, or comprising the amino acid sequences provided herein are part of the instant invention, regardless of the glycosylation of the antibodies.

By the term "effective amount", or "therapeutically effective amount," as used herein, is meant an amount that when administered to a mammal, preferably a human, mediates a detectable therapeutic response compared to the response detected in the absence of the compound. A therapeutic response, such as, but not limited to, inhibition of and/or decreased tumor growth, tumor size, metastasis, and the like, can be readily assessed by a plethora of art-recognized methods, including, *e.g.*, such methods as disclosed herein.

The skilled artisan would understand that the effective amount of the compound or composition administered herein varies and can be readily determined based on a number of factors such as the disease or condition being treated, the stage of the disease, the age and health and physical condition of the mammal being treated, the severity of the disease, the particular compound being administered, and the like.

By the term "compete", as used herein with regard to an antibody, is meant that a first antibody, or an antigen-binding portion thereof, competes for binding with a second antibody, or an antigen-binding portion thereof, where binding of the first antibody with its cognate epitope is detectably decreased in the presence of the second antibody compared to the binding of the first antibody in the absence of the second antibody. The alternative, where the binding of the second antibody to its epitope is also detectably decreased in the presence of

the first antibody, can, but need not be the case. That is, a first antibody can inhibit the binding of a second antibody to its epitope without that second antibody inhibiting the binding of the first antibody to its respective epitope. However, where each antibody detectably inhibits the binding of the other antibody with its cognate epitope or ligand, whether to the same, greater, or lesser extent, the antibodies are said to "cross-compete" with each other for binding of their respective epitope(s). For instance, cross-competing antibodies can bind to the epitope, or potion of the epitope, to which the antibodies used in the invention bind. Use of both competing and cross-competing antibodies is encompassed by the present invention. Regardless of the mechanism by which such competition or cross-competition occurs (*e.g.*, steric hindrance, conformational change, or binding to a common epitope, or portion thereof, and the like), the skilled artisan would appreciate, based upon the teachings provided herein, that such competing and/or cross-competing antibodies are encompassed and can be useful for the methods disclosed herein.

The term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and nonconformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

"Instructional material," as that term is used herein, includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the compound, combination, and/or composition of the invention in the kit for affecting, alleviating or treating the various diseases or disorders recited herein. Optionally, or alternately, the instructional material can describe one or more methods of alleviating the diseases or disorders in a cell, a tissue, or a mammal, including as disclosed elsewhere herein.

The instructional material of the kit may, for example, be affixed to a container that contains the compound and/or composition of the invention or be shipped together with a container which contains the compound and/or composition. Alternatively, the instructional material may be shipped separately from the container with the intention that the recipient uses the instructional material and the compound cooperatively.

Except when noted, the terms "patient" or "subject" are used interchangeably and refer to mammals such as human patients and non-human primates, as well as veterinary subjects such as rabbits, rats, and mice, and other animals. Preferably, patient refers to a human.

Conventional notation is used herein to portray polypeptide sequences: the left-hand end of a polypeptide sequence is the amino-terminus; the right-hand end of a polypeptide sequence is the carboxyl-terminus.

By the phrase "specifically binds," as used herein, is meant a compound, *e.g.*, a protein, a nucleic acid, an antibody, and the like, which recognizes and binds a specific molecule, but does not substantially recognize or bind other molecules in a sample. For instance, an antibody or a peptide inhibitor which recognizes and binds a cognate ligand (*e.g.*, an anti-CTLA4 antibody that binds with its cognate antigen, CTLA4) in a sample, but does not substantially recognize or bind other molecules in the sample. Thus, under designated assay conditions, the specified binding moiety (*e.g.*, an antibody or an antigen-binding portion thereof) binds preferentially to a particular target molecule and does not bind in a significant amount to other components present in a test sample. A variety of assay formats may be used to select an antibody that specifically binds a molecule of interest. For example, solid-phase ELISA immunoassay, immunoprecipitation, BIAcore, FACS, and Western blot analysis are among many assays that may be used to identify an antibody that specifically reacts with CTLA4. Typically, a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 times background, even more specifically, an antibody is said to "specifically bind" an antigen when the equilibrium dissociation constant (K_D) is ≤ 1 μM, preferably ≤ 100 nM and most preferably ≤ 10 nM.

The term "K_D" refers to the equilibrium dissociation constant of a particular antibody-antigen interaction.

As used herein, "substantially pure" means an object species is the predominant species present (*i.e.*, on a molar basis it is more abundant than any other individual species in the composition), and preferably a substantially purified fraction is a composition wherein the object species (*e.g.*, an anti-CTLA4 antibody) comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, more preferably more than about 85%, 90%, 95%, and 99%. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

As used herein, to "treat" means reducing the frequency with which symptoms of a disease (*i.e.*, tumor growth and/or metastasis, or other effect mediated by the numbers and/or activity of immune cells, and the like) are experienced by a patient. The term includes the administration of the compounds or agents of the present invention to prevent or delay the onset of the symptoms, complications, or biochemical indicia of a disease, alleviating the

symptoms or arresting or inhibiting further development of the disease, condition, or disorder. Treatment may be prophylactic (to prevent or delay the onset of the disease, or to prevent the manifestation of clinical or subclinical symptoms thereof) or therapeutic suppression or alleviation of symptoms after the manifestation of the disease.

"Combination therapy" embraces the administration of an immunostimulatory anti-CTLA4 antibody, preferably, CP-675,206, and another therapeutic agent as part of a specific treatment regimen optionally including a maintenance phase, intended to provide a beneficial effect from the co-action of these therapeutic agents. The beneficial effect of the combination includes, but is not limited to, pharmacokinetic or pharmacodynamic co-action resulting from the combination of therapeutic agents. Administration of these therapeutic agents in combination typically is carried out over a defined time period (usually minutes, hours, days or weeks depending upon the combination selected). "Combination therapy" generally is not intended to encompass the administration of two or more of these therapeutic agents as part of separate monotherapy regimens that incidentally and arbitrarily result in the combinations of the present invention.

"Combination therapy" embraces administration of these therapeutic agents in a sequential manner, that is, wherein each therapeutic agent is administered at a different time, as well as administration of these therapeutic agents, or at least two of the therapeutic agents, in a substantially simultaneous manner. Sequential or substantially simultaneous administration of each therapeutic agent can be effected by any appropriate route including, but not limited to, oral routes, intravenous routes, intramuscular, subcutaneous routes, and direct absorption through mucous membrane tissues. The therapeutic agents can be administered by the same route or by different routes. For example, a first therapeutic agent (*e.g.*, a chemotherapeutic agent) can be administered orally, and a second agent (e.g., anti-CTLA4 antibody) can be administered intravenously. Further, a first therapeutic agent of the combination selected may be administered by intravenous injection while the other therapeutic agents of the combination may be administered orally. Alternatively, for example, both the therapeutic agents may be administered by intravenous or subcutaneous injection.

In the present specification the term "sequential" means, unless otherwise specified, characterized by a regular sequence or order, *e.g.*, if a dosage regimen includes the administration of an anti-CTLA4 antibody and a chemotherapeutic agent, a sequential dosage regimen could include administration of the anti-CTLA4 antibody before, simultaneously, substantially simultaneously, or after administration of the chemotherapeutic agent, but both agents will be administered in a regular sequence or order. The term "separate" means, unless otherwise specified, to keep apart one from the other. The term "simultaneously" means, unless otherwise specified, happening or done at the same time, *i.e.*, the compounds

of the invention are administered at the same time. The term "substantially simultaneously" means that the compounds are administered within minutes of each other (*e.g.*, within 10 minutes of each other) and intends to embrace joint administration as well as consecutive administration, but if the administration is consecutive it is separated in time for only a short period (*e.g.*, the time it would take a medical practitioner to administer two compounds separately). As used herein, concurrent administration and substantially simultaneous administration are used interchangeably. Sequential administration refers to temporally separated administration of the anti-CTLA4 antibody and the chemotherapeutic agent.

"Combination therapy" also can embrace the administration of the therapeutic agents as described above in further combination with other biologically active ingredients (such as, but not limited to, a second and different antineoplastic agent, a dendritic cell vaccine or other tumor vaccine) and non-drug therapies (such as, but not limited to, surgery or radiation treatment). Where the combination therapy further comprises radiation treatment, the radiation treatment may be conducted at any suitable time so long as a beneficial effect from the co-action of the combination of the therapeutic agents and radiation treatment is achieved. For example, in appropriate cases, the beneficial effect is still achieved when the radiation treatment is temporally removed from the administration of the therapeutic agents, perhaps by days or even weeks.

As used herein, the term "adjuvant therapy" refers to treatment given after the primary treatment, including, without limitation, radiation, chemotherapy, hormone therapy, etc. The goal of adjuvant therapy is to increase the patients' chances of remission or cure, to increase the patients' overall survival benefit, and to help decrease the risk of recurrence. Therefore, it will be understood that if the anti-CTLA4 antibody and therapeutic agent combination is administered as adjuvant therapy, the combination may be administered to the patient after the primary treatment, *e.g.*, the patient is given a regimen of surgery, radiation and/or chemotherapy, followed by a course of a combination of an anti-CTLA4 antibody and a therapeutic agent. In this regard, the dose of anti-CTLA4 antibody and therapeutic agent may be considered a therapeutic dose or a maintenance dose, depending on the goals of the adjuvant therapy. The term "neoadjuvant therapy" refers to treatment given before the primary treatment, including, without limitation, surgery, radiation, chemotherapy, etc. In the neoadjuvant setting, the dose of anti-CTLA4 antibody and therapeutic agent is considered a therapeutic dose.

The term "first-line therapy" refers to the first type of therapy given for a condition or disease, or the first therapy of choice for the treatment of a particular type of cancer. It necessarily follows that the term "second-line therapy" therefore refers to the treatment given when the initial or first-line therapy is unsuccessful, and "third-line therapy" refers to a

treatment or treatment regimen that is given when both the initial treatment and the subsequent treatment are unsuccessful.

I. Combination Therapy

A. Cancer

CTLA4 antibodies described herein can be used to treat a wide variety of cancers. Without wishing to be bound by any particular theory, administration of a therapeutic agent, when administered with an immunoenhancing anti-CTLA4 antibody, may provide a synergistic effect. Without wishing to be bound by any theory of the invention, the agent that mediates an anti-tumor effect may mediate a decrease in tumor load, may mediate an increase of tumor antigens in the host antigen-presentation route, may decrease inflammation such that the antibody and/or other therapeutic agents may better penetrate the tumor, and/or may mediate a decrease in immune-suppressive tumor factors. Thus, the combination of an anti-CTLA4 antibody and at least one therapeutic agent may mediate a synergistic therapeutic effect thereby providing a benefit to a patient in need thereof which is greater than either compound alone.

Cancers that may be treated include, but are not limited to human sarcomas and carcinomas, *e.g.*, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, Kaposi's sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, erythroblastoma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, a primary or secondary brain tumor, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, uterine cancer, cervical cancer, prostate cancer, skin cancer, bone cancer, cancer of the small intestine, cancer of the anal region, cancer of the head or neck, gastrointestinal (gastric, colorectal, and duodenal) cancer, esophageal cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, cutaneous or intraocular melanoma, carcinoma of the endometrium, carcinoma of the vagina, carcinoma of the fallopian tubes, carcinoma of the vulva, carcinoma of the cervix, neoplasms of the central nervous system (CNS), including primary or secondary CNS tumors, spinal axis tumors, primary CNS lymphoma, brain stem glioma, pituitary adenoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, stomach cancer, colon cancer, cancer of the rectum, renal cell carcinoma (RCC), hepatoma, bile duct carcinoma, gall bladder cancer, liver cancer, kidney cancer, gastro-esophageal cancer, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, testicular tumor, cancer of the penis, lung carcinoma, small cell lung carcinoma (SCLC), non-small cell lung cancer (NSCLC), thyroid cancer, cancer of the parathyroid gland,

carcinoid tumors, cancer of the urethra, cancer of the ureter, cancer of the renal pelvis, cancer of the endocrine system, cancer of the adrenal gland, pancreatic endocrine tumors (such as pheochromocytoma, insulinoma, vasoactive intestinal peptide tumor, islet cell tumor and glucagonoma), bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, pituitary adenoma, adrenocortical cancer, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, tumors of the blood vessels (including benign and malignant tumors such as hemangioma, hemangiosarcoma, hemangioblastoma and lobular capillary hemangioma), acoustic neuroma, oligodendroglioma, meningioma, melanoma, cholangiocarcinoma, neuroblastoma, retinoblastoma, leukemias, acute lymphocytic leukemia, acute myelocytic leukemia, myeloblastic leukemia, promyelocytic leukemia, myelomonocytic leukemia, monocytic leukemia, erythroleukemia, chronic leukemia, lymphocytic leukemia, chronic myeloid leukemia, chronic myelogenous leukemia, chronic myelocytic leukemia, chronic granulocytic leukemia, chronic lymphocytic leukemia, lymphocytic lymphomas, polycythemia vera, lymphoma, Hodgkin's disease lymphoma, non-Hodgkin's disease lymphoma, cutaneous T-cell lymphoma (CTCL), cutaneous B-cell lymphoma, multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, soft tissue sarcomas, gastrointestinal stromal tumors (GIST), glioblastomas, or a combination of one or more of the foregoing cancers.

In preferred embodiments, the cancers include non-Hodgkin's lymphoma, non-small cell lung cancer, colorectal carcinoma, chronic myeloid leukemia, chronic lymphocytic leukemia, pancreatic cancer, breast cancer and ovarian cancer.

Various preferred combination therapies are described herein for a variety of cancers; however, in certain embodiments described herein, the invention is not limited to these, or any other, combination therapies.

1. Non-Hodgkin's lymphoma (NHL)

In one embodiment, the invention includes administering CP-675,206 to treat NHL, in combination with at least one therapeutic agent, preferably, rituximab (RITUXAN; Genentech, San Francisco, CA).

The combination can be administered to treat a patient afflicted with indolent NHL or aggressive NHL. In one embodiment, the NHL is indolent NHL, and the combination of CP-675,206 and rituximab comprises first-line therapy. In a further embodiment, the combination therapy is administered as second-line therapy following cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) and/or CHOP-R (cyclophosphamide, doxorubicin, vincristine, and prednisone with rituximab). In yet a further embodiment, the combination is followed by administration of rituximab as a single agent (SA) therapy. The combination of rituximab and CP-675,206 may increase and/or prolong an anti-tumor cell response thereby providing a therapeutic benefit to a patient suffering from NHL.

In one embodiment, a combination of CP-675,206 and rituximab is administered as a second-line therapy to treat aggressive NHL refractory to CHOP-R. In another embodiment, where the treatment is second-line therapy for aggressive NHL refractory to CHOP-R, the method further comprises administering rituximab as a single agent therapy following administration of the CP-675,206/rituximab combination.

In another aspect, the combination of CP-675,206 and rituximab is administered to a patient as a second-line therapy following another treatment including, but not limited to, a chemotherapeutic therapy or a bone marrow transplant. In one aspect, the CP-675,206 rituximab combination is administered following high-dose bone marrow ablative chemotherapy and bone marrow transplant (BMT). In another aspect, the combination is administered as a first-line therapy prior to bone marrow ablation chemotherapy and BMT. Methods well-known in the art for assessing therapeutic strategies for treatment of NHL, which consider various known factors such as age and condition of the patient, stage of the disease, and the like, are available to determine when the combination therapy is indicated to effectively treat a patient afflicted with aggressive NHL.

2. Non-small cell lung cancer (NSCLC)

In one embodiment, the invention includes a method for treating NSCLC comprising administering a combination comprising CP-675,206 and a therapeutic agent to a patient in need of treatment. In one aspect, the agent is a non-immunesuppressive chemotherapeutic agent, such as, but not limited to, gefitinib (IRESSA). The combination of CP-675,206 and therapeutic agent may increase the immune response to the tumor due to, *inter alia*, increased release of tumor antigen associated with the cytotoxic effect of the chemotherapeutic agent. Thus, the skilled artisan, based upon the disclosure provided herein, would appreciate that while any therapeutic agent that mediates cell death and/or release of tumor antigen can be used with an anti-CTLA4 antibody to provide an increased immune response to the tumor and, thereby, providing a therapeutic benefit to the patient. Moreover, the skilled artisan would further understand once armed with the teachings provided herein, that the therapeutic effect can be further enhanced wherein the therapeutic agent does not decrease the immune enhancing effect of the antibody. While gefitinib is a chemotherapeutic agent known not to decrease the immune response in a patient, the invention is not limited to this, or any other, particular therapeutic agent. Rather, in an embodiment, the invention encompasses administration of CP-675,206 and a therapeutic agent that may mediate a therapeutic response to a NSCLC tumor.

In one embodiment of the invention, treatment comprises administration combination therapy comprising CP-675,206 and platinum-based chemotherapy. In one aspect, CP-675,206 is administered to a NSCLC patient following a course of platinum-based therapy as

an adjuvant therapy. That is, a patient that has been administered a course of platinum-based therapy and where the disease has responded or remained stable is administered CP-675,206. In one aspect, the patient NSCLC is Stage IIIb (with effusion) or Stage IV disease that has responded or remained stable after about six cycles of a platinum-containing regimen. In a further aspect, the patient is administered CP-675,206 at least about three weeks after the last dose of platinum-based chemotherapy. In yet another aspect, CP-675,206 is administered within about six weeks after the last dose of platinum-based chemotherapy. In another aspect, CP-675,206 is administered at least about three weeks after but within six weeks of the last dose of platinum-based therapy. In one aspect of the invention, platinum-based chemotherapy comprises a platinum-based compound selected from such compounds as cisplatin, carboplatin (PARAPLATIN), eptaplatin, lobaplatin, nedaplatin, oxaliplatin (ELOXATIN, Sanofi), streptozocin, satraplatin (JM-126).

In another embodiment of the present invention, treatment for lung cancer comprises administration of bevacizumab (AVASTIN) and CP-675,206. In one aspect, the lung cancer comprises non-small cell and small cell lung cancer. In another aspect, the lung cancer comprises small cell lung cancer.

In another embodiment, the cancer is NSCLC and the treatment encompasses administration of CP-675,206 in combination with a taxane, where a taxane can include, but is not limited to, docetaxel (TAXOTERE), and paclitaxel (TAXOL), in further combination with gemcitabine (GEMZAR).

In another embodiment of the invention, the invention encompasses a method for treating NSCLC comprising administration of a CP-675,206, a taxane (*e.g.*, docetaxel and paclitaxel), and a platinum compound. In one aspect, the method for treating NSCLC is a first line therapy comprising administering a combination of CP-675,206, a taxane and a platinum compound where the taxane is paclitaxel and the platin is carboplatin (carbo/paclitaxel). In another aspect, the combination of CP-675,206, paclitaxel and carboplatin is administered contemporaneously, or in sequence. In yet a further aspect, the paclitaxel/carboplatin combination is administered prior to or following administration of CP-675,206. That is, the antibody is contemporaneously administered in combination with the carbo/paclitaxel therapy or it is administered following carbo/paclitaxel therapy. The interval between administration of carbo/paclitaxel therapy and administration of the antibody may be readily determined by one skilled in the art based upon well-known methods. In one aspect, the cancer is locally advanced Stage IIIb NSCLC. In another aspect, the NSCLC is metastatic Stage IV NSCLC. In another aspect, the CP-675,206-carboplatin-paclitaxel combination is administered with bevacizumab. In another aspect, the CP-675,206-carboplatin-paclitaxel combination is

administered with sunitinib (SU11248). In a further aspect, the CP-675,206-carboplatin-paclitaxel combination is administered with PF03512676 (CpG-7909).

In another embodiment of the invention, the therapy is administration of docetaxel (TAXOTERE) and CP-675,206 as second line therapy for NSCLC for patients with locally advanced Stage IIIb or metastatic Stage IV disease after failure of prior platinum-based chemotherapy. In one aspect, CP-675,206 is administered contemporaneously with docetaxel therapy. In another aspect, CP-675,206 is administered following docetaxel therapy. In yet another aspect, the NSCLC comprises locally advanced Stage IIIb or metastatic Stage IV non-small cell lung cancer after failure of prior platinum-based chemotherapy.

In yet another embodiment, the invention includes treatment for NSCLC where the therapy comprises administration of CP-675,206 and erlotinib (TARCEVA) as second line therapy. In one aspect, CP-675,206 is contemporaneously administered with erlotinib. In another aspect, CP-675,206 is administered following erlotinib therapy.

In a further embodiment, the invention includes a second line therapy for NSCLC comprising administration of CP-675,206 and pemetrexed (ALIMTA). In one aspect, CP-675,206 is administered contemporaneously with pemetrexed. In another aspect, CP-675,206 is administered following pemetrexed therapy. In yet another aspect, the NSCLC is locally advanced Stage IIIb. In yet a further aspect, the NSCLC is metastatic Stage IV non-small cell lung cancer after failure of prior platinum-based chemotherapy.

In another embodiment, the invention includes treatment of locally advanced Stage IIIb or metastatic Stage IV NSCLC after failure of prior platinum-based chemotherapy and epidermal growth factor receptor inhibition-based therapy where the method is a third line treatment. In one aspect, CP-675,206 is administered as a single agent. In another aspect, CP-675,206 is administered in combination with a therapeutic agent.

3. Colorectal carcinoma (CRC)

In one embodiment, the invention includes a method for treating cancer of the colon and/or rectum. In one embodiment, the method comprises administering a combination comprising CP-675,206 and fluorouracil (5FU) as first line therapy for CRC patients as first line therapy of CRC patients intolerant of oxaliplatin (ELOXATIN) or irinotecan (CAMPTO). In another embodiment, the treatment comprises administering a combination of CP-675,206 and capecitabine (XELODA) as first line therapy for patients intolerant of oxaliplatin or irinotecan.

In another embodiment, the therapy comprises first line treatment of patients with metastatic carcinoma of the colon or rectum comprising administration of CP-675,206 and FOLFOX (fluorouracil, leucovorin, and oxaliplatin). In one aspect, CP-675,206 is

administered contemporaneously with FOLFOX therapy. In another aspect, CP-675,206 is administered following FOLFOX therapy.

In another aspect, the treatment comprises adjuvant therapy of patients with Stage III colon cancer who have undergone complete resection of the primary tumor. In a further aspect , the therapy comprises administration of CP-675,206 and FOLFOX (fluorouracil, leucovorin, and oxaliplatin). In one aspect, CP-675,206 is administered contemporaneously with FOLFOX therapy. In another aspect, CP-675,206 is administered following FOLFOX therapy.

In another embodiment, the therapy comprises first line treatment of patients with metastatic CRC comprising administration of CP-675,206 and FOLFIRI (fluorouracil, leucovorin, and irinotecan). In one aspect, CP-675,206 is administered contemporaneously with FOLFIRI therapy. In another aspect, CP-675,206 is administered following FOLFIRI therapy.

In another embodiment of the invention, the therapy comprises treatment of CRC comprising administration of CP-675,206 and cetuximab (ERBITUX, ImClone). In one aspect, CP-675,206 is administered contemporaneously with cetuximab. In another aspect, CP-675,206 is administered following cetuximab.

In another embodiment, the invention encompasses a method of treating CRC comprising administering a combination of CP-675,206 and fluorouracil and capecitabine as adjuvant therapy. Coadministration of a chemotherapeutic agent, which can mediate increased release of a tumor antigen, and CP-675,206 may mediate an increased immune response to the tumor thereby providing a therapeutic benefit to a patient afflicted therewith.

4. Leukemia

In one embodiment, the invention includes a method of treating chronic myeloid leukemia (CML). In one aspect, the method comprises administering a combination of CP-675,206 and imatinib mesylate (GLEEVEC, Novartis) as first line therapy of CML.

In another embodiment, the invention includes a method for treating chronic lymphocytic leukemia (CLL). In one aspect, the treatment comprises administration of CP-675,206 and imatinib mesylate as first line therapy.

5. Pancreatic cancer

In one embodiment, the invention includes a method for treating pancreatic cancer comprising administering a combination of CP-675,206 and gemcitabine (GEMZAR). In one aspect, the therapy is a first line treatment for patients with locally advanced (non-resectable Stage II or Stage III) adenocarcinoma of the pancreas. In another aspect, the cancer is metastatic (Stage IV) adenocarcinoma of the pancreas. In yet another aspect, gemcitabine is

administered contemporaneously with CP-675,206. In yet a further aspect, CP-675,206 is administered following administration of gemcitabine.

In one embodiment of the invention, CP-675,206 is administered after at least one course of gemcitabine. In one aspect, gemcitabine is administered intravenously at a dose of about 1000 mg/m^2 once per week for up to seven (7) weeks or until toxicity necessitates reducing or withholding a dose. The administration phase is followed by a one week resting period during which gemcitabine is not administered. Subsequent cycles of gemcitabine administration consist of i.v. infusions once weekly for three consecutive weeks followed by one week resting period. In a further aspect, CP-675,206 is administered after a full course (*i.e.*, seven weeks administration and one week resting period) of gemcitabine. In another embodiment, CP-675,206 is administered every three weeks thereafter. In yet another aspect, the combination of gemcitabine (*e.g.*, three weeks administration followed by one week rest) and CP-675,206 (*e.g.*, every three weeks) is continued until disease progression or intolerable toxicity.

6. Breast cancer

In one embodiment, the invention includes a method to treat locally advanced or metastatic triple receptor negative breast cancer. In one aspect, the method is a first line therapy. In another aspect, the therapy comprises administering CP-675,206 and a taxane (*e.g.*, docetaxel and paclitaxel). In one aspect, CP-675,206 taxane are administered contemporaneously. In another aspect, CP-675,206 is administered following administration of the taxane.

In another embodiment, the therapy comprises adjuvant treatment for breast cancer comprising administration of CP-675,206 and cyclophosphamide, doxorubicin and a taxane. In one aspect, the therapy comprises adjuvant therapy.

In another embodiment, therapy comprises administration of CP-675,206 and tamoxifen (NOVALDEX), anastrazole (ARIMIDEX), letrozole (FEMARA), exemestane (AROMASIN), or fulvestrant (FASLODEX), or a combination thereof. In one aspect, the therapy is adjuvant therapy for metastatic breast cancer.

In another embodiment of the present invention, the therapy for breast cancer comprises administration of CP-675,206 and trastuzumab (HERCEPTIN). In one aspect, the antibodies are administered contemporaneously. In another aspect, trastuzumab is administered followed by administration of CP-675,206. In a further aspect, the CP-675,206-trastuzumab combination therapy comprises adjuvant therapy and first line therapy for metastatic breast cancer.

In yet another embodiment, the therapy comprises administering CP-675,206 and bevacizumab. In one aspect, CP-675,206 and bevacizumab are administered

contemporaneously. In another aspect, CP-675,206 is administered following administration of bevacizumab.

In one embodiment of the invention, the therapy for treating breast cancer comprises administering CP-675,206 and cetuximab. In one aspect, CP-675,206 and cetuximab are administered contemporaneously. In another aspect, CP-675,206 is administered following administration of cetuximab.

In another embodiment of the invention, the therapy for treating breast cancer comprises administering CP-675,206 and axitinib. In one aspect, CP-675,206 and axitinib are administered contemporaneously. In another aspect, CP-675,206 is administered following administration of axitinib.

7. Ovarian cancer

In one embodiment, the invention includes a method for treatment carcinoma of the ovary. In one embodiment, therapy comprises administration of CP-675,206 and carboplatin and paclitaxel. In one aspect, CP-675,206 is administered contemporaneously with carboplatin and paclitaxel. In another aspect, CP-675,206 is administered following administration of carboplatin and paclitaxel. In yet another aspect, the treatment is a first line treatment for advanced carcinoma of the ovary.

In another embodiment of the invention, therapy comprises second line treatment for patients who have progressed (*e.g.*, as indicated by tumor assessment, CA-125 doubling value, CT scan, and the like) following paclitaxel-based therapy. In one aspect, therapy comprises administration of CP-675,206 as a single agent. In another aspect, therapy comprises administration of CP-675,206 in combination with an agent selected from the group consisting of altretamine, anastrozole, bevacizumab, carboplatin, cisplatin, cyclophosphamide, liposomal doxorubicin, docetaxel, gemcitabine, ifosfamide, irinotecan, letrozole, melphalan, oral etoposide, oxaliplatin, tamoxifen, topotecan, and vinorelbine, and any combination thereof.

8. Gastric cancer

In one embodiment of the present invention, the treatment comprises gastric cancer and therapy comprises administering CP-675,206 and irinotecan. In one aspect, CP-675,206 and irinotecan are administered contemporaneously. In another aspect, CP-675,206 is administered following administration of irinotecan.

In another embodiment, treatment comprises administration of CP-675,206, fluorouracil and leucovorin. In one aspect, CP-675,206, fluorouracil and leucovorin are administered contemporaneously. In another aspect, CP-675,206 is administered following administration of fluorouracil and leucovorin.

9. Liver cancer

In one embodiment of the invention, the cancer is liver cancer and therapy comprises administering CP-675,206 and doxorubicin, ifosfamide and vincristine. In one aspect, CP-675,206, and doxorubicin, ifosfamide and vincristine are administered contemporaneously. In another aspect, CP-675,206 is administered following administration of doxorubicin, ifosfamide and vincristine.

In another embodiment, the cancer is liver cancer and therapy comprises administering CP-675,206, doxorubicin, and vincristine. In one aspect, CP-675,206, and doxorubicin, ifosfamide and vincristine are administered contemporaneously. In another aspect, CP-675,206 is administered following administration of doxorubicin and vincristine.

10. Metastatic lesions

In one embodiment of the present invention, CP-675,206 is administered to treat metastases where the metastases is to the brain and/or bone metastasis. In one aspect, the brain and bone metastases are treated using CP-675,206 regardless of the site of the primary tumor from which the cancer metastasized.

In one aspect, brain metastases are treated using a combination of surgical resection and/or radiotherapeutic ablation in further combination with an anti-CTLA4 antibody, preferably, CP-675,206. That is, where multiple metastases and/or lesions greater than about 3 centimeters in diameter are present, whole brain radiation may be administered followed by administration of anti-CTLA4 antibody. In a further aspect, the antibody is administered preceding radiation. In yet a further aspect, the antibody is administered at least about 3 mg/kg, more preferably, at least about 6 mg/kg, even more preferably, at least about 10 mg/kg, and more preferably, at least about 15 mg/kg. In another aspect, the antibody is administered every three weeks, more preferably, every four weeks, and even more preferably, every three months.

In one embodiment, where there are fewer than about 5 brain lesions and all lesions are less than about three centimeres in maximal diameter, stereotactic radiosurgery (SRS) is administered followed by administration of anti-CTLA4 antibody. In one aspect, the antibody is administered before SRS. In another aspect, the antibody is administered before and after SRS.

In another embodiment, surgery, whole brain radiation and SRS may be combined in any order to treat brain metastases, which treatment is further combined with antibody therapy. In one aspect, the antibody therapy is administered after radiation/surgery/SRS. Alternatively, antibody surgery is administered before radiation/surgery/SRS and may be administered before and after such therapy.

This is because, as would be understood by the skilled artisan, brain metastases are common in melanoma. While SRS may achieve local control of the brain metastases, most

patients die of systemic metastases and/or reseeding of the CNS occurs due to poor management of the systemic disease. Accordingly, in one embodiment, the invention provides a combination therapy to control the local brain metastases and to prevent reseeding of the CNS and control the systemic disease thereby providing a therapeutic benefit to a patient in need thereof.

11. Bladder cancer

In one embodiment, CP-675,206 is administered in combination with BCG to treat bladder cancer. In one aspect, CP-675,206 and BCG are administered contemporaneously. In another aspect, CP-675,206 is administered following administration of BCG.

In another embodiment, treatment of bladder cancer comprises administering CP-675,206 and gemcitabine and cisplatin. In one aspect, CP-675,206, gemcitabine and cisplatin are administered contemporaneously. In another aspect, CP-675,206 is administered following administration of gemcitabine and cisplatin.

12. Melanoma

The present invention encompasses combination of CP-675,206 and interferon alpha for treating melanoma. In one aspect, CP-675,206 and interferon alpha are administered contemporaneously. In another aspect, CP-675,206 is administered following administration of interferon alpha. In a further aspect, interferon alpha is administered as a high dose.

13. Multiple myeloma

In one embodiment of the present invention, treatment for multiple myeloma comprises administration of CP-675,206 and bortezomib. In one aspect, CP-675,206 and bortezomib are administered contemporaneously. In another aspect, CP-675,206 is administered following administration of bortezomib.

In another embodiment, therapy comprises administration of CP-675,206 and dexamethasone and thalidomide. In one aspect, CP-675,206, dexamethasone and thalidomide are administered contemporaneously. In another aspect, CP-675,206 is administered following administration of dexamethasone and thalidomide.

In alternate embodiments of the invention discussed above, anti-CTLA4 antibodies other than CP-675,206 may be used in the methods of the invention. Alternate antibodies are described herein, including the antibodies described in U.S. Patent Application No. 09/472,087, now issued as U.S. Patent No. 6,682,736; Int. Appl. No. PCT/US99/30895 (published June 29, 2000, as WO 00/37504); U.S. Pat. Appl. No. 10/612,497 (published November 18, 2004, as US 2004/0228858); U.S. Pat. Appl. No. 10/776,649 (published November 18, 2004, as US 2004/0228861); Int. Appl. No. Int. Appl. No. PCT/US00/23356 (published March 1, 2001, as WO 01/14424) (*e.g.*, antibody 10D1, also known as MDX-010, and referred to herein as ipilimumab, Medarex, Princeton, NJ); Int. Appl. No. PCT/US99/28739

(published June 8, 2000, as WO 00/32231); U.S. Pat. Nos. 5,811,097, 5,855,887, 6,051,227, and 6,207,156; U.S. Pat. No. 5,844,095, to Linsley et al.; Int. Appl. No. PCT/US92/05202 (published Jan. 7, 1993, as WO 93/00431); U.S. Patent Appl. No. 10/153,382 (published May 8, 2003, as US 2003/0086930); U.S. Pat. Appl. No. 10/673,738 (published February 24, 2005 as US 2005/0042223); U.S. Pat. Appl. No. 11/085,368 (published October 13, 2005, as US 2005/0226875); U.S. Pat. Appl. No. 60/624,856 (filed Nov. 4, 2004); U.S. Pat. Appl. No. 60/664,364 (filed March 23, 2005); U.S. Pat. Appl. No. 60/664,653 (filed March 23, 2005); U.S. Pat. Appl. No. 60/697,082 (filed July 7, 2005); U.S. Pat. Appl. No. 60/711,707 (filed Aug. 26, 2005). More preferably, the antibodies are described in U.S. Patent No. 6,682,736, and WO 01/14424. Even more preferably, the antibodies include an antibody having the heavy and light chain amino acid sequences of an antibody selected from the group consisting of 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1 (CP-675,206 or ticilimumab), 11.6.1, 11.7.1., 12.3.1.1, 12.9.1.1, and ipilimumab.

B. Pathogens

Because of the immune-enhancing effects of CTLA4-blockade, it would be understood by the skilled artisan armed with the disclosure provided herein that the present invention encompasses administration of anti-CTLA4 antibody in combination with standard of care therapy to treat a variety of pathogen-associated diseases, disorders or conditions, where an immune response to the pathogen would provide a therapeutic benefit. These include, but are not limited to, bacterial, viral, fungal, parasitic, and other pathogenic disease. Treatment of infectious disease comprising anti-CTLA4 antibody blockade is discussed in, *e.g.*, WO 03/086459, published on Oct. 23, 2003.

Similar to use of CTLA4 blockade in combination to treat a tumor discussed previously, anti-CTLA4 antibody blockade can be used in combination with vaccines and other therapeutic agents, including standard of care therapy, to treat a disease, disorder or condition associated with a pathogens, or a toxin derived therefrom. That is, previous studies have demonstrated that anti-CTLA4 antibody blockade can be useful in treatment of infectious parasites (McCoy et al., *J. Exp. Med.* **186**:183-187 (1997); Murphy et al., *J. Immunol.* **161**:4153-4160 (1998)). Examples of pathogens for which this therapeutic approach may be particularly useful, include, but are not limited to HIV, Hepatitis (A, B, & C), Influenza, Herpes, Giardia, Malaria, Leishmania, Staphylococcus aureus, Pseudomonas aureginosa, and Helicobacter pylori.

Some examples of viruses treatable by methods of the invention include hepatitis (A, B, or C), herpes virus (e.g., VZV, HSV-1, HAV-6, HSV-II, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, ebola virus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus,

parvovirus, vaccinia virus, poxvirus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus.

In one embodiment, bacterial infection treatable by methods of the invention include chlamydia, rickettsial bacteria, mycobacteria, staphylococci, streptococci, pneumonococci, meningococci and conococci, klebsiella, proteus, serratia, pseudomonas, legionella, diphtheria, salmonella, bacilli, helicobacter, cholera, tetanus, botulism, anthrax, plague, leptospirosis, and Lyme Disease bacteria (e.g., Borrelia burgdorferi).

In another embodiment, fungal infection treatable according to the methods of the invention include, but are not limited to, Candida (albicans, krusei, glabrata, tropicalis, etc.), Cryptococcus neoformans, Aspergillus (fumigatus, niger, etc.), Genus Mucorales (mucor, absidia, rhizophus), Sporothrix schenkii, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Coccidioides immitis and Histoplasma capsulatum.

In yet another embodiment, parasitic infection treatable by methods of the invention include, among others, Entamoeba histolytica, Balantidium coli, Naegleriafowleri, Acanthamoeba sp., Giardia lambia, Cryptosporidium sp., Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondi, Nippostrongylus brasiliensis.

1. HIV/AIDS

Studies suggest that CTLA4 blockade may be useful for treatment of viral disease, more specifically, studies in rhesus macaques demonstrated that anti-CTLA4 (*i.e.*, ipilimumab), combined with antiretroviral therapy ("ART"), was associated with increased antiviral response. *See* Hryniewicz et al., *Blood* 108:3834-3842 (2006). More particularly, CTLA4 blockade not only decreased viral load and increased virus-specific effector T cells, it also decreased the level of immunosuppressive molecules (*e.g.*, transforming growth factor beta [TGF-β] and indoleamine 2,3-dioxygenase [IDO]), in SIV_{mac251}-infected macaques also receiving ART (didanosine, stavudine and tenofovir [PMPA]). These data suggest that anti-CTLA4 antibody, in combination with antiviral therapy, may provide a therapeutic benefit in treatment of HIV infection in humans.

In one embodiment of the invention, anti-CTLA4 antibodies, in particular CP-675,206, and their pharmaceutically acceptable salts, solvates and derivatives, may be administered alone or as part of a combination therapy. Thus included within the scope of the present invention are embodiments comprising co-administration of, and compositions which contain, in addition to a compound of the invention, one or more additional therapeutic agents.

Such multiple drug regimens, often referred to as combination therapy, may be used in the treatment and prevention of infection and multiplication of the human immunodeficiency

virus, HIV, and related pathogenic retroviruses within a patient in need of treatment or one at risk of becoming such a patient. The ability of such retroviral pathogens to evolve within a relatively short period of time into strains resistant to any monotherapy which has been administered to said patient is well known in the literature. A recommended treatment for HIV is a combination drug treatment called Highly Active Anti-Retroviral Therapy ("HAART"). HAART combines three or more HIV drugs. Thus, the methods of treatment and pharmaceutical compositions of the present invention may employ a compound of the invention in the form of monotherapy, but said methods and compositions may also be used in the form of combination therapy in which anti-CTLA4 antibody is co-administered in combination with one or more additional therapeutic agents such as those described in detail further herein.

The therapeutic agents that may be used in combination with the anti-CTLA4 antibody include, but are not limited to, those useful as HIV protease inhibitors (PIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), CCR5 antagonists, agents which inhibit the interaction of gp120 with CD4, other agents which inhibit the entry of HIV into a target cell (such as fusion inhibitors), inhibitors of HIV integrase, RNaseH inhibitors, prenylation inhibitors, maturation inhibitors which act by interfering with production of the HIV capsid protein, compounds useful as anti-infectives, and others as described below.

It will be appreciated by a person skilled in the art, that a combination drug treatment, as described herein above, may comprise two or more compounds having the same, or different, mechanism of action. Thus, by way of illustration only, a combination may comprise a compound of the invention and: one or more NRTIs; one or more NRTIs and a PI; one or more NRTIs and another CCR5 antagonist; a PI; a PI and an NNRTI; an NNRTI; and so on.

Examples of PIs include, but are not limited to, amprenavir (141W94), CGP-73547, CGP-61755, DMP-450 (mozenavir), nelfinavir, ritonavir, saquinavir (invirase), lopinavir, TMC-126, atazanavir, palinavir, GS-3333, KN I-413, KNI-272, LG-71350, CGP-61755, PD 173606, PD 177298, PD 178390, PD 178392, U-140690, ABT-378, DMP-450, AG-1776, MK-944, becanavir (formerly known as VX-478, GW640385), indinavir, tipranavir, TMC-114, DPC-681, DPC-684, fosamprenavir calcium (Lexiva), benzenesulfonamide derivatives disclosed in WO 03/053435, R-944, Ro-03-34649, VX-385, GS-224338, OPT-TL3, PL-100, PPL-100, SM-309515, AG-148, DG-35-VIII, DMP-850, GW-5950X, KNI-1039, L-756423, LB-71262, LP-130, RS-344, SE-063, UIC-94-003, Vb-19038, A-77003, BMS-182193, BMS-186318, SM-309515, JE-2147, GS-9005.

Examples of NRTIs include, but are not limited to, abacavir, GS-840, lamivudine, adefovir dipivoxil, beta-fluoro-ddA, zalcitabine, didanosine, stavudine, zidovudine, tenofovir

(9-[9(R)-2-(phosphonomethoxy)propyl]adenine; PMPA), tenofovir disoproxil fumarate (Viread; Gliead Sciences), amdoxovir (DAPD), SPD-754, SPD-756, racivir, reverset (DPC-817), MIV-210 (FLG), beta-L-Fd4C (ACH-126443), MIV-310 (alovudine, FLT), dOTC, DAPD, entecavir, GS-7340, emtricitabine (FTC).

Examples of NNRTIs include, but are not limited to, efavirenz, HBY-097, nevirapine, TMC-120 (dapivirine), TMC-125, etravirine, delavirdine, DPC-083, DPC-961, capravirine, rilpivirine, 5-{[3,5-Diethyl-1-(2-hydroxyethyl)-1*H*-pyrazol-4-yl]oxy}isophthalonitrile or pharmaceutically acceptable salts, solvates or derivatives thereof; GW-678248, GW-695634, MIV-150, calanolide, and tricyclic pyrimidinone derivatives as disclosed in WO 03/062238.

Examples of CCR5 antagonists include, but are not limited to, TAK-779, SC-351125, ancriviroc (formerly known as SCH-C), vicriviroc (formerly known as SCH-D), maraviroc, NCB-9471, CCR5mAb004, PRO-140, aplaviroc (also known as GW-873140, Ono-4128, AK-602), AMD-887 CMPD-167, methyl 1-*endo*-{8-[(3*S*)-3-(acetylamino)-3-(3-fluorophenyl)propyl]-8-azabicyclo[3.2.1]oct-3-yl}-2-methyl-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-5-carboxylate or pharmaceutically acceptable salts, solvates or derivatives thereof, methyl 3-*endo*-{8-[(3*S*)-3-(acetamido)-3-(3-fluorophenyl)propyl]-8-azabicyclo[3.2.1]oct-3-yl}-2-methyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-*c*]pyridine-5-carboxylate or pharmaceutically acceptable salts, solvates or derivatives thereof, ethyl 1-*endo*-{8-[(3S)-3-(acetylamino)-3-(3-fluorophenyl)propyl]-8-azabicyclo[3.2.1]oct-3-yl}-2-methyl-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-c]pyridine-5-carboxylate or pharmaceutically acceptable salts, solvates or derivatives thereof, and *N*-{(1*S*)-3-[3-*endo*-(5-Isobutyryl-2-methyl-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridin-1-yl)-8-azabicyclo[3.2.1]oct-8-yl]-1-(3-fluorophenyl)propyl}acetamide) or pharmaceutically acceptable salts, solvates or derivatives thereof.

In one embodiment, the anti-CTLA4 antibody, preferably CP-675,206, is administered in combination with maraviroc. In another embodiment, anti-CTLA4 antibody/maraviroc combination therapy comprises assessing whether is expressed on the surface of the patient's cells. In yet another embodiment, the therapy comprises assessing the co-receptor (*i.e.*, CXCR4 or CCR5) tropism of the HIV virus to determine whether the patient is infected with an HIV that uses the CCR5 co-receptor, the CXCR4 co-receptor, or both (dual/mixed tropic) to determined whether the patient is a candidate for maraviroc therapy. In another embodiment, the co-receptor tropism assay is TROFILE (Monogram Biosciences, Inc., San Francisco, CA). This is because treatment with maraviroc inhibits CCR5-mediated entry into cells. Thus, assessing CCR5 expression by a patient's cell and/or assessing the co-receptor tropism of the virus optimizes the treatment options for the patient.

Examples of entry and fusion inhibitors include, but are not limited to, BMS-806, BMS-488043, 5-{(1S)-2-[(2R)-4-Benzoyl-2-methyl-piperazin-1-yl]-1-methyl-2-oxo-ethoxy}-4-

methoxy-pyridine-2-carboxylic acid methylamide and 4-{(1S)-2-[(2R)-4-Benzoyl-2-methyl-piperazin-1-yl]-1-methyl-2-oxo-ethoxy}-3-methoxy-N-methyl-benzamide, enfuvirtide (T-20), sifuvirtide SP-01A, T1249, PRO 542, AMD-3100, soluble CD4, compounds disclosed in JP 2003171381, and compounds disclosed in JP 2003119137.

Examples of inhibitors of HIV integrase include, but are not limited to, L-000870810 GW-810781, 1,5-naphthyridine-3-carboxamide derivatives disclosed in WO 03/062204, compounds disclosed in WO 03/047564, compounds disclosed in WO 03/049690, and 5-hydroxypyrimidine-4-carboxamide derivatives disclosed in WO 03/035076, MK-0518 (5-(1,1-dioxo-1,2-thiazinan-2-yl)-N- (4-fluorobenzyl)-8-hydroxy-1,6-naphthyridine-7-carboxamide-disclosed in WO 03016315), GS-9137 (JTK-303).

Examples of prenylation inhibitors include, but are not limited to, HMG CoA reductase inhibitors, such as statins (e.g., atorvastatin).

Examples of maturation inhibitors include 3-O-(3'3'-dimethylsuccinyl) betulic acid (otherwise known as PA-457) and alphaHGA.

II. Dosage Regimens

Dosage regimens may be adjusted to provide the optimum desired response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the antibody and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non limiting range for a therapeutically effective amount of ticilimumab administered according to the invention is at least about 1 mg/kg, at least about 5 mg/kg, at least about 10 mg/kg, more than about 10 mg/kg, or at least about 15 mg/kg, for example about 1-30 mg/kg, or for example about 1-25 mg/kg, or for example about 1-20 mg/kg, or for example about 5-20 mg/kg, or for example about 10-20 mg/kg, or for example about 15-20 mg/kg, or for example, about 15 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated, and may include single or multiple doses. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted

over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. Determining appropriate dosages and regiments for administration of the antibody are well-known in the relevant art and would be understood to be encompassed by the skilled artisan once provided the teachings disclosed herein.

In one embodiment, CP-675,206 is administered in an intravenous formulation as a sterile aqueous solution containing about 5 to 20 mg/ml of antibody, in an appropriate buffer system.

In one embodiment, for administration of low doses, part of the dose is administered by an intravenous bolus and the rest by infusion of the antibody formulation. For example, a 0.01 mg/kg intravenous injection of the antibody may be given as a bolus, and the rest of a predetermined antibody dose may be administered by intravenous injection. In another embodiment, the entire low dose is administered as a single bolus injection. For higher doses, *e.g.*, 3 mg/kg, the antibody is not administered as a bolus, but the entire amount is administered by infusion. A predetermined dose of the antibody may be administered, for example, over a period of about an hour and a half to about five hours.

The present invention relates to administering a combination of an anti-CTLA4 antibody and at least one therapeutic agent. In one embodiment, the antibody is CP-675,206. In alternative embodiments of the invention, the antibody is selected from various anti-CTLA4 antibodies, including, but not limited to, ipilimumab. In one aspect, the antibody is ipilimumab and the dose is about 3 mg/kg. In another aspect, the dose of ipilimumab is at least about 10 mg/kg.

In one embodiment of the present invention, the antibody (*e.g.*, CP-675,206, ipilimumab, and the like) is administered approximately every three weeks, more preferably, for about four cycles followed by every three months thereafter. In one aspect of this embodiment, the antibody is administered at about 10 mg/kg.

The skilled artisan would appreciate that the combination of CP-675,206 and a therapeutic agent can be administered simultaneously or the antibody and the therapeutic agent can be administered at different times. For instance, in one embodiment, the antibody is administered as a single injection and/or infusion and the therapeutic agent (*e.g.*, gemcitabine) is administered once per day commencing before, during, or after administration of the antibody. However, the present invention is not limited to any particular dosage or administration regimen for a therapeutic agent. Rather, the optimal dose, route and regimen for administration of the antibody and the therapeutic agent can be readily determined by one of ordinary skill in the relevant art using well-known methods.

For instance, a single dose or multiples doses of the antibody may be administered. Alternatively, at least one dose, or at least three, six or 12 doses may be administered. The doses may be administered, for example, every two weeks, every three weeks, monthly, every twenty days, every 25 days, every 28 days, every 30 days, every 40 days, every 6 weeks, every 50 days, every two months, every 70 days, every 80 days, every three months, every six months or yearly. In one aspect, the antibody is administered once every three weeks, preferably for four cycles, and then every three months thereafter. In addition, the second therapeutic agent can be administered daily, several times or once per day, weekly, every other week, every third week, every fourth week, monthly, every three months, every six months, once per year, or any other period that provides a therapeutic benefit to the patient as determined by the skilled practitioner.

In one aspect, CP-675,206 is administered once per month. In another aspect, CP-675,206 is administered every three months. In yet another aspect, ipilimumab is administered once per month.

The antibody can be administered until disease progression, or intolerable toxicity, or up to 12 consecutive cycles, whichever time is shorter. The antibody can also be administered using a regimen comprising administration of a loading dose followed by a lower dose. Repeat courses of at least one, and more preferably, several cycles of antibody and a therapeutic agent can be administered to a patient that experiences a tumor recurrence who had previously derived benefit from administration of the combination of an anti-CTLA4 antibody and another therapeutic agent, or the antibody or therapeutic agent not administered in combination previously.

In one embodiment, a single injection comprising CP-675,206 is administered to a patient intravenously at a dose of about 3 mg/kg every twenty-eight days. In another embodiment, an anti-CTLA4 antibody is administered to a patient intravenously at a dose of about 3 mg/kg, preferably, about 6 mg/kg, more preferably, about 10 mg/kg, preferably, about 15 mg/kg, every three weeks. After four administrations, the antibody is then administered every three months.

The invention encompasses administration of an anti-CTLA4 antibody, preferably CP-675,206, in combination with a wide plethora of agents. The skilled artisan would appreciate, once armed with the teachings provided herein, that any art-recognized dosing regimen for the agent may be used. Dosing regimens for chemotherapeutic and other agents described herein may be found in treatises well known in the art, including, but not limited to, Cancer: Principles and Practice of Oncology, 7th edition, DeVita, Hellman, and Rosenberg, editors, Lippincott, Williams & Wilkins, 2004, and Cancer Chemotherapy and Biotherapy Principles and Practice, 4th edition. Chabner, Longo, editors, Lippincott, Williams & Wilkins, 2005. Additionally,

administration of many agents disclosed herein may be performed based upon the FDA-approved drug label instructions. Such label instructions are readily and publicly available from the website of the Department of Health and Sciences, U.S. Food and Drug Administration, Center for Drug Evaluation and Research website, which may be searched according to drug name or active ingredient. Thus, the skilled artisan would understand the dosing regimen to be used in combination therapy for various diseases comprising administration of an anti-CTLA4 antibody and a therapeutic agent. The dose may be adjusted as known in the art based on, among other factors, toxicity, if any, and therapeutic effectiveness. Thus, dosing regimens for many chemotherapeutic, and other agents related to cancer treatment, are well known in the art and are described in numerous publications, including those described herein. *See also* International Publication No. PCT/US03/12802 (published Nov. 6, 2003, as WO 03/090686), which is incorporated by reference herein for all purposes, for a list of exemplary dosing regimens for various chemotherapeutic agents (*e.g.*, at pages 72 through 74).

In some embodiments, the antibody-therapeutic agent combination can be administered as an adjuvant therapy, or it can be administered to the patient as a neoadjuvant therapy prior to surgery, radiation therapy, or any other treatment, in order to sensitize the tumor cells or to otherwise confer a therapeutic benefit to the patient.

Further, in some embodiments, the combination can be administered as a first line therapy, or as a second line or third line therapy, such as, but not limited to, once previous therapy(ies) has failed. Alternatively, the combination can be administered concurrently with a first line therapy, and or at any point during therapy, following initial treatment. Thus, a combination of an anti-CTLA4 antibody and a therapeutic agent can provide a therapeutic benefit once a previous therapy has failed, once systemic adjuvant therapy using another therapeutic agent (*e.g.*, temozolomide, leuprolide, paclitaxel, imatinib mesylate, gefitinib, and the like) has failed. Therefore, the invention encompasses administration of an anti-CTLA4 antibody and a therapeutic agent in combination with or following additional therapy, including, but not limited to, a different therapeutic agent (*e.g.*, a chemotherapeutic, an antibody, an immunomodulator, a cytokine, and the like) as would be appreciated by one skilled in the art based upon the disclosure provided herein.

III. Anti-CTLA4 Antibodies

The invention encompasses alternate embodiments employing CTLA4 antibodies in addition to CP-675,206. In one embodiment, the CTLA4 antibody comprises a heavy chain wherein the amino acid sequence of the V_H comprises the amino acid sequences set forth in SEQ ID NOs:3, 15 and 27. In yet another embodiment, the V_L of the CTLA4 antibody comprises the amino acid sequences set forth in SEQ ID NOs:9, 21 and 33. More preferably,

the V_H and V_L regions of the antibody comprise the amino acid sequences set forth in SEQ ID NO:3 (V_H 4.1.1) and SEQ ID NO:9 (V_L 4.1.1), respectively; the amino acid sequences set forth in SEQ ID NO:15 (V_H 4.13.1) and SEQ ID NO:21 (V_L 4.13.1), respectively; and the amino acid sequences set forth in SEQ ID NO:27 (V_H 11.2.1) and SEQ ID NO:33 (V_L 11.2.1), respectively. Most preferably, the antibody is CP-675,206, which has the heavy and light chain amino acid sequences of antibody 11.2.1 (ticilimumab).

In yet another embodiment, the amino acid sequence of the heavy chain comprises the amino acid sequence encoded by a nucleic acid comprising the nucleic acid sequences set forth in SEQ ID NOs:1, 13, and 25. In yet another embodiment, the light chain comprises the amino acid sequence encoded by a nucleic acid comprising the nucleic acid sequences set forth in SEQ ID NOs:7, 19 and 31. More preferably, the heavy and light chains comprise the amino acid sequences encoded by nucleic acids comprising the nucleic acid sequences set forth in SEQ ID NO:1 (heavy chain 4.1.1) and SEQ ID NO:7 (light chain 4.1.1), respectively; the nucleic acid sequences set forth in SEQ ID NO:13 (heavy chain 4.13.1) and SEQ ID NO:19 (light chain 4.13.1), respectively; and the nucleic acid sequences set forth in SEQ ID NO:25 (heavy chain 11.2.1) and SEQ ID NO:31 (light chain 11.2.1), respectively.

Furthermore, the antibody can comprise a heavy chain amino acid sequence comprising human CDR amino acid sequences derived from the V_H 3-30 or 3-33 gene, or conservative substitutions or somatic mutations therein. It is understood that the V_H 3-33 gene encodes from FR1 through FR3 of the heavy chain variable region of an antibody molecule. Thus, the invention encompasses an antibody that shares at least 85%, more preferably, at least 90%, yet more preferably, at least 91%, even more preferably, at least 94%, yet more preferably, at least 95%, more preferably, at least 97%, even more preferably, at least 98%, yet more preferably, at least 99%, and most preferably, 100% identity, with the sequence from FR1 through FR3 of an antibody selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1 (CP-675,206), 11.6.1, 11.7.1, 12.3.1.1, 2.9.1.1, ipilimumab, and DP-50.

The antibody can further comprise CDR regions in its light chain derived from the A27 or the O12 gene or it may comprise the CDR regions of an antibody selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1 (CP-675,206), 11.6.1, 11.7.1, 12.3.1.1, 2.9.1.1, ipilimumab.

In other embodiments of the invention, the antibody inhibits binding between CTLA4 and B7-1, B7-2, or both. Preferably, the antibody can inhibit binding with B7-1 with an IC_{50} of about 100 nM or lower, more preferably, about 10 nM or lower, for example about 5 nM or lower, yet more preferably, about 2 nM or lower, or even more preferably, for example, about 1 nM or lower. Likewise, the antibody can inhibit binding with B7-2 with an IC_{50} of about 100

nM or lower, more preferably, 10 nM or lower, for example, even more preferably, about 5 nM or lower, yet more preferably, about 2 nM or lower, or even more preferably, about 1 nM or lower.

Further, in another embodiment, the anti-CTLA4 antibody has a binding affinity for CTLA4 of about 10^{-8}, or greater affinity, more preferably, about 10^{-9} or greater affinity, more preferably, about 10^{-10} or greater affinity, and even more preferably, about 10^{-11} or greater affinity.

The anti-CTLA4 antibody includes an antibody that competes for binding with an antibody having heavy and light chain amino acid sequences of an antibody selected from the group consisting of 4.1.1, 6.1.1, 11.2.1 (CP-675,206), 4.13.1 and 4.14.3. Further, the anti-CTLA4 antibody can compete for binding with antibody ipilimumab.

In another embodiment, the antibody preferably cross-competes with an antibody having a heavy and light chain sequence, a variable heavy and a variable light chain sequence, and/or the heavy and light CDR sequences of antibody 4.1.1, 4.13.1, 4.14.3, 6.1.1. or 11.2.1 (CP-675,206). For example, the antibody can bind to the epitope to which an antibody that has heavy and light chain amino acid sequences, variable sequences and/or CDR sequences, of an antibody selected from the group consisting of 4.1.1, 4.13.1, 4.14.3, 6.1.1, or 11.2.1 (CP-675,206) binds. In another embodiment, the antibody cross-competes with an antibody having heavy and light chain sequences, or antigen-binding sequences, of MDX-D010.

In another embodiment, the invention is practiced using an anti-CTLA4 antibody that comprises a heavy chain comprising the amino acid sequences of CDR-1, CDR-2, and CDR-3, and a light chain comprising the amino acid sequences of CDR-1, CDR-2, and CDR-3, of an antibody selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1 (CP-675,206), 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1, or sequences having changes from the CDR sequences selected from the group consisting of conservative changes, wherein the conservative changes are selected from the group consisting of replacement of nonpolar residues by other nonpolar residues, replacement of polar charged residues other polar uncharged residues, replacement of polar charged residues by other polar charged residues, and substitution of structurally similar residues; non-conservative substitutions, wherein the non-conservative substitutions are selected from the group consisting of substitution of polar charged residue for polar uncharged residues and substitution of nonpolar residues for polar residues, additions and deletions.

In a further embodiment of the invention, the antibody contains fewer than 10, 7, 5, or 3 amino acid changes from the germline sequence in the framework or CDR regions. In another embodiment, the antibody contains fewer than 5 amino acid changes in the

framework regions and fewer than 10 changes in the CDR regions. In one preferred embodiment, the antibody contains fewer than 3 amino acid changes in the framework regions and fewer than 7 changes in the CDR regions. In a preferred embodiment, the changes in the framework regions are conservative and those in the CDR regions are somatic mutations.

In another embodiment, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 94%, preferably, at least 95%, more preferably, at least 99%, sequence(*e.g.*, amino acid, nucleic acid, or both) identity or sequence similarity over the heavy and light chain full-length sequences, or over the heavy or the light chain, separately, with the sequences of antibody 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1 (CP-675,206), 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, ipilimumab. Even more preferably, the antibody shares 100% sequence identity or sequence similarity over the heavy chain and the light chain, or with the heavy chain or the light chain, separately, of an antibody selected from antibody 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1 (CP-675,206), 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, ipilimumab.

In yet another embodiment, the antibody has heavy and light chain regions having the amino acid sequences of ipilimumab (previously known as MDX-010).

In another embodiment, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 94%, more preferably, at least 95%, even more preferably, at least 99%, sequence identity or sequence similarity over the heavy and light chain full-length sequences, or over the heavy or the light chain, separately, with the sequences of germline V_κ A27, germline V_κ O12, and germline DP50 (which is an allele of the V_H 3-33 gene locus). Even more preferably, the antibody shares 100% sequence identity or sequence similarity over the heavy chain sequence of germline DP50 and/or with the light chain sequence of germline A27, or germline O12.

In one embodiment, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 94%, preferably, at least 95%, more preferably, at least 99%, sequence(*e.g.*, amino acid, nucleic acid, or both) identity or sequence similarity over the heavy and light chain variable region sequences, or over the heavy or the light chain variable region sequence, separately, with the sequences of antibody 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1 (CP-675,206), 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, ipilimumab. Even more preferably, the antibody shares 100% sequence identity or sequence similarity over the heavy chain and the light chain variable region sequences, or with the heavy chain or the light chain sequence, separately, of an antibody selected from antibody 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1 (CP-675,206), 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, ipilimumab.

In another embodiment, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 94%, more preferably, at least 95%, even more preferably, at least 99%, sequence identity or sequence similarity over heavy chain variable region sequence with the heavy chain variable sequence of heavy germline DP50 (which is an allele of the V_H 3-33 gene locus) or with the light chain variable sequence of germline V_κ A27, or germline V_κ O12. Even more preferably, the antibody heavy chain region sequence shares 100% sequence identity or sequence similarity with the sequence of germline DP50 or with the light chain sequence of germline A27, or germline O12.

In one embodiment of the present invention, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 95%, more preferably, at least 99%, sequence identity or sequence similarity with the heavy chain, the light chain, or both, sequences from FR1 through FR4 with the FR1 through FR4 region sequences of antibody 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1 (CP-675,206), 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, ipilimumab. Even more preferably, the antibody shares 100% sequence identity or sequence similarity over the heavy, light, or both, sequences from FR1 through FR4 with antibody 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, CP-675,206, and ipilimumab.

In another embodiment of the present invention, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 95%, more preferably, at least 99%, and most preferably, about 100%, sequence identity or sequence similarity with the heavy chain sequences from FR1 through FR3 with the FR1 through FR3 region sequences of germline DP50.

In yet another embodiment of the present invention, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 95%, more preferably, at least 99%, and most preferably, about 100%, sequence identity or sequence similarity with the light chain sequences from FR1 through FR4 with the FR1 through FR4 region sequences of germline V_κ A27, or germline V_κ O12.

In one embodiment of the present invention, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 95%, more preferably, at least 99%, sequence identity or sequence similarity with the heavy chain, the light chain, or both, CDR-1, CDR-2 and CDR-3 sequences of antibody 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1 (CP-675,206), 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, ipilimumab. Even more preferably, the antibody shares 100% sequence identity or sequence similarity over the heavy, light, or both, CDR-1, CDR-2 and CDR-3 sequences with antibody

3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, CP-675,206, and ipilimumab.

In another embodiment of the present invention, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 95%, more preferably, at least 99%, and most preferably, about 100%, sequence identity or sequence similarity with the heavy chain CDR-1 and CDR-2 sequences with the CDR-1 and CDR-2 sequences of germline DP50.

In yet another embodiment of the present invention, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 95%, more preferably, at least 99%, and most preferably, about 100%, sequence identity or sequence similarity with the light chain CDR-1, CDR-2 and CDR-3 sequences with the CDR-1, CDR-2 and CDR-3 sequences of germline V_κ A27, or germline V_κ O12.

Examples of antibodies employable in the present invention, and methods of producing them, are described in, among others, U.S. Patent Application No. 09/472,087, now issued as U.S. Patent No. 6,682,736; Int. Appl. No. PCT/US00/23356 (published March 1, 2001, as WO 01/14424) (*e.g.*, antibody ipilimumab, also known as MDX-010, Medarex, Princeton, NJ); Int. Appl. No. PCT/US99/28739 (published June 8, 2000, as WO 00/32231); U.S. Pat. Nos. 5,811,097, 5,855,887, 6,051,227, and 6,207,156; each of which is incorporated by reference herein. While information on the amino and nucleic acid sequences relating to these antibodies is provided herein, further information can be found in U.S. Patent No. 6,682,736, as well as WO 00/37504; the sequences set forth in those applications are hereby incorporated herein by reference.

Certain uses for these antibodies to treat various cancers were discussed in U.S. Patent Application No. 10/153,382, now published as U.S. Patent Application Publication No. 2003/0086930, which is incorporated by reference as if set forth in its entirety herein.

Characteristics of human anti-CTLA4 antibodies useful in the methods of the invention are extensively discussed in, *e.g.*, U.S. Patent No. 6,682,736, and include antibodies having amino acid sequences of an antibody such as, but not limited to, antibody 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, CP-675,206, and ipilimumab. The invention also relates to methods using antibodies comprising the amino acid sequences of the CDRs of the heavy and light chains of these antibodies, as well as those comprising changes in the CDR regions, as described in the above-cited applications and patent. The invention also concerns antibodies comprising the variable regions of the heavy and light chains of those antibodies. In another embodiment, the antibody is selected from an antibody comprising the full length, variable region, or CDR, amino acid sequences of the heavy and light

chains of antibodies 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1, CP-675,206, and ipilimumab.

While the anti-CTLA4 antibodies discussed previously herein may be preferred, the skilled artisan, based upon the disclosure provided herein, would appreciate that the invention encompasses a wide variety of anti-CTLA4 antibodies and is not limited to these particular antibodies. More particularly, while human antibodies are preferred, the invention is in no way limited to human antibodies; rather, the invention encompasses useful antibodies regardless of species origin, and includes, among others, chimeric, humanized and/or primatized antibodies. Also, although the antibodies exemplified herein were obtained using a transgenic mammal, *e.g.*, a mouse comprising a human immune repertoire, the skilled artisan, based upon the disclosure provided herein, would understand that the present invention is not limited to an antibody produced by this or by any other particular method. Instead, the invention includes an anti-CTLA4 antibody produced by any method, including, but not limited to, a method known in the art (*e.g.,* screening phage display libraries, and the like) or to be developed in the future for producing an anti-CTLA4 antibody of the invention. Based upon the extensive disclosure provided herein and in, *e.g.*, U.S. Patent No. 6,682,736, to Hanson et al., and U.S. Pat. App. Pub. No. 2002/0088014, one skilled in the art can readily produce and identify an antibody useful for treatment of prostate cancer in combination with a hormonal therapeutic agent using the novel methods disclosed herein.

The present invention encompasses human antibodies produced using a transgenic non-human mammal, *i.e.*, XenoMouse™ (Abgenix, Inc., Fremont, CA) as disclosed in the U.S. 6,682,736, to Hanson et al.

Another transgenic mouse system for production of "human" antibodies is referred to as "HuMAb-Mouse™" (Medarex, Princeton, NJ), which contains human immunoglobulin gene miniloci that encodes unrearranged human heavy (mu and gamma) and kappa light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous mu and kappa chain loci (Lonberg et al. *Nature* **368**:856-859 (1994), and U.S. Pat. No. 5,770,429).

However, the invention uses human anti-CTLA4 antibodies produced using any transgenic mammal such as, but not limited to, the Kirin TC Mouse™ (Kirin Beer Kabushiki Kaisha, Tokyo, Japan) as described in, *e.g.*, Tomizuka et al., *Proc Natl Acad Sci USA* **97**:722 (2000); Kuroiwa et al., *Nature Biotechnol* **18**:1086 (2000); U.S. Patent Application Publication No. 2004/0120948, to Mikayama et al.; and the HuMAb-Mouse™ (Medarex, Princeton, NJ) and XenoMouse™ (Abgenix, Inc., Fremont, CA), supra. Thus, the invention encompasses using an anti-CTLA4 antibody produced using any transgenic or other non-human animal.

In another embodiment, the antibodies employed in methods of the invention are not fully human, but "humanized". In particular, murine antibodies or antibodies from other species can be "humanized" or "primatized" using techniques well known in the art. *See, e.g.,* Winter and Harris *Immunol. Today* **14**:43-46 (1993), Wright et al. *Crit. Reviews in Immunol.* **12**:125-168 (1992), and US Patent No. 4,816,567, to Cabilly et al, and Mage and Lamoyi *in Monoclonal Antibody Production Techniques and Applications* pp. 79-97, Marcel Dekker, Inc., New York, NY (1987). Thus, humanized, chimeric antibodies, anti-CTLA4 antibodies derived from any species (including single chain antibodies obtained from camelids as described in, *e.g.,* U.S. Pat. Nos. 5,759,808 and 6,765,087, to Casterman and Hamers), as well as any human antibody, can be combined with a therapeutic agent to practice the novel methods disclosed herein.

As will be appreciated based upon the disclosure provided herein, antibodies for use in the invention can be obtained from a transgenic non-human mammal, and hybridomas derived therefrom, but can also be expressed in cell lines other than hybridomas.

Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, NS0, Sp2, HEK, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), and human hepatocellular carcinoma cells (*e.g.*, Hep G2). Non-mammalian prokaryotic and eukaryotic cells can also be employed, including bacterial, yeast, insect, and plant cells.

Nucleic acid molecules encoding an anti-CTLA4 antibody, and expression vectors comprising these nucleic acid molecules, may be used for transfection of a suitable mammalian, plant, bacterial or yeast host cell. Transformation may be by any known method for introducing polynucleotides into a host cell. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei. In addition, nucleic acid molecules may be introduced into mammalian cells by viral vectors. Methods of transforming plant cells are well known in the art, including, e.g., Agrobacterium-mediated transformation, biolistic transformation, direct injection, electroporation and viral transformation. Methods of transforming bacterial and yeast cells are also well known in the art.

An expression vector may also be delivered to an expression system using DNA biolistics, wherein the plasmid is precipitated onto microscopic particles, preferably gold, and the particles are propelled into a target cell or expression system. DNA biolistics techniques are well-known the art and devices, *e.g.*, a "gene gun", are commercially available for delivery

of the microparticles in to a cell (*e.g.*, Helios Gene Gun, Bio-Rad Labs., Hercules, CA) and into the skin (PMED Device, PowderMed Ltd., Oxford, UK).

Various expression systems can be used as well known in the art, such as, but not limited to, those described in, *e.g.*, Sambrook and Russell, *Molecular Cloning, A Laboratory Approach*, Cold Spring Harbor Press, Cold Spring Harbor, NY (2001), and Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (2002). These expression systems include dihydrofolate reductase (DHFR)-based systems, among many others. The glutamine synthetase system of expression is discussed in whole or part in connection with European Patents No. 0216846B1, No. 0256055B1, and No. 0323997B1, and European Patent Application No. EP89303964. In one embodiment, the antibody used is made in NS0 cells using a glutamine synthetase system (GS-NS0). In another embodiment, the antibody is made in CHO cells using a DHFR system. Both systems are well-known in the art and are described in, among others, Barnes et al. *Biotech & Bioengineering* **73**:261-270 (2001), and references cited therein.

Site directed mutagenesis of the antibody CH2 domain to eliminate glycosylation may be preferred in order to prevent changes in either the immunogenicity, pharmacokinetic, and/or effector functions resulting from non-human glycosylation. Further, the antibody can be deglycosylated by enzymatic (*see, e.g.*, Thotakura et al. *Meth. Enzymol.* 138:350 (1987)) and/or chemical methods (*see, e.g.*, Hakimuddin et al., *Arch. Biochem. Biophys.* **259**:52 (1987)).

Further, the invention encompasses using an anti-CTLA4 antibody comprising an altered glycosylation pattern. The skilled artisan would appreciate, based upon the disclosure provided herein, that an anti-CTLA4 antibody can be modified to comprise additional, fewer, or different glycosylations sites compared with the naturally-occurring antibody. Such modifications are described in, *e.g.*, U.S. Patent Application Publication Nos. 2003/0207336, and 2003/0157108, and International Patent Publication Nos. WO 01/81405 and 00/24893.

Additionally, the invention comprises using an anti-CTLA4 antibody regardless of the glycoform, if any, present on the antibody. Moreover, methods for extensively remodeling the glycoform present on a glycoprotein are well-known in the art and include, *e.g.*, those described in International Patent Publication Nos. WO 03/031464, WO 98/58964, and WO 99/22764, and US Patent Application Publication Nos. 2004/0063911, 2004/0132640, 2004/0142856, 2004/0072290, and US Patent No. 6,602,684 to Umaña et al.

Further, the invention encompasses using an anti-CTLA4 antibody with any art-known covalent and non-covalent modification, including, but not limited to, linking the polypeptide to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in, for example, U.S. Patent Application Publication

Nos. 2003/0207346 and 2004/0132640, and U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192; 4,179,337.

Additionally, the invention encompasses using an anti-CTLA4 antibody, or antigen-binding portion thereof, chimeric protein comprising, *e.g.,* a human serum albumin polypeptide, or fragment thereof. Whether the chimeric protein is produced using recombinant methods by, *e.g.,* cloning of a chimeric nucleic acid encoding the chimeric protein, or by chemical linkage of the two peptide portions, the skilled artisan would understand once armed with the teachings provided herein that such chimeric proteins are well-known in the art and can confer desirable biological properties such as, but not limited to, increased stability and serum half-life to the antibody of the invention and such molecules are therefore included herein.

Antibodies that are generated for use in the invention need not initially possess a particular desired isotype. Rather, the antibody as generated can possess any isotype and can be isotype switched thereafter using conventional techniques. These include direct recombinant techniques (*see*, *e.g.*, U.S. Patent 4,816,397), and cell-cell fusion techniques (see e.g., U.S. Patent No. 5,916,771).

The effector function of the antibodies of the invention may be changed by isotype switching to an IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM for various therapeutic uses. Furthermore, dependence on complement for cell killing can be avoided through the use of bispecifics, immunotoxins, or radiolabels, for example.

Although antibody 4.1.1, 4.13.1 and 11.2.1 (CP-675,206) are IgG2 antibodies and the sequences of the variable regions of the antibodies are provided herein (Figures 1-3), and in the applications and patents referenced and incorporated herein, it is understood that the full-length sequences of these antibodies are encompassed herein, as well as the use of any antibody comprising the sequences set forth in SEQ ID NOs:1-36, and further comprising any constant region, regardless of isotype as more fully discussed elsewhere herein. Likewise, any antibody comprising the full-length sequence of ipilimumab, or any portion thereof, including a sequence encoding an antigen-binding portion of ipilimumab, can be used according to the methods of the invention.

Thus, the skilled artisan, once provided with the teachings provided herein, would readily appreciate that the anti-CTLA4 antibody-therapeutic agent combination of the invention can comprise a wide plethora of anti-CTLA4 antibodies.

Further, one skilled in the art, based upon the disclosure provided herein, would understand that the invention is not limited to administration of only a single antibody; rather, the invention encompasses administering at least one anti-CTLA4 antibody, *e.g.*, one of 4.1.1, 4.13.1, or 11.2.1 (CP-675,206), in combination with a therapeutic agent. Further, any

combination of anti-CTLA4 antibodies can be combined with at least one therapeutic agent and the present invention encompasses any such combination and permutation thereof.

IV. Therapeutic Agents

The present invention relates to combinations comprising anti-CTLA4 antibody and at least one therapeutic agent, which may be further combined with additional agents and/or therapeutic modalities, *e.g.*, chemotherapy, surgery, radiotherapy, transplantation, and the like, to treat cancer. That is, the patient may be subjected to additional chemotherapy with agents well-known, such as, but not limited to, growth factor inhibitors, biological response modifiers, alkylating agents, intercalating antibiotics, vinca alkaloids, immunomodulators, taxanes, platinum compounds, signal transduction inhibitors, selective estrogen receptor modulators (SERMs), such as, but not limited to, lasofoxifene, and angiogenesis inhibitors.

Co-administration of the antibody with an additional therapeutic agent (combination therapy) encompasses administering a pharmaceutical composition comprising both the anti-CTLA4 antibody and one or more additional therapeutic agents, and administering two or more separate pharmaceutical compositions, one comprising the anti-CTLA4 antibody and the other(s) comprising the additional therapeutic agent(s). Further, although co-administration or combination (conjoint) therapy generally mean that the antibody and additional therapeutic agents are administered at the same time as one another, it also encompasses simultaneous, sequential or separate dosing of the individual components of the treatment. Additionally, where an antibody is administered intravenously and the anti-cancer agent is administered orally (*e.g.*, imatinib mesylate, gemcitabine, capecitabine, temozolomide, and the like), it is understood that their combination is preferably administered as two separate pharmaceutical compositions.

Therapeutic agents are numerous and have been described in, for instance, U.S. Patent Application Publication No. 2004/0005318, No. 2003/0086930, No. 2002/0086014, and International Publication No. WO 03/086459, all of which are incorporated by reference herein, among many others. Such therapeutic agents include, but are not limited to, topoisomerase I inhibitors; other antibodies (rituximab, bevacizumab, trastuzumab, anti-IGF 1R antibody [*e.g.*, CP-751,871], anti-CD40 antibody [*e.g.*, CP-870,893], and the like); chemotherapeutic agents such as, but not limited to, imatinib (GLEEVEC), SU11248 (SUTENT; sunitinib), SU12662, SU14813; BAY 43-9006, AG-013736 (axitinib), immunomodulators, including toll-like receptor agonists (*e.g.*, TLR-9 agonist; such as, but not limited to, CPG-7909, also referred to as PF03512676 or PROMUNE), and other immunomodulators, *e.g.*, indoleamine-2,3,-dioxygenase (IDO) inhibitors; selective estrogen receptor modulators (SERMs; *e.g.*, lasofoxifene); taxanes; vinca alkaloids; temozolomide;

angiogenesis inhibitors; EGFR inhibitors; VEGF inhibitors; erbB2 receptor inhibitors; anti-proliferative agents (*e.g.*, farnesyl protein transferase inhibitors, and αvβ3 inhibitors, αvβ5 inhibitors, p53 inhibitors, and the like); immunomodulators; biological response modifiers; cytokines; tumor vaccines; tumor-specific antigens; heat shock protein-based tumor vaccines; dendritic and stem cell therapies; alkylating agents; folate antagonists; pyrimidine antagonists; anthracycline antibiotics; platinum compounds; immune costimulatory molecules (*e.g.*, CD4, CD25, PD-1, B7-H3, 4-1BB, OX40, ICOS, CD30, HLA-DR, MHCII, and LFA), and agonist antibodies thereto; among many others.

In one embodiment, the methods of the invention may be further combined with transplantation, *e.g.*, stem cell transplantation, to provide a therapeutic benefit to a patient afflicted with breast cancer. Stem cell transplantation may be performed according to the methods known in the art and may be allogeneic or autologous stem cell transplantation. Additionally, one skilled in the art would appreciate, based upon the disclosure provided herein, that transplantation encompasses adoptive transfer of lymphocytes, either autologous or obtained from an HLA-matched donor. Where the method comprises stem cell transplant, the first dose of the antibody-AI therapy agent combination can be administered after the immune system of the mammal has recovered from transplantation, for example, in the period of from one to 12 months post transplantation. In certain embodiments, the first dose is administered in the period of from one to three, or one to four months post transplantation. Transplantation methods are described many treatises, including Appelbaum *in Harrison's Principles of Internal Medicine*, Chapter 14, Braunwald *et al.*, Eds., 15th ed., McGraw-Hill Professional (2001), which is hereby incorporated herein by reference.

As pointed out previously herein, there are many chemotherapeutic agents currently available for the treatment of tumors that are suitable for use in the combination therapy of the present invention. For example, alkylating agents are a class of drugs that alkylate DNA, restricting uncoiling and replication of strands. A preferred alkylating agent for use in the methods of the present invention is cyclophosphamide (CYTOXAN). In one embodiment, CP-675,206 is administered with low-dose cyclophosphamide. Without wishing to be bound by any particular theory, this is because such dose may mediate depletion of Tregs and because CTLA4 blockade appears not to affect Tregs such that these two anti-tumor mechanisms may provide a synergistic therapeutic effect.

Folate antagonists bind to dihydrofolate reductase (DHFR) and interfere with pyrimidine (thymidine) synthesis. Methotrexate and pemetrexed (ALIMTA) are folate antagonists suitable for use in the methods of the present invention. In addition to DHFR, pemetrexed also inhibits thymidylate synthase and glycinamide ribonucleotide formyl transferase, two other folate-dependant enzymes involved in thymidine synthesis.

Pyrimidine antagonists inhibit enzymes involved in pyrimidine synthesis. As pyrimidine analogs, they also interfere with DNA production by competing with normal nucleotides for incorporation into the DNA molecule. Pyrimidine antagonists suitable for use in the methods of the present invention include 5-fluorouracil (5-FU); capecitabine (XELODA), a prodrug of 5'-deoxy-5-fluorouridine (5'-FDUR), which is enzymatically converted to 5-FU *in vivo*; and gemcitabine (GEMZAR).

Anthracycline antibiotics inhibit the uncoiling of DNA by intercalation between DNA strands. Anthracycline antibiotics include doxorubicin hydrochloride (ADRIAMYCIN), epirubicin hydrochloride (ELLENCE, PHARMORUBICIN), daunorubicin (CERUBIDINE, DAUNOXOME), and idarubicin hydrochloride (IDAMYCIN PFS, ZAVEDOS). Preferred anthracyclines for use with the present invention include doxorubicin and epirubicin.

Platinum compounds exert their anti-neoplastic effect by intercalation and intracalation between DNA strands, which inhibits uncoiling of the DNA. Platinum compounds useful in the methods of the present invention include cisplatin (PLATINOL), oxaliplatin (ELOXATIN), and carboplatin (PARAPLATIN).

Taxanes promote assembly of microtubules while inhibiting their disassembly into tubulin, thereby blocking a cell's ability to break down the mitotic spindle during mitosis. They have demonstrated significant activity against many solid tumors as single agent therapy and in combination with other chemotherapy agents. One embodiment of the combination therapy of the present invention includes the use of one or more taxanes in combination with an IGF-1R antibody. Suitable taxanes for use in combination with the IGF-1R antibody include docetaxel (TAXOTERE) and paclitaxel (TAXOL).

Taxane-derivatives, which may be active in cells resistant to doxorubicin, vinblastine, paclitaxel, docetaxel, and the like, include XRP-9981 (Sanofi Aventis), and are encompassed in the invention.

Vinca alkaloids, like taxanes, are "spindle poisons," acting on the microtubules that form the mitotic spindle. They inhibit mitosis by interfering with microtubule assembly, keeping the spindle from being formed. Vinca alkaloids include vindesine (ELDISINE), vinblastine sulfate (VELBAN), vincristine sulfate (ONCOVIN) and vinorelbine tartrate (NAVELBINE). A preferred vinca alkaloid for use in the methods of the present invention is vinorelbine.

BMS-247550 (ixabepilone) promotes tubulin polymerization and microtubule stabilization, thereby arresting cells in the G2-M phase and inducing tumor cell apoptosis. This agent demonstrates activity against taxane-resistant cells.

Analogs of rapamycin, which bind and inhibit the mammalian target of rapamycin (mTOR), are also useful and include, among others, CCI-779 (temsirolimus; Wyeth) and RAD-001 (everolimus, CERTICAN; Novartis).

The camptothecin analogs act through inhibition of topoisomerase I, an enzyme critical for DNA replication and packaging. Levels of topoisomerase I are higher in tumor cells than in normal tissue. A camptothecin analog useful in the methods of the present invention is irinotecan (CAMPTOSAR). Topoisomerase I inhibitors useful in the embodiments of the present invention include 9-aminocamptothecin, belotecan, BN-80915 (Roche), camptothecin, diflomotecan, edotecarin, exatecan (Daiichi), gimatecan, 10-hydroxycamptothecin, lurtotecan, Orathecin (rubitecan, Supergen), SN-38, topotecan, and combinations thereof.

Camptothecin derivatives are of particular interest in the combination embodiments of the invention and include camptothecin, 10-hydroxycamptothecin, 9-aminocamptothecin, irinotecan, SN-38, edotecarin, topotecan and combinations thereof.

A particularly preferred topoisomerase I inhibitor is irinotecan (CAMPTOSAR)

Topoisomerase II inhibitors useful in the combination embodiments of the present invention include aclarubicin, adriamycin, amonafide, amrubicin, annamycin, daunorubicin, doxorubicin, elsamitrucin, epirubicin, etoposide, idarubicin, galarubicin, hydroxycarbamide, nemorubicin, novantrone (mitoxantrone), pirarubicin, pixantrone, procarbazine, rebeccamycin, sobuzoxane, tafluposide, valrubicin, and Zinecard (dexrazoxane).

Particularly preferred toposimerase II inhibitors include epirubicin (Ellence), doxorubicin, daunorubicin, idarubicin and etoposide.

Alkylating agents that may be used in the embodiments of the invention include, but are not limited to, nitrogen mustard N-oxide, cyclophosphamide, AMD-473, altretamine, AP-5280, apaziquone, brostallicin, bendamustine, busulfan, carboquone, carmustine, chlorambucil, dacarbazine, estramustine, fotemustine, glufosfamide, ifosfamide, KW-2170, lomustine, mafosfamide, mechlorethamine, melphalan, mitobronitol, mitolactol, mitomycin C, mitoxatrone, nimustine, ranimustine, temozolomide, thiotepa, and platinum-coordinated alkylating compounds such as cisplatin, carboplatin (PARAPLATIN), eptaplatin, lobaplatin, nedaplatin, oxaliplatin (ELOXATIN, Sanofi), streptozocin, satraplatin and combinations thereof.

Antimetabolites that may be used in combination therapy with CTLA4 antibodies, optionally with one or more other agents include, but are not limited to dihydrofolate reductase inhibitors (such as methotrexate and NeuTrexin (trimetresate glucuronate)), purine antagonists (such as 6-mercaptopurine riboside, mercaptopurine, 6-thioguanine, cladribine, clofarabine (Clolar), fludarabine, nelarabine, and raltitrexed), pyrimidine antagonists (such as 5-fluorouracil (5-FU), pemetrexed disodium (Alimta, LY231514, MTA), capecitabine (Xeloda),

cytosine arabinoside, gemcitabine (Gemzar, Eli Lilly), Tegafur (UFT Orzel or Uforal and including TS-1 combination of tegafur, gimestat and otostat), doxifluridine, carmofur, cytarabine (including ocfosfate, phosphate stearate, sustained release and liposomal forms), enocitabine, 5-azacitidine (Vidaza), decitabine, and ethynylcytidine) and other antimetabolites such as eflornithine, hydroxyurea, leucovorin, nolatrexed (Thymitaq), triapine, trimetrexate,or for example, one of the preferred anti-metabolites disclosed in European Patent Application No. 239362 such as N-(5-[N-(3,4-dihydro-2-methyl-4-oxoquinazolin-6-ylmethyl)-N-methylamino]-2-thenoyl)-L-glutamic acid and combinations thereof.

In another embodiment the anti-cancer agent is a poly(ADP-ribose) polymerase-1 (PARP-1) inhibitor such as AG-014699, ABT-472, INO-1001, KU-0687 and GPI 18180.

Microtubulin inhibitors that may be used in combination therapy with CTLA4 antibodies, optionally with one or more other agents include, but are not limited to ABI-007, Albendazole, Batabulin, CPH-82, EPO 906 (Novartis), discodermolide (XAA-296), vinfunine and ZD-6126 (AstraZeneca).

Antibiotics that may be used in combination therapy with CTLA4 antibodies, optionally with one or more other agent including, but are not limited to, intercalating antibiotics such as actinomycin D, bleomycin, mitomycin C, neocarzinostatin (Zinostatin), peplomycin, and combinations thereof.

Plant derived anti-tumor substances (also known as spindle inhibitors) that may be used in combination therapy with CP-675,206, but are not limited to, mitotic inhibitors, for example vinblastine, vincristine, vindesine, vinorelbine (NAVELBINE), docetaxel (TAXOTERE), Ortataxel, paclitaxel (including Taxoprexin a DHA/paciltaxel conjugate) and combinations thereof.

Platinum-coordinated compounds include but are not limited to, cisplatin, carboplatin, nedaplatin, oxaliplatin (ELOXATIN), Satraplatin (JM-216), and combinations thereof.

Other agents include alitretinoin, l-asparaginase, AVE-8062 (Aventis), calcitriol (Vitamin D derivative), Canfosfamide (Telcyta, TLK-286), Cotara (131I chTNT 1/b), DMXAA (Antisoma), exisulind, ibandronic acid, miltefosine, NBI-3001 (IL-4), pegaspargase, RSR13 (efaproxiral), Targretin (bexarotene), tazarotne (Vitamin A derivative), Tesmilifene (DPPE), Theratope, tretinoin, Trizaone (tirapazamine), Xcytrin (motexafin gadolinium) and Xyotax (polyglutamate paclitaxel), and combinations thereof.

In another embodiment of the present invention statins may be used in combination with CP-675,206. Statins (HMG-CoA reductase inhibitors) may be selected from the group consisting of Atorvastatin (Lipitor, Pfizer Inc.), Provastatin (Pravachol, Bristol-Myers Squibb), Lovastatin (Mevacor, Merck Inc.), Simvastatin (Zocor, Merck Inc.), Fluvastatin (Lescol,

Novartis), Cerivastatin (Baycol, Bayer), Rosuvastatin (Crestor, AstraZeneca), Lovostatin and Niacin (Advicor, Kos Pharmaceuticals), derivatives and combinations thereof.

In a preferred embodiment the statin is selected from the group consisting of atovorstatin and lovastatin, derivatives and combinations thereof.

Other agents useful as anti-tumor agents include CADUET, Lipitor and torcetrapib.

In certain embodiments of the invention, the above described methods are combined with a cancer vaccine described, *e.g.*, in Rosenberg, S., *Development of Cancer Vaccines, ASCO Educational Book* Spring: 60-62 (2000); Logothetis, C. *ASCO Educational Book* Spring: 300-302 (2000); Khayat, D. 2000, *ASCO Educational Book* Spring: 414-428; Foon, K. 2000, *ASCO Educational Book* Spring: 730-738; *see also* Restifo, N. and Sznol, M., in, 1997, *in Cancer: Principles and Practice of Oncology*, pp. 3023-3043, Fifth Edition, DeVita, V. et al., eds. (1997), including a vaccine using autologous or allogeneic tumor cells. Cellular vaccines have been shown to be effective especially when the tumor cells are transduced to express GM-CSF, which is a potent activator of antigen presentation for tumor vaccination (Dranoff et al. (1993) Proc. Natl. Acad. Sci U.S.A. 90 (80: 3539-43).

Numerous tumor-specific antigens have been identified to date. The study of gene expression and large scale gene expression patterns in various tumors has led to the definition of so called tumor specific antigens (Rosenberg, SA *Immunity* **10**:281-287 (1999)). In many cases, these tumor specific antigens are differentiation antigens expressed in the tumors and in the cell from which the tumor arose, for example melanocyte antigens gp 100, MAGE antigens, Trp-2. The tumor antigen may also include the protein telomerase, which is required for the synthesis of telomeres of chromosomes and which is expressed in more than 85% of human cancers and in only a limited number of somatic tissues (Kim, N et al. *Science* **266**:2011-2013 (1994)). These somatic tissues may be protected from immune attack by various means. More importantly, many of these antigens can be shown to be the targets of tumor specific T cells found in the host, and such tumor-specific antigens can be combined with an anti-CTLA4 antibody to treat cancer by stimulating an anti-tumor response in the host. More particularly, CTLA4 inhibition can render these otherwise non-immunogenic proteins immunogenic thereby mediating an immune response to the tumor cell expressing them. That is, these proteins are normally viewed by the immune system as self antigens and are therefore tolerogenic but such tolerization can be overcome by anti-CTLA4 antibodies.

Tumor-specific antigen can also be "neo-antigens" expressed in cancer cells because of somatic mutations that alter protein sequence or create fusion proteins between two unrelated sequences (*i.e.* bcr-abl in the Philadelphia chromosome mutation for CML), or idiotype from B cell tumors. Other tumor vaccines can include the proteins from viruses implicated in human cancers such a Human Papilloma Viruses (HPV), Hepatitis Viruses (HBV

and HCV) and Kaposi's Herpes Sarcoma Virus (KHSV). Regardless of the source of the tumor-specific antigen, where it is expressed by a tumor cell, co-administration of the antigen with an anti-CTLA4 antibody can render the tumor cell subject to immune attack thus providing a therapeutic benefit.

Another form of tumor-specific antigen which can be used in conjunction with CTLA4 antibody administration is a purified heat shock protein (HSP) isolated from the tumor tissue. These heat shock proteins contain fragments of proteins from the tumor cells and are highly efficient at delivery to antigen presenting cells for eliciting tumor immunity (Suot, R & Srivastava, P *Science* **269**:1585-1588 (1995); Tamura, Y. et al. *Science* **278**:117-120 (1997). Thus, administration of an anti-CTLA4 antibody in combination with a tumor HSP can provide an important benefit by mediating an immune response to the tumor. HSP-based tumor vaccines, and methods for producing the same, include, *e.g.*, ONCOPHAGE (HSPPC-96), and AG-858 (HSPPC-70), both from Antigenics (Lexington, MA), among others.

Another therapeutic agent that can be combined with an anti-CTLA4 antibody is a dendritic cell (DC). DCs are potent antigen-presenting cells that can be used to prime antigen-specific responses. DCs can be produced ex vivo and can be loaded with various protein and peptide antigens, as well as with tumor cell extracts (Nestle, F. et al. *Nature Medicine* **4**:328-332 (1998)). Where the DCs are loaded with tumor cell extracts, the precise nature of the tumor antigen need not be established; rather, the DC cell processes the tumor extract and presents the antigen(s) in the context of MHC. Thus, the nature of the antigen need not be elucidated and the ability of the DCs to present it can be exploited. DCs can also be transduced by genetic means to express desired tumor antigens as well. DCs have also been fused directly to tumor cells for the purposes of immunization (Kugler, A. et al. *Nature Medicine* **6**:332-336 (2000)). As a method of tumor vaccination, DC immunization can be effectively combined with an anti-CTLA4 antibody to mediate a more potent anti-tumor response. *See also* Oh et al., *Cancer Res.* **64**:2610-2618 (2004) (TARP epitopes and breast cancer); Kontani et al., *Int J Molec Med* **12**:493-502 (2003) (dendritic cell vaccine targeting MUC1 mucin); Holmberg & Sandmaier *Expert Rev Vaccines* **3**:269-277 (2004)(vaccination for breast or ovarian cancer).

As suggested by data disclosed herein, an immune response to a vaccine-specific antigen may be enhanced by coadministration of anti-CTLA4 antibody. Therefore, the present invention includes a method of treating cancer comprising administering to a patient in need thereof a therapeutically effective amount of an anti-CTLA4 antibody and a therapeutically effective amount of at least one therapeutic agent where the agent comprises a vaccine and/or antigen, or combination of more than one antigen, or a cell presenting an antigen and/or expressing a cytokine, *e.g.*, GM-CSF.

That is, useful vaccines may be, without limitation, those comprised of breast cancer-associated antigens (*e.g.*, HER-2/neu, mammaglobin, prostate and breast tumor-associated protein [TARP], MUC1, CEA, sialyl-Tn and other carbohydrate antigens), other tumor cancer-associated antigens (*e.g.*, p53, telomerase), anti-idiotype antibodies such as 11D10, as well as vaccines comprising GM-CSF (*see, e.g.*, GVAX, Cell Genesys, Inc., Lapuleucel-T [APC8024] and Sipuleucel-T [APC8015], Dendreon Corp.; and TVAX, Geron Corp.), DNA and cell-based vaccines, dendritic cell vaccines (*reviewed in* Banchereau & Palucka, *Nature Revs. Immunol.* **5**:296-306 (2005)), recombinant viral (*e.g.*, vaccinia virus) vaccines, and heat shock protein (HSP) vaccines (*e.g.*, vitespen [ONCOPHAGE], Antigenics Inc.).

In one embodiment, the present invention encompasses a combination of CP-675,206 and a tumor antigen. Tumor antigens or tumor-associated antigens (TAAs) include, *e.g.*, cancer-germ cell (CG) antigens (MAGE, NY-ESO-1), mutational antigens (MUM-1, p53, CDK-4), over-expressed self-antigens (p53, HER2/NEU), viral antigens (from Papilloma Virus, Epstein-Barr Virus), tumor proteins derived from non-primary open reading frame mRNA sequences (NY-ESO1, LAGE1), Melan A, MART-1, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, tyrosinase, gp100, gp75, HER-2/*neu*, c-erb-B2, CEA, PSA, MUC-1, CA-125, Sialyl-Tn (STn), STn-KLH (THERATOPE, Biomira Inc.), TAG-72, KSA (17-1A), PSMA, p53 (point mutated and/or overexpressed), RAS (point mutated), EGF-R, VEGF, GD2, GM2, GD3, Anti-Id, CD20, CD19, CD22, CD36, Aberrant class II, B1, CD25 30 (IL-2R) (anti-TAC), or HPV. TAAs are discussed, for example, in Palena et al., *Adv. Cancer Res.* 95:115-145 (2006), and other art-recognized treatises, and include use of gp100 from a non-human species, *e.g.*, mouse gp100, to increase or enhance an anti-tumor response.

In one embodiment, the TAA comprises at least one characteristic likely to be related to generating or enhancing an anti-tumor immune response. Such characteristics may include, but are not limited to, prevalence, specificity, immunogenicity, and necessity for cell viability or growth. More particularly, prevalence means the antigen is present in most patients with a certain type of cancer. In another embodiment, the more types of cancer cells that express the antigen compared with expression of the antigen by normal cells the more desirable the prevalence would be. In yet another embodiment, the TAA is specific for the cancer cells in that the expression of the TAA is significantly greater on a cancer cell compared with the level of expression of the TAA on an otherwise identical but non-cancer cell, more preferably, the TAA is not detectably expressed on a normal cell but is expressed on most, if not all, cancer cells in a patient. Most preferably, the TAA is unique to a tumor cell and is not expressed in a normal cell.

In yet another embodiment, the TAA is immunogenic, *e.g.*, it induces a detectable immune response to a tumor cell. More specifically, the higher the magnitude of the

response, the more preferred the TAA. Even more preferably, the TAA induces, increases and/or prolongs a cellular immune response.

In another embodiment, the TAA is vital to cell growth or survival. That is, the TAA is needed by a tumor cell to survive, grow and/or proliferate. Even more preferably, the TAA is vital to a tumor cell such that affecting or inhibiting a biological activity of the TAA prevents or inhibits cell survival or proliferation. Even more preferably, the TAA is vital such that inhibiting or reducing a biological activity of the antigen inhibits development or selection of a tumor cell variant not expressing the antigen or a variant cell expressing a mutant antigen not affected by the anti-tumor immune response to the original TAA. That is, inducing an immune response to the antigen prevents or inhibits cell survival such that a variant antigen that avoids the immune response is not selected.

In one embodiment, CP-675,206 is administered to a patient (*e.g.*, a melanoma patient) previously vaccinated using a tumor vaccine (*e.g.*, a MART-1 antigen), thereby inducing, enhancing and/or prolonging an immune response to the vaccine, thereby providing a therapeutic benefit to the patient. In one aspect, CP-675,206 can be administered with at least one additional therapeutic agent, such as, but not limited to, a chemotherapeutic agent, an immunomodulatory agent (a TLR-9 agonist), an immune enhancing antibody (*e.g.*, an agonist anti-CD40 antibody), among many additional agents.

In another aspect, a vaccines is be administered prior to, or subsequent to, administration of the antibody-therapeutic agent combination, and when chemotherapy is part of the regimen, a vaccine may be administered prior to chemotherapy. In certain embodiments, the antibody-therapeutic agent combination of the invention may also be administered prior to chemotherapy. In yet other embodiments, the treatment can be combined with stem cell transplant. That is, the antibody-chemotherapeutic agent combination can be administered before or after stem cell transplant. A vaccine may also be administered before or after stem cell transplantation and, in certain embodiments, concomitantly with the antibody.

Stem cell transplantation may be performed according to the methods known in the art. Some such methods are described in Appelbaum *in Harrison's Principles of Internal Medicine*, Chapter 14, Braunwald *et al.*, Eds., 15th ed., McGraw-Hill Professional (2001), which is hereby incorporated herein by reference. Thus, the methods of the present invention relate to the treatment of cancer in a mammal who has undergone stem cell transplantation, which methods comprise administering to the mammal an amount of a human anti-CTLA4 antibody in combination with a therapeutic agent, which combination is effective in treating the cancer in further combination with stem cell transplantation.

Where the method comprises stem cell transplant, the first dose of the antibody-therapeutic agent combination can be administered after the immune system of the mammal has recovered from transplantation, for example, in the period of from one to 12 months post transplantation. In certain embodiments, the first dose is administered in the period of from one to three, or one to four months post transplantation. The patient may undergo stem cell transplantation and preparatory treatment(s).

Where an anti-CTLA4 antibody is combined with a standard cancer treatment, such as, *inter alia*, chemotherapeutic regimes, it may be possible to reduce the dose of chemotherapeutic reagent administered (Mokyr, M. et al. *Cancer Research* **58**: 5301-5304 (1998)). This is because combined use of an anti-CTLA4 antibody and chemotherapy can mediate cell death that is a consequence of the cytotoxic action of most chemotherapeutic compounds. Such cell death likely results in increased levels of tumor-specific antigen in the antigen presentation pathway, and the anti-CTLA4 antibody mediates an increased immune response thereto. Other combination therapies that can result in synergy with anti-CTLA4 enhancement of the immune response through cell death release of tumor antigens are radiation, surgery, and hormone deprivation, among many others. Each of these protocols, and others described elsewhere herein, creates a source of tumor antigen in the host. Likewise, angiogenesis and signal inhibitors can also be combined with an anti-CTLA4 antibody to increase an immune response to a tumor cell since inhibition of angiogenesis or signal transduction can lead to tumor cell death which may feed tumor antigen into host antigen presentation pathways. Therefore, the combination therapies disclosed herein can provide an increased source of tumor-specific antigens thereby providing an increased immune response to the tumor which, in turn, provides a therapeutic benefit to the patient.

The present invention, as noted previously, encompasses a combination of an anti-CTLA4 antibody and at least one signal transduction inhibitor, such as agents that can inhibit EGFR (epidermal growth factor receptor) responses (*e.g.*, EGFR antibodies, EGF antibodies, and molecules that are EGFR inhibitors); VEGF (vascular endothelial growth factor) inhibitors (*e.g.*, VEGF receptors and molecules that can inhibit VEGF); and erbB2 receptor (HER2) inhibitors (*e.g.*, small molecules or antibodies that bind to the erbB2 receptor, where exemplary antibodies include trastuzumab and pertuzumab, which is a HER dimerization inhibitor (HDI)).

EGFR inhibitors are described in, for example in International Patent Publication Nos. WO 95/19970, WO 98/14451, WO 98/02434, and U.S. Patent No. 5,747,498, and such substances can be used in the present invention as described herein. EGFR-inhibiting agents include, but are not limited to, the monoclonal antibodies C225, anti-EGFR 22Mab (ImClone Systems Inc., New York, NY), and ABX-EGF (Abgenix Inc., remont, CA), the compounds ZD-

1839 (AstraZeneca), BIBX-1382 (Boehringer Ingelheim), MDX-447 (Medarex,Inc., Annandale, NJ), and OLX-103 (Merck & Co., Whitehouse Station, NJ), TP-38 (IVAX), EGFR fusion protein, EGF-vaccine, anti-EGFR immunoliposomes (Hermes Biosciences Inc.), VRCTC-310 (Ventech Research) and EGF fusion toxin (Seragen Inc., Hopkinton, MA). These and other EGFR-inhibiting agents can be used in the present invention in combination with anti-CTLA4 antibody to treat various cancers.

Compounds directed at inhibition of epidermal growth factor receptor (EGFR) tyrosine kinase (TK) represent a relatively new class of antineoplastic drugs that are useful in the method of the present invention. Many human cancers express members of the EGFR family on the cell surface. When a ligand binds to EGFR, it sets off a cascade of cellular reactions that result in increased cell division and influence other aspects of cancer development and progression, including angiogenesis, metastatic spread, and inhibition of apoptosis. EGFR-TK inhibitors may selectively target one of the members of the EGFR family (EGFR (also known as HER1 or ErbB-1), HER2/neu (also known as ErbB-2), HER3 (also known as ErbB-3), or HER4 (also known as ErbB-4)), or may target two or more of them. EGFR-TK inhibitors suitable for use in the present invention include gefitinib (IRESSA), erlotinib (TARCEVA), CI-1033 (Pfizer), GW2016 (GlaxoSmithKline), EKB-569 (Wyeth), PKI-166 (Novartis), CP-724,714 (Pfizer), CI-1033 (canertinib, Pfizer Inc), and BIBX-1382 (Boeringer-Ingelheim). Additional EGFR-TK inhibitors are described in U.S. Patent No. 6,890,924.

VEGF inhibitors, for example SU-5416, SU-6668, SU-11248, SU-12662, SU-14813 (Sugen/Pfizer), as well as AG-013736 (Pfizer) and CP-547,632 (Pfizer, NY) can also be employed in combination with the anti-CTLA4 antibody. VEGF inhibitors are described, for example, in International Patent Application No. PCT/IB99/00797 (filed May 3, 1999), International Patent Publication Nos. WO 95/21613; WO 97/22596 (published June 26, 1997); WO 97/32856 (published Sept. 12, 1997); WO 98/02437 and WO 98/02438 (both published Jan. 22, 1998); WO 98/50356; WO 98/54093 (published Dec. 3, 1998); WO 99/10349 (published March 4, 1999); WO 99/16755 (published April 8, 1999); WO 99/24440; WO 99/61422; U.S. Patent Nos. 5,792,783, 5,834,504; 5,883,113; 5,886,020; 6,177,401; 6,235,764; 6,316,429; 6,395,734; 6,492,383; 6,534,524; and 6,653,308. Other examples of specific VEGF inhibitors useful in the present invention are vandetanib (Zactima), sorafenib (Bayer/Onyx), AEE788 (Novartis), AZD-2171, VEGF Trap (Regeneron/Aventis), vatalinib (also known as PTK-787, ZK-222584; Novartis/Schering AG as described in U.S. Pat. No. 6,258,812), MACUGEN (pegaptanib octasodium, NX-1838, EYE-001; Pfizer Inc./Gilead/Eyetech), IM862 (Cytran Inc., Kirkland, WA); neovastat (Aeterna), IMC-1C11 ImClone antibody, bevacizumab (AVASTIN, Genentech, Inc., San Francisco, CA); cetuximab

(ERBITUX, ImClone); and ANGIOZYME, a synthetic ribozyme that cleaves mRNA producing VEGF1 from Ribozyme (Boulder, CO) and Chiron (Emeryville, CA).

ErbB2 receptor inhibitors, such as GW-282974, GW-572016 (lapatinib) (Glaxo Wellcome plc), and the monoclonal antibodies AR-209 (Aronex Pharmaceuticals Inc., Woodlands, TX), mapatumumab (HGS-ETR1, Human Genome Sciences, Inc.) an agonist of TRAIL Receptor 1), trastuzumab (HERCEPTIN; Genentech, Inc., San Francisco, CA), pertuzumab (OMNITARG; 2C4; Genentech, a HER2 dimerization inhibitor HDI)); TAK-165 (Takeda), GW-572016 (lapatinib, GlaxoSmithKline), pelitinib (EKB-569 Wyeth), BMS-599626, PKI-166 (Novartis), dHER2 (HER2 Vaccine, Corixa and GlaxoSmithKline), Osidem (IDM-1), APC8024 (HER2 Vaccine, Dendreon), anti-HER2/neu bispecific antibody (Decof Cancer Center), B7.her2.IgG3 (Agensys), AS HER2 (Research Institute for Rad Biology & Medicine), trifunctional bispecific antibodies (University of Munich), mAB AR-209 (Aronex Pharmaceuticals Inc), and 2B-1 (Chiron), can be combined with the anti-CTLA4 antibody to provide effective combination therapy. Other erbB2 receptor inhibitors are described in, for example, International Patent Publication Nos. WO 95/19970 (published July 27, 1995); WO 97/13760 (published April 17, 1997); WO 98/02434 (published January 22, 1998); WO 98/02437 (published January 22, 1998); WO 99/35132 (published July 15, 1999); WO 99/35146 (published July 15, 1999); U.S. Patent Nos. 5,587,458, and 5,877,305. ErbB2 receptor inhibitors useful in the present invention are also described in EP1029853 (published August 23, 2000) and in International Patent Publication No. WO 00/44728, (published August 3, 2000). ErbB2 receptor inhibitors useful in the present invention are also described in United States Patent Nos. 6,465,449, and 6,284,764, and International Application No. WO 2001/98277 each of which are herein incorporated by reference in their entirety. The erbB2 receptor inhibitor compounds and substances described in the aforementioned PCT applications, U.S. patents, and U.S. provisional applications, as well as other compounds and substances that inhibit the erbB2 receptor, can be used with the anti-CTLA4 antibody in combination therapy in accordance with the present invention.

Various other compounds, such as styrene derivatives, have also been shown to possess tyrosine kinase inhibitory properties, and some of tyrosine kinase inhibitors have been identified as erbB2 receptor inhibitors. Other erbB2 Inhibitors are described in European patent publications EP 566,226 A1 (published October 20, 1993), EP 602,851 A1 (published June 22, 1994), EP 635,507 A1 (published January 25, 1995), EP 635,498 A1 (published January 25, 1995), and EP 520,722 A1 (published December 30, 1992). These publications refer to certain bicyclic derivatives, in particular quinazoline derivatives possessing anti-cancer properties that result from their tyrosine kinase inhibitory properties. Also, WO 92/20642 (published November 26, 1992), refers to certain bis-mono and bicyclic

aryl and heteroaryl compounds as tyrosine kinase inhibitors that are useful in inhibiting abnormal cell proliferation. WO 96/16960 (published June 6, 1996), WO 96/09294 (published March 6, 1996), WO 97/30034 (published August 21, 1997), WO 98/02434 (published January 22, 1998), WO 98/02437 (published January 22, 1998), and WO 98/02438 (published January 22, 1998), also refer to substituted bicyclic heteroaromatic derivatives as tyrosine kinase inhibitors that are useful for the same purpose. Other patent applications that refer to anti-cancer compounds are World Patent Application WO 00/44728 (published August 3, 2000), EP 1029853A1 (published August 23, 2000), and WO 01/98277 (published December 12, 2001), all of which are incorporated herein by reference in their entirety.

BAY 43-9006 (Onyx Pharmaceuticals) is an inhibitor of both the RAF/MEK/ERK signaling pathway and the VEGFR-2/PDGFR-β signaling cascade. RAF kinase is an enzyme in the RAS pathway and mutations in the RAS gene are associated with approximately 20% of all human cancers, including 90% of pancreatic cancer, 50% of colon cancer and about 30% of non-small cell lung cancer (NSCLC), and BRAF, a specific RAF kinase, has been demonstrated to be mutated in about two-thirds of melanomas and some colorectal cancer (CRC) and other solid tumors. In addition, VEGFR and PDGFR-β play key roles in angiogenesis such that their inhibition decreases angiogenesis. Thus, the present invention encompasses administering a combination comprising an anti-CTLA4 antibody and BAY 43-9006 to treat cancer.

PDGFR inhibitors include but not limited to those disclosed in Int. Pat. Pub. No. WO 01/40217, published June 7, 2001 and Int. Pat. Pub. No. WO 2004/020431, published March 11, 2004, the contents of which are incorporated in their entirety for all purposes.

Preferred PDGFR inhibitors include Pfizer's CP-673,451 and CP-868,596 and their pharmaceutically acceptable salts.

TIE-2 inhibitors include GlaxoSmithKline's benzimidazoles and pyridines including GW-697465A such as described in Int. Pat. Pub. Nos. WO 02/044156 published June 6, 2002, WO 03/066601 published August 14, 2003, WO 03/074515 published September 12, 2003, WO 03/022852 published March 20, 2003 and WO 01/37835 published May 31, 2001. Other TIE-2 inhibitors include Regeneron's biologicals such as those described in Int. Pat. Pub. No. WO 09/611269 published April 18, 1996, Amgen's AMG-386, and Abbott's pyrrolopyrimidines such as A-422885 and BSF-466895 described in Int. Pat. Pub. Nos. WO 09/955335, WO 09/917770, WO 00/075139, WO 00/027822, WO 00/017203 and WO 00/017202.

Use of an angiogenesis inhibitor in combination with an anti-CTLA4 antibody has been discussed previously elsewhere herein. Moreover, an angiogenesis inhibitor includes, but is not limited to, bevacizumab (AVASTIN) which is a humanized antibody to VEGF.

Bevacizumab can be used in combination with 5FU, and is indicated as a first-line treatment of patients with metastatic carcinoma of the colon or rectum. Agents that directly target angiogenic factors or their receptors offer the prospect for greater activity in receptor-competent hematologic malignancies by interrupting autocrine receptor signaling. Bevacizumab produces sustained neutralization of circulating VEGF and may be useful for treatment of myelodysplastic syndrome (MDS), lymphoma, acute myeloid leukemia (AML), and solid tumors. Thus, an anti-CTLA4 antibody and bevacizumab combination therapy, in further combination with 5FU and additional chemotherapeutic agents, for treatment of CRC, and other malignancies, is encompassed by the present invention.

In another embodiment of the present invention the anti-angiogenesis agent is a protein kinase C ß such as enzastaurin, midostaurin, perifosine, staurosporine derivative (such as RO 318425, RO317549, RO318830 or RO 318220 (Roche)), teprenone (Selbex) and UCN-01 (Kyowa Hakko).

The receptor tyrosine kinase inhibitors (RTKI) represent a class of synthetic, small molecule inhibitors of angiogenic receptor signaling. The first receptor antagonist to enter clinical testing in hematologic malignancies is SU5416 (Sugen), which impairs ligand-induced autophosphorylation of the VEGFR-1 and VEGFR-2 receptors and c-Kit. SU5416 inhibits VEGF-induced clonogenic response in leukemia cell lines and promotes apoptosis in myeloblasts from AML patients. Other RTKIs, including SU11248 (sunitinib), SU12662, SU14813, (Pfizer Inc.), vatalanib (also known as PTK787/ZK222584; Novartis), and AG-013736 (Pfizer), can be used in combination with an anti-CTLA4 antibody to treat AML and other receptor-competent hematologic malignancies. CP-675,206 may be combined with sunitinib (SU-11248), among other RTKIs. Thus, the present invention encompasses a combination of an anti-CTLA4 antibody and at least one antiangiogenic and/or signal transduction inhibitor, including, *e.g.*, SU-11248, SU-12662, SU-14813, AG-013736, as well as other angiogenesis and signal transduction inhibitors that are well-known in the art or developed in the future.

As noted previously, the combination therapy methods of the invention can be used with other agents useful in treating abnormal cell growth or cancer, including, but not limited to other agents capable of enhancing antitumor immune responses, such as additional, different, CTLA4 antibodies, and other agents also capable of blocking CTLA4; and anti-proliferative agents such as farnesyl protein transferase inhibitors, and αvβ3 inhibitors, such as the αvβ3 antibody VITAXIN, αvβ5 inhibitors, p53 inhibitors, and the like.

Where the antibody of the invention is administered in combination with another immunomodulatory agent, the immunomodulatory agent can be selected for example from the group consisting of a dendritic cell activator such as CD40 ligand and anti-CD40 agonist

antibodies, as well as enhancers of antigen presentation, enhancers of T-cell tropism, inhibitors of tumor-related immunosuppressive factors, such as TGF-β (transforming growth factor beta), and IL-10. Preferred anti-CD40 agonist antibodies encompass antibodies disclosed in International Patent Application No. PCT/US02/36107, filed November 8, 2002, now published as International Patent Publication No. WO 03/040170, and U.S. Patent Application No. 10/292,088, filed November 8, 2002, now published as U.S. Patent Publication No. US2003/0211100, including, but not limited to, an antibody having the heavy and light chain amino acid sequence of antibody 3.1.1, 3.1.1.H-A78T, 3.1.1H-A78T-V88A-V97A, 3.1.1L-L4M-L83V, 3.1.1H-A78T-V88A-V97A/3.1.1L-L4M-L83V, 7.1.2, 10.8.3, 15.1.1, 21.2.1, 21.4.1, 22.1.1, 22.1.1H-C109A, 23.5.1, 23.25.1, 23.28.1, 23.28.1H-D16E, 23.29.1, and 24.2.1.

The present treatment regimens may also be combined with antibodies or other ligands that inhibit tumor growth by binding to IGF-1R (insulin-like growth factor 1 receptor). Specific anti-IGF-1R antibodies that can be used in the present invention include those described in International Patent Application No. PCT/US01/51113, filed 12/20/01, and published as International Patent Publication No. WO02/053596, International Patent Application No. PCT/IB2004/002555, filed August 3, 2004, and published as International Patent Publication No. WO 2005/016967. Preferred anti-IGFR-1R antibodies encompass an antibody having the heavy and light chain amino acid sequence of, *e.g.*, antibody 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2 and 4.17.3.

Ligands that inhibit signaling via the IGF-1R also encompass small molecules, and other ligands including, *inter alia*, somavert (PEGVISOMANT), which is a growth hormone analog that inhibits IGF-1 signaling. PEGVISOMANT is conjugated with polyethylene glycol and can be used, among other things, to treat acromegaly. PEGVISOMANT can be co-administered with anti-CTLA4 antibody to treat cancer in that the combination can inhibit tumor growth. Thus, PEGVISOMANT, similarly with anti-IGF-1R antibodies, can be used in combination with an anti-CTLA4 antibody to treat cancer as disclosed herein.

Additional exemplary therapeutic and/or prophylactic antibodies include, but are not limited to, SYNAGIS (MedImmune, MD) which is a humanized anti-respiratory syncytial virus (RSV) monoclonal antibody; REMICADE (infliximab) (Centocor, PA) which is a chimeric anti-TNFα monoclonal antibody; REOPRO (abciximab) (Centocor) which is an anti-glycoprotein IIb/IIIa receptor; ZENAPAX (daclizumab) (Roche Pharmaceuticals, Switzerland) which is a humanized anti-CD25 monoclonal antibody; AVASTIN (bevacizumab); matuzumab (Merck AG), nimotuzumab, panitumumab, EGFR inhibitor antibody (Amgen/Abgenix); ERBITUX (cetuximab). Other examples are a humanized anti-CD18 $F(ab')_2$ (Genentech); CDP860 which is a humanized anti CD18 $F(ab')_2$ (Celltech, UK); PRO542 which is an anti-HIV gp120

antibody fused with CD4 (Progenics/Genzyme Transgenics); OSTAVIR which is a human anti Hepatitis B virus antibody (Protein Design Lab/Novartis); PROTOVIR which is a humanized anti-CMV IgG1 antibody (Protein Design Lab/Novartis); MAK-195 (SEGARD) which is a murine-anti-TNF-α $F(ab')_2$ (Knoll Pharma/BASF); IC14 which is an anti-CD14 antibody (ICOS Pharm); a humanized anti-VEGF IgG1 antibody (Genentech); OVAREX which is a murine anti-CA 125 antibody (Altarex); PANOREX which is a murine anti-17-IA cell surface antigen IgG2a antibody (Glaxo SmithKline/Centocor); BEC2 which is a murine 5 anti-idiotype (GD3 epitope) IgG antibody (ImClone System); IMC-C225 which is a chimeric anti-EGFR IgG antibody (ImClone System); VITAXIN which is a humanized anti-αVβ3 integrin antibody (Applied Molecular Evolution/MedImmune); Campath 1H/LDP-03 which is a humanized anti CD52 IgG1 antibody (Leukosite); Smart M195 which is a humanized anti-CD33 IgG antibody (Protein Design Lab/Kanebo); rituximab (RITUXAN) which is a chimeric anti-CD20 IgG1 antibody (Biogen IDEC/Genentech, Roche/Zettyaku); BEXXAR (131-I-tositumomab); belimumab (LymphoStat-B); HuMax-CD20 (HuMax, Genmab); R 1594 (Roche/Genentech); TRU-015 (Trubion Pharmaceuticals); ocrelizumab (PRO 70769); LYMPHOCIDE which is a humanized anti-CD22 IgG antibody (Immunomedics); Smart ID10 which is a humanized anti-HLA antibody (Protein Design Lab); ONCOLYM (Lym-1) is a radiolabelled murine anti-HLA diagnostic reagent antibody (Techniclone); ABX-IL8 is a human anti-IL8 antibody (Abgenix); anti-CD11a is a humanized IgG1 antibody (Genentech/Xoma); ICM3 is a humanized anti-ICAM3 antibody (ICOS Pharm); IDEC-114 is a primatized anti-CD80 antibody (IDEC Pharm/Mitsubishi); SGN14, chimeric anti-CD40 antibody (Seattle Genetics); SGN40, humanized anti-CD40 antibody (Seattle Genetics); CHIR-12.12, antagonist anti-CD40 antibody (Chiron); ISF-154 (Ad-CD154, Tragen); toralizumab; ABI-793 (Novartis); IDEC-131 is a humanized anti-CD40L antibody (IDEC/Eisai); IDEC-151 is a primatized anti-CD4 antibody (IDEC); IDEC-152 is a primatized anti-CD23 antibody (IDEC/Seikagaku); SMART anti-CD3 is a humanized anti-CD3 IgG (Protein Design Lab); 5G1.1 is a humanized anti-complement factor 5 (C5) antibody (Alexion Pharm); adalimumab is a human anti-TNF-α antibody (HUMIRA, CAT/Abbott); CDP870 is a humanized anti-TNF-α Fab fragment (Celltech); MDX-CD4 is a human anti-CD4 IgG antibody (Medarex/Eisai/Genmab); CDP571 is a humanized anti-TNF-α IgG4 antibody (Celltech); LDP-02 is a humanized anti—α4β7 antibody (LeukoSite/Genentech); OrthoClone OKT4A is a humanized anti-CD4 IgG antibody (Ortho Biotech); ANTOVA is a humanized anti-CD40L IgG antibody (Biogen); ANTEGREN is a humanized anti VLA-4 IgG antibody (Elan); MDX-33 is a human anti-CD64 (FcγR) antibody 30 (Medarex/Centeon); SCH55700 is a humanized anti-IL-5 IgG4 antibody (Celltech/Schering); SB-240563 and SB-240683 are humanized anti-IL-5 and IL-4 antibodies, respectively (SmithKline Beecham); rhuMab-E25 is a humanized anti-IgE IgG1 antibody

(Genentech/Norvartis/Tanox Biosystems); ABX-CBL is a murine anti CD-147 IgM antibody (Abgenix); BTI- 322 is a rat anti-CD2 IgG antibody (Medimmune/Bio Transplants); Orthoclone/OKT3 is a murine anti-CD3 IgG2a antibody (Ortho Biotech); SIMULECT is a chimeric anti-CD25 IgG1 antibody (Novartis Pharm); LDP-01 is a humanized anti-β2-integrin IgG antibody (LeukoSite); Anti-LFA-1 is a murine anti CD18 $F(ab')_2$ (Pasteur-Merieux/Immunotech); CAT-152 is a human anti-TGF-β_2 antibody (Cambridge Ab Tech); and CORSEVIN M is a chimeric anti-Factor VII antibody (Centocor). The above-listed immunoreactive reagents, as well as any other immunoreactive reagents, may be administered according to any regimen known to those of skill in the art, including the regimens recommended by the suppliers of the immunoreactive reagents.

In another embodiment the agent used in conjunction with CP-675,206 is a hepatocyte growth factor receptor (HGFR or c-MET) antagonist.

In another embodiment of the invention, CP-675,206 or ipilimumab is administered with an antibody to MAdCAM to treat cancer.

The nucleotide sequence encoding an antibody or other immunoreactive reagent may be obtained from any information available to those of skill in the art (i.e., from Genbank, the literature, or by routine cloning). If a clone containing a nucleic acid encoding a particular antibody or an epitope-binding fragment thereof or other immunoreactive reagent is not available, but the sequence of the antibody molecule or epitope-binding fragment thereof or other immunoreactive reagent is known, a nucleic acid encoding the immunoglobulin or other immunoreactive reagent may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody) by any method, including, *e.g.*, PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g. a cDNA clone from a cDNA library that encodes the antibody.

The anti-CTLA4 antibody, as well as any other second antibody administered therewith, may also be administered with a cytokine such as, *e.g.*, interleukin (*e.g.*, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-15, IL-18), interferon (*e.g.*, IFNα, IFNβ, IFNγ), tumor necrosis factor (*e.g.*, TNFα, TNFβ), G-CSF, GM-CSF, TGF-β, SLC, endothelial monocyte activating protein-2 (EMAP2), MIP-3α, MIP-3β, or an MHC gene, such as HLA-B7.

In one embodiment, CTLA4 blockade is co-administered with IL-15 or an agonist thereof, to increase, induce or prolong an immune response. This is because studies demonstrated that while CTLA4 blockade induced rejection of engrafted islet cells, inhibition

of IL-15/IL-15R processes inhibited activation of CD8+ T cells thereby increasing immunogenic tolerance and preventing graft rejection. That is, anti-CTLA4 blockade leads to graft rejection and IL-15-blockade had the opposite effect. *See* Ferrari-Lacraz et al., *Transplantation* **82**:1510-1517 (2006). Thus, where decreasing immunogenic tolerance is desired, *e.g.,* where inducing an anti-tumor immune response in a patient is desired, co-administration of a CTLA4 antagonist in combination with IL-15, or an agonist of IL-15/IL-15R, may enhance a therapeutic anti-tumor response, and may provide a greater immune response than either agent alone, and more preferably, may provide a synergistic effect greater than the additive effect of each agent combined. Thus, in an embodiment of the invention, combination therapy comprising an anti-CTLA4 antibody and an agonist of the IL-15/IL-15R signaling pathway (*e.g.*, IL-15, an agonist anti-IL15 antibody, an agonist anti-IL-15R antibody, among others), is provided to induce, increase and/or prolong an immune response.

Additional exemplary cytokines include other members of the TNF family, including, but not limited to, TNF-α-related apoptosis-inducing ligand (TRAIL), TNF-α-related activation-induced cytokine (TRANCE), TNF-α-related weak inducer of apoptosis (TWEAK), CD40 ligand (CD40L), LT-α, LT-β, OX40L, FasL, CD27L, CD30L, 4-1BBL, APRIL, LIGHT, TL1, TNFSF16, TNFSF17, and AITR-L, or a functional portion thereof. *See, e.g.*, Kwon et al., *Curr. Opin. Immunol.* **11**:340-345 (1999) for a general review of the TNF family.

In one embodiment, interferons and numerous other immune enhancing agents that may be used in combination therapy with CP-675,206 include, but are not limited to interferon alpha, interferon alpha-2a, interferon, alpha-2b, interferon beta, interferon gamma-1a, interferon gamma-1b (Actimmune), or interferon gamma-n1, PEG Intron A, and combinations thereof. Other agents include interleukin 2 agonists (such as aldesleukin, BAY-50-4798, Ceplene (histamine dihydrochloride), EMD-273063, MVA-HPV-IL2, HVA-Muc-1-IL2, interleukin 2, teceleukin and Virulizin), Ampligen, Canvaxin, CeaVac (CEA), denileukin, filgrastim, Gastrimmune (G17DT), gemtuzumab ozogamicin, Glutoxim (BAM-002), GMK vaccine (Progenics), Hsp 90 inhibitors (such as HspE7 from Stressgen, AG-858, KOS-953, MVJ-1-1 and STA-4783), imiquimod, krestin (polysaccharide K), lentinan, Melacine (Corixa), MelVax (mitumomab), molgramostim, Oncophage (HSPPC-96), OncoVAX (including OncoVAX-CL and OncoVAX-Pr), oregovomab, sargramostim, sizofiran, tasonermin, TheraCys, thymalfasin, pemtumomab (Y-muHMFG1), picibanil, Provenge (Dendreon), ubenimex, WF-10 (Immunokine), Z-100 (Ancer-20 from Zeria), Lenalidomide (REVIMID, Celegene), thalomid (Thalidomide), and combinations thereof.

The present invention includes coadministration of an anti-CTLA4 antibody, or combination of such antibodies, with at least one T cell costimulatory molecule (also referred to herein as a "costim"), including, but not limited to, CD4, CD25, and the like. Costimulatory

molecules further include, *e.g.*, PD-1, B7-H3, OX40, ICOS, CD30, HLA-DR, MHCII, and LFA, or agonist antibodies thereto. These and other costims are well-known in the art and have been well characterized as described in, *e.g.*, Schwartz et al., *Nature* **410**:604-608 (2001); Schwartz et al., *Nature Immunol.* **3**:427-434 (2002); and Zhang et al., *Immunity* **20**:337-347 (2004).

In one alternative embodiment of the invention, CP-675,206 or ipilimumab is administered with an antibody to human 4-1BB to treat cancer. Suitable anti-4-1BB antibodies are described in, *e.g.*, Int. Appl. No. PCT/US2004/033587 (published April 21, 2005, as WO 2005/035584). One such antibody is BMS66513.

In another embodiment of the invention, CP-675,206 or ipilimumab is administered with an antibody to human mucosal addressin cell adhesion molecule (MAdCAM) to treat cancer. Suitable anti-MAdCAM antibodies are described in, *e.g.*, Int. Appl. No. PCT/US2005/000370 (published July 28, 2005, as WO 2005/067620). Preferably, the anti-MAdCAM antibody is an antibody comprising a heavy and light chain variable region amino acid sequence of antibody 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, and 9.8.2.

In addition to combination of anti-CTLA4 and immunostimulatory compounds to increase an anti-tumor immune response, it would be understood that certain immunosuppressive molecules may inhibit or decrease an anti-tumor response. Accordingly, in another embodiment of the invention, anti-CTLA antibody is administered in combination with an inhibitor of an immunosuppressive molecule. Such immunosuppressive molecules include, but are not limited to, IDO, TGF-β, PD-1, among others. Thus, a combination of anti-CTLA4 blockade and inhibition of another immunoregulatory pathway is encompassed in the present invention, thereby further enhancing the anti-tumor response provided by anti-CTLA4 blockade.

The treatment regimens described herein may be combined with anti-angiogenesis agents, such as MMP-2 (matrix-metalloproteinase 2) inhibitors, MMP-9 (matrix-metalloproteinase 9) inhibitors, and COX-II (cyclooxygenase II) inhibitors, can be used in conjunction with the antibody in the method of the invention. Examples of useful COX-II inhibitors include CELEBREX (celecoxib), parecoxib, deracoxib, ABT-963, COX-189 (Lumiracoxib), BMS 347070, RS 57067, NS-398, Bextra (valdecoxib), Vioxx (rofecoxib), SD-8381, 4-methyl-2-(3,4-dimethylphenyl)-1-(4-sulfamoyl-phenyl)-1H-pyrrole, 2-(4-ethoxyphenyl)-4-methyl-1-(4-sulfamoylphenyl)-1H-pyrrole, T-614, JTE-522, S-2474, SVT-2016, CT-3, SC-58125 and Arcoxia (etoricoxib). Additonally, COX-II inhibitors are disclosed in U.S. Patent Application Nos. 10/801,446 and 10/801,429, the contents of which are incorporated in their entirety for all purposes.

In one embodiment the agent is celecoxib as disclosed in U.S. Patent No. 5,466,823, the contents of which are incorporated by reference in its entirety for all purposes. In another embodiment the agent is deracoxib as disclosed in U.S. Patent No. 5,521,207, the contents of which are incorporated by reference in its entirety for all purposes.

Other useful anti-angiogenic inhibitors used in conjunction with CP-675,206 include aspirin, and non-steroidal anti-inflammatory drugs (NSAIDs) which nonselectively inhibit the enzymes that make prostaglandins (cyclooxygenase I and II), resulting in lower levels of prostaglandins. Such agents include, but are not limited to, Aposyn (exisulind), Salsalate (Amigesic), Diflunisal (Dolobid), Ibuprofen (Motrin), Ketoprofen (Orudis), Nabumetone (Relafen), Piroxicam (Feldene), Naproxen (Aleve, Naprosyn), Diclofenac (Voltaren), Indomethacin (Indocin), Sulindac (Clinoril), Tolmetin (Tolectin), Etodolac (Lodine), Ketorolac (Toradol), Oxaprozin (Daypro) and combinations thereof.

Preferred nonselective cyclooxygenase inhibitors include ibuprofen (Motrin), nuprin, naproxen (Aleve), indomethacin (Indocin), nabumetone (Relafen) and combinations thereof.

Other anti-angiogenic compounds include acitretin, angiostatin, aplidine, cilengtide, COL-3, combretastatin A-4, endostatin, fenretinide, halofuginone, Panzem (2-methoxyestradiol), PF03446962 (ALK-1 inhibitor), rebimastat, removab, Revlimid, squalamine, thalidomide, ukrain, Vitaxin (alpha-v/beta-3 integrin), and zoledronic acid.

Examples of useful matrix metalloproteinase inhibitors are described in International Patent Publication Nos. WO 96/33172; WO 96/27583; WO 98/07697, WO 98/03516, WO 98/34918, WO 98/34915, WO 98/33768, WO 98/30566, WO 90/05719, WO 99/52910, WO 99/52889, WO 99/29667, European Patent Application Nos. 780386 (published June 25, 1997), 97304971 (filed July 8, 1997), 99308617 (filed October 29, 1999), 606046 (published July 13, 1994), 931788 (published July 28, 1999), 99302232 (filed March 25, 1999), International Application PCT/IB98/01113 (filed July 21, 1998), Great Britain patent application number 9912961 (filed June 3, 1999), United States Provisional Patent Application No. 60/148,464 (filed August 12, 1999), and U.S. Patent Nos. 5,863,949, and 5,861,510.

Preferred MMP-2 and MMP-9 inhibitors are those that have little or no activity inhibiting MMP-1. More preferred are those that selectively inhibit MMP-2 and/or MMP-9 relative to the other matrix-metalloproteinases (*i.e.*, MMP-1, MMP-3, MMP-4, MMP-5, MMP-6, MMP-7, MMP-8, MMP-10, MMP-11, MMP-12, and MMP-13).

Some specific examples of MMP inhibitors useful in the present invention are AG-3340, RO 32-3555, RS 13-0830, ABT-510 (Abbott), ABT 518 (Abbott), Apratastat (Amgen), AZD 8955 (AstraZeneca), Neovostat (AE-941), COL 3 (CollaGenex Pharmaceuticals), doxycycline hyclate, MPC 2130 (Myriad) and PCK 3145 (Procyon), and the compounds recited in the following list:

3-[[4-(4-fluoro-phenoxy)-benzenesulfonyl]-(1-hydroxycarbamoyl-cyclopentyl)-amino]-propionic acid;

3-exo-3-[4-(4-fluoro-phenoxy)-benzenesulfonylamino]-8-oxa-bicyclo[3.2.1]octane-3-carboxylic acid hydroxyamide;

(2R, 3R) 1-[4-(2-chloro-4-fluoro-benzyloxy)-benzenesulfonyl]-3-hydroxy-3-methyl-piperidine-2-carboxylic acid hydroxyamide;

4-[4-(4-fluoro-phenoxy)-benzenesulfonylamino]-tetrahydro-pyran-4-carboxylic acid hydroxyamide;

3-[[4-(4-fluoro-phenoxy)-benzenesulfonyl]-(1-hydroxycarbamoyl-cyclobutyl)-amino]-propionic acid;

4-[4-(4-chloro-phenoxy)-benzenesulfonylamino]-tetrahydro-pyran-4-carboxylic acid hydroxyamide;

(R) 3-[4-(4-chloro-phenoxy)-benzenesulfonylamino]-tetrahydro-pyran-3-carboxylic acid hydroxyamide;

(2R, 3R) 1-[4-(4-fluoro-2-methyl-benzyloxy)-benzenesulfonyl]-3-hydroxy-3-methyl-piperidine-2-carboxylic acid hydroxyamide;

3-[[4-(4-fluoro-phenoxy)-benzenesulfonyl]-(1-hydroxycarbamoyl-1-methyl-ethyl)-amino]-propionic acid;

3-[[4-(4-fluoro-phenoxy)-benzenesulfonyl]-(4-hydroxycarbamoyl-tetrahydro-pyran-4-yl)-amino]-propionic acid;

3-exo-3-[4-(4-chloro-phenoxy)-benzenesulfonylamino]-8-oxa-bicyclo[3.2.1]octane-3-carboxylic acid hydroxyamide;

3-endo-3-[4-(4-fluoro-phenoxy)-benzenesulfonylamino]-8-oxa-bicyclo[3.2.1]octane-3-carboxylic acid hydroxyamide; and

(R) 3-[4-(4-fluoro-phenoxy)-benzenesulfonylamino]-tetrahydro-furan-3-carboxylic acid hydroxyamide;

and pharmaceutically acceptable salts and solvates of said compounds.

In another embodiment, the agent is a signal transduction inhibitor. Such inhibitors include small molecules, antibodies, and antisense molecules, and encompass tyrosine kinase inhibitors, serine/threonine kinase inhibitors. More specifically, signal transduction inhibitors include farnesyl protein transferase inhibitors, EGF inhibitors, ErbB-1 (EGFR), ErbB-2, pan erb, IGF1R inhibitors, MEK, c-Kit inhibitors, FLT-3 inhibitors, K-Ras inhibitors, PI3 kinase inhibitors, JAK inhibitors, STAT inhibitors, Raf kinase inhibitors, Akt inhibitors, mTOR inhibitor, P70S6 kinase inhibitors and inhibitors of the WNT pathway and so called multi-targeted kinase inhibitors

In another embodiment, the signal transduction inhibitor is a farnesyl protein transferase inhibitor. Farnesyl protein transferase inhibitors include the compounds disclosed and claimed in United States Patent 6,194,438, issued February 27, 2002; United States Patent 6,258,824, issued July 10, 2001; United States Patent 6,586,447, issued July 1, 2003; United States Patent 6,071,935, issued June 6, 2000; and United States Patent 6,150,377, issued November 21, 2000. Other farnesyl protein transferase inhibitors include AZD-3409 (AstraZeneca), BMS-214662 (Bristol-Myers Squibb), Lonafarnib (Sarasar) and RPR-115135 (Sanofi-Aventis). Each of the foregoing patent applications and provisional patent applications is herein incorporated by reference in their entirety.

In another embodiment the signal transduction inhibitor is a GARF inhibitor. Preferred GARF inhibitors (glycinamide ribonucleotide formyltransferse inhibitors) include Pfizer's AG-2037 (pelitrexol) and its pharmaceutically acceptable salts. GARF inhibitors useful in the practice of the present invention are disclosed in US Patent No. 5,608,082, which is incorporated in its entirety for all purposes.

In another embodiment the signal transduction inhibitor is a MEK inhibitor. MEK inhibitors include Pfizer's MEK1/2 inhibitor PD325901, Array Biopharm's MEK inhibitor ARRY-142886, and combinations thereof.

In another embodiment the anti-cancer signal transduction inhibitor is an mTOR inhibitor. mTOR inhibitors include everolimus (RAD001, Novartis), zotarolimus, temsirolimus (CCI-779, Wyeth), AP 23573 (Ariad), AP23675, Ap23841, TAFA 93, rapamycin (sirolimus), and combinations thereof.

In another embodiment the anti-cancer signal transduction inhibitor is an Aurora 2 inhibitor such as VX-680 and derivatives thereof (Vertex), R 763 and derivatives thereof (Rigel), and ZM 447439 and AZD 1152 (AstraZeneca), or a Checkpoint kinase 1/2 inhibitors such as XL844 (Exilixis).

In another embodiment the anti-cancer signal transduction inhibitor is an Akt inhibitor (Protein Kinase B) such as API-2, perifosine and RX-0201.

Additionally, other targeted anti-cancer agents include the raf inhibitors sorafenib (BAY-43-9006, Bayer/Onyx), GV-1002, ISIS-2503, LE-AON and GI-4000, BMS 184476, CCI 779, DTIC, ISIS 2503, ONYX 015, and flavopyridol.

The invention also relates to methods comprising CP-675,206 and cell cycle inhibitors such as the CDK2 inhibitors ABT-751 (Abbott), AZD-5438 (AstraZeneca), alvocidib (flavopiridol, Aventis), BMS-387,032 (SNS 032 Bristol Myers), EM-1421 (Erimos), indisulam (Esai), seliciclib (Cyclacel), BIO 112 (Onc Bio), UCN-01 (Kyowa Hakko), and AT7519 (Astex Therapeutics) and Pfizer's multitargeted CDK inhibitors PD0332991 and AG24322.

The invention also relates to the use of CP-675,206 together with telomerase inhibitors such as transgenic B lymphocyte immunotherapy (Cosmo Bioscience), GRN 163L (Geron), GV1001 (Pharmexa), RO 254020 (and derivatives thereof), and diazaphilonic acid.

The invention also relates to the use of CP-675,206 with hormonal, anti-hormonal, anti-androgenal therapeutic agents such as anti-estrogens including, but not limited to toremifene, raloxifene, anti-androgens such as finasteride, mifepristone, ABARELIX (Praecis), TRELSTAR, ATAMESTANE (Biomed-777), ATRASENTAN (Xinlay), Bosentan, doxercalciferol, and combinations thereof.

The invention also contemplates the use of CP-675,206 together with gene silencing agents or gene activating agents such as histone deacetylase (HDAC) inhibitors such as suberolanilide hydroxamic acid (SAHA, Merck Inc./Aton Pharmaceuticals), depsipeptide (FR901228 or FK228), G2M-777, MS-275, pivaloyloxymethyl butyrate and PXD-101.

The invention also contemplates the use of CP-675,206 with gene therapeutic agents such as ADVEXIN (ING 201), TNFerade (GeneVec, a compound which express TNFalpha in response to radiotherapy), RB94 (Baylor College of Medicine).

The invention also contemplates CP-675,206 together with ribonucleases such as Onconase (ranpirnase).

The invention also contemplates CP-675,206 with antisense oligonucleotides such as bcl-2 antisense inhibitor Genasense (Oblimersen, Genta).

The invention also contemplates CP-675,206 together with proteosomics such as PS-341 (MLN-341) and VELCADE (bortezomib).

The invention also contemplates CP-675,206 together with anti-vascular agents such as Combretastatin A4P (Oxigene).

The invention also contemplates combination of CP-675,206 with traditional cytotoxic agents including DNA binding agents, mitotic inhibitors, alkylating agents, anti-metabolites, intercalating antibiotics, topoisomerase inhibitors and microtubulin inhibitors.

In an embodiment of the invention, an anti-CTLA4 antibody is administered to a patient receiving a chemotherapeutic agent for treatment of cancer. Such chemotherapeutic agents are known in the art and include, but are not limited to: methotrexate, taxol, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclophosphamide, ifosfamide, nitrosoureas, cisplatin, carboplatin, mitomycin, dacarbazine, procarbizine, etoposides, camptothecins, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, plicamycin, mitoxantrone, asparaginase, vinblastine, vincristine, vinorelbine, paclitaxel, and docetaxel, doxorubicin, epirubicin, 5-fluorouracil, taxanes such as docetaxel and paclitaxel, leucovorin, levamisole, irinotecan, estramustine, etoposide, nitrosoureas such as carmustine and lomustine, L-asparaginase, topotecan, nitrogen mustards, cytoxan, etoposide, BCNU, vinca

alkaloids, platinum compounds, mitomycin, gemcitabine, hexamethylmelamine, temsirolimus (CCI-779); lapatinib (GW 572016); RAD-001 (everolimus); XRP-9881; ixabepilone (BMS-247550); pertuzumab (OMNITARG, 2C4); topotecan, tyrosine kinase inhibitors, tyrphostins, imatinib mesylate (GLEEVEC), herbimycin A, genistein, erbstatin, and lavendustin A.

In other embodiments, suitable chemotherapeutics include, but are not limited to, alkylating agents: nitrogen mustards (*e.g.*, cyclophosphamide, ifosfamide, trofosfamide, chlorambucil); nitrosoureas (*e.g.*, carmustine (BCNU), lomustine (CCNU)); alkylsulphonates (*e.g.*, busulfan, treosulfan); triazenes (*e.g.*, dacarbazine); Platinum containing compounds (*e.g.*, cisplatin, carboplatin, aroplatin, oxaliplatin); Plant Alkaloids: Vinca alkaloids (*e.g.*, vincristine, vinblastine, vindesine, vinorelbine); Taxoids (*e.g.*, paclitaxel, docetaxel; DNA Topoisomerase Inhibitors: epipodophyllins (*e.g.*, etoposide, teniposide, topotecan, 9-aminocamptothecin, camptothecin, crisnatol); mitomycins (*e.g.*, mitomycin C, anti-metabolites); anti-folates: DHFR inhibitors (*e.g.*, methotrexate, trimetrexate) IMP dehydrogenase Inhibitors (*e.g.*, mycophenolic acid, tiazofurin, ribavirin, EICAR); Ribonuclotide reductase Inhibitors (*e.g.*, hydroxyurea, deferoxamine); pyrimidine analogs: uracil analogs (*e.g.*, 5-fluorouracil, floxuridine, doxifluridine, ratitrexed); cytosine analogs (*e.g.*, cytarabine (ara C), cytosine arabinoside, fludarabine); purine analogs (*e.g.*, mercaptopurine, thioguanine); DNA antimetabolites (*e.g.*, 3-HP, 2'-deoxy-5-fluorouridine, 5-HP, alpha-TGDR, aphidicolin glycinate, ara-C, 5-aza-2'-deoxycytidine, beta-TGDR, cyclocytidine, guanazole, inosine glycodialdehyde, macebecin II, pyrazoloimidazole); Hormonal therapies: Receptor antagonists: Anti-estrogen (*e.g.*, tamoxifen, raloxifene, megestrol); aromatase inhibitors (*e.g.*, exemestane, anastrozole, letrozole); GnRH antagonists (*e.g.*, abarelix, histrelin); selective estrogen receptor modulators (SERMs) (*e.g.*, lasofoxifene); LH-RH agonists (*e.g.*, goserelin, tryptorelin, buserelin, leuprolide acetate); Anti-androgens (*e.g.*, flutamide, bicalutamide, nilutamide, megestrol, cyproterone); Retinoids/Deltoids cis-retinoic acid; vitamin A derivative (*e.g.*, all-trans retinoic acid (ATRA-IV)); vitamin D3 analogs (*e.g.*, EB 1089, CB 1093, KH 1060); Photodynamic therapies (*e.g.*, vertoporfin (BPD-MA), phthalocyanine, photosensitizer Pc4, demethoxy-hypocrellin A (2BA-2-DMHA); Cytokines, *e.g.*, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-15, IL-18, IFNα, IFNβ, IFNγ, TNFα, TNFβ, G-CSF, GM-CSF, TGF-β, SLC, EMAP2, MIP-3α, MIP-3β, HLA-B7, other members of the TNF family (*e.g.*, TRAIL, TRANCE, TWEAK, CD40L, LT-α, LT-β, OX40L, CD40L, FasL, CD27L, CD30L, 4-1BBL, APRIL, LIGHT, TL1, TNFSF16, TNFSF17, and AITR-L, or a functional portion thereof); Angiogenesis Inhibitors: angiostatin (plasminogen fragment), antiangiogenic antithrombin III, angiozyme, ABT-627, Bay 12-9566, benefin, bevacizumab, BMS-275291, cartilage-derived inhibitor (CDI), CAI, CD59 complement fragment, CEP-7055, Col 3, combretastatin A-4, endostatin (collagen XVIII fragment), fibronectin fragment, Gro-beta,

halofuginone, heparinases, heparin hexasaccharide fragment, HMV833, human chorionic gonadotropin (hCG), IM-862, interferon alpha/beta/gamma, interferon inducible protein (IP-10), interleukin-12, Kringle 5 (plasminogen fragment), marimastat, metalloproteinase inhibitors (TIMPs), 2-methoxyestradiol, MMI 270 (CGS 27023A), MoAb IMC-1C11, neovastat (Aeterna), NM-3, panzem, PI-88, placental ribonuclease inhibitor, plasminogen activator inhibitor, platelet factor-4 (PF4), prinomastat, prolactin 16kD fragment, proliferin-related protein (PRP), PTK 787/ZK 222594, retinoids, solimastat, squalamine, SS 3304, SU 5416, SU6668, SU11248, SU12662, SU14813, BAY 43-9006, AG-013736, tetrahydrocortisol-S, tetrathiomolybdate, thalidomide, thrombospondin-1 (TSP-1), TNP-470, transforming growth factor-beta (TGF-b), vasculostatin, vasostatin (calreticulin fragment), ZD6126, ZD 6474, farnesyl transferase inhibitors (FTI), bisphosphonates; Antimitotic agents (*e.g.*, allocolchicine, halichondrin B, colchicine, colchicine derivative, dolstatin 10, maytansine, rhizoxin, thiocolchicine, trityl Cysteine); Other agents: isoprenylation inhibitors; dopaminergic neurotoxins (*e.g.*, 1-methyl-4-phenylpyridinium ion); cell cycle inhibitors (*e.g.*, staurosporine): actinomycins (*e.g.*, actinomycin D, dactinomycin); bleomycins (*e.g.*, bleomycin A2, bleomycin B2, peplomycin); anthracyclines (*e.g.*, daunorubicin, doxorubicin (adriamycin), idarubicin, epirubicin, pirarubicin, zorubicin, mitoxantrone); mTOR inhibitors (*e.g.*, temsirolimus, everolimus); MDR inhibitors (*e.g.*, verapamil); Ca^{2+} ATPase inhibitors (*e.g.*, thapsigargin); toll-like receptor agonists (*e.g.*, CpG-7909, also known as PF03512676 or PROMUNE; Coley Pharm; reviewed in Krieg, 1998, *In: Applied Oligonucleotide Technology*, pp. 431-448, C.A. Stein and A.M. Krieg, (Eds.), John Wiley and Sons, Inc., New York, NY); costimulatory molecules (*e.g.*, CD4, CD25, PD-1, B7-H3, 4-1BB, OX40, ICOS, CD30, HLA-DR, MHCII, and LFA, and agonist antibodies thereto); among many other agents known in the art.

Additional anti-cancer agents that may be used in the methods of the present invention include, but are not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; amifostine trihydrate; aminoglutethimide; amsacrine; anastrozole; anthramycin; arsenic trioxide; asparaginase; asperlin; azacitidine; azetepa; azotomycin; Bacillus Calmette-Guerin; batimastat; benzodepa; bevacizumab; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; bortezomib; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; capecitabine; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cisplatin; chlorambucil; cladribine; clodronate; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; darbepoietin; daunorubicin hydrochloride; decitabine; dexormaplatin; dexrazoxane; dezaguanine; dezaguanine mesylate; diaziquone; diethylstilbestrol; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene

citrate; dromostanolone propionate; duazomycin; farmorubicin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; erlotinib; erythropoietin; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; everolimus; exemestane; fadrozole hydrochloride; fazarabine; fenretinide; filgastrim (G-CSF); floxuridine; fludarabine phosphate; fludrocortisone; fluorouracil; fluoxymesterone; flurocitabine; fosquidone; fostriecin sodium; fulvestrant; gefitinib; gemcitabine; gemcitabine hydrochloride; gemtuzumab; goserelin; hydroxyurea; ibritumomab tiuxetan; idarubicin hydrochloride; ifosfamide; ilmofosine; imatinib; interleukin II (including recombinant interleukin II, or rIL2), interferon alfa-2a; interferon alfa-2b; interferon alfa-n1; interferon alfa-n3; interferon beta-1a; interferon gamma-1b; iproplatin; irinotecan hydrochloride; ixabepilone; ketoconazole; lanreotide acetate; lapatinib; letrozole; leucovorin; leuprolide acetate; levamisole; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; medroxyprogesterone; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; mesna; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; octreotide; ormaplatin; oxaliplatin; oxisuran; paclitaxel; pamidronate; pegaspargase; PEG-L-asparaginase; PEG-filgastrim; peliomycin; pentamustine; pentostatin; peplomycin sulfate; perfosfamide; pertuzumab; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfimer; porfiromycin; prednimustine; pemetrexed; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; raltitrexed; riboprine; rituximab; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; somavert (PEGVISOMANT); sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; sunitinib; streptozocin; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; temozolomide; temsirolimus; teniposide; teroxirone; testolactone; thalidomide; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; topotecan; toremifene citrate; trastuzumab; tretinoin; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; topotecan; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zolendronate; zorubicin hydrochloride.

Other anti-cancer drugs that can be used include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol;

adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorlns; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; docetaxel; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7;

lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anti-cancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras famesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofuran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium;

telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

In another specific embodiment, a combination comprising an anti-CTLA4 antibody and a second therapeutic agent is administered to a subject receiving radiation therapy for treatment of cancer. For radiation treatment, the radiation can be gamma rays or X-rays. The methods encompass treatment of cancer comprising radiation therapy, such as external-beam radiation therapy, interstitial implantation of radioisotopes (I-125, palladium, iridium), radioisotopes such as strontium-89, thoracic radiation therapy, intraperitoneal P-32 radiation therapy, and/or total abdominal and pelvic radiation therapy. In preferred embodiments, the radiation treatment is administered as external beam radiation or teletherapy wherein the radiation is directed from a remote source. In various preferred embodiments, the radiation treatment is administered as internal therapy or brachytherapy wherein a radiaoactive source is placed inside the body close to cancer cells or a tumor mass. Radiation therapy can be administered in accordance to well-known radiotherapy methods for treatment of cancer. The dose and regimen for radiotherapy can be readily determined by one skilled in the art and is based on the stage of the disease, and other factors well-known in the art. For a general overview of radiation therapy, *see* Hellman, In: Principles of Cancer Management: Radiation Therapy, Chapter 16, DeVita et al., eds., 6th edition, 2001, J. B. Lippencott Company, Philadelphia.

Co-administration of the antibody with at least one therapeutic agent (combination therapy) encompasses administering a pharmaceutical composition comprising both the anti-CTLA4 antibody and one or more therapeutic agent(s), and administering two or more separate pharmaceutical compositions, one comprising the anti-CTLA4 antibody and the other(s) comprising the therapeutic agent(s). Further, although co-administration or combination (conjoint) therapy generally mean that the antibody and additional therapeutic agents are administered at the same time as one another, it also encompasses simultaneous, sequential or separate dosing of the individual components of the treatment. Additionally, where an antibody is administered intravenously and the therapeutic agent is administered

orally (*e.g.*, exemestane), it is understood that their combination is preferably administered as two separate pharmaceutical compositions.

The present invention also encompasses the administration of other therapeutic agents in addition to the antibody and first therapeutic agent, either concurrently with one or more of those components, or sequentially before and/or after. Such therapeutic agents include cancer vaccines, anti-vascular agents, anti-proliferative agents, antiagionesis agents, signal transduction inhibitors, immunomodulatory agents, cytokines, and palliative agents to provide supportive care, such as, but not limited to, analgesics, anti-emetic agents, anti-diarrheal agents, and steroids. Preferred anti-emetic agents include ondansetron hydrochloride, granisetron hydrochloride, and metoclopramide. Preferred anti-diarrheal agents include diphenoxylate and atropine (LOMOTIL), loperamide (IMMODIUM), octreotide (SANDOSTATIN), olsalazine (DIPENTUM), and mesalamine (ASACOL). Preferred steroids include the non-absorbable steroid budesonide (ENTOCORT), and the steroids for systemic administration dexametasone (DECADRON) and prednisone (METICORTEN).

Each administration may vary in its duration from a rapid administration to a continuous perfusion. As a result, for the purposes of the present invention, the combinations are not exclusively limited to those that are obtained by physical association of the constituents, but also to those that permit a separate administration, which can be simultaneous or spaced out over a period of time. The compositions according to the invention are preferably compositions which can be administered parentally. However, these compositions may be administered orally or intraperitoneally in the case of localized regional therapies.

As will be appreciated by one of skill in the art, the choice of therapeutic agents to be used in combination with anti-CTLA4 antibodies, and the timing of their use, will be determined in part by the type and stage of the cancer that is being treated.

The methods of the present invention are suitable for use both as first line therapy and second line therapy. Treatment of both early stage and advanced (metastatic) cancers are within the scope of the present invention.

V. Pharmaceutical Compositions

The invention encompasses the preparation and use of pharmaceutical compositions comprising a human anti-CTLA4 antibody of the invention as an active ingredient in combination with a therapeutic agent, *e.g.*, a chemotherapeutic agent, another antibody, an immunostimulatory agent, a signal transduction inhibitor, an angiogenesis inhibitor, preferably, the therapeutic agent is a chemotherapeutic agent. Such a pharmaceutical composition may consist of each active ingredient alone, as a combination of at least one

active ingredient (*e.g.*, an effective dose of an anti-CTLA4, an effective does of a therapeutic agent) in a form suitable for administration to a subject, or the pharmaceutical composition may comprise the active ingredient and one or more pharmaceutically acceptable carriers, one or more additional (active and/or inactive) ingredients, or some combination of these.

In one embodiment, the antibody is administered parenterally (*e.g.*, intravenously) in an aqueous solution while the therapeutic agent (*e.g.*, a chemotherapeutic agent, a signal transduction inhibitor, an immunomodulator, an angiogenesis inhibitor, and the like) is administered orally in pill/capsule form. However, the skilled artisan would understand, based upon the disclosure provided herein, that the invention is not limited to these, or any other, formulations, doses, routes of administration, and the like. Rather, the invention encompasses any formulation or method of administering an antibody in combination with a therapeutic agent, including, but not limited to, administering each agent separately in a different formulation via a different route of administration, and administering the antibody and the therapeutic agent in a single composition (*e.g.* where the therapeutic agent is a protein, such as, another antibody, a cytokine, a costim, and the like), in an aqueous composition administered, *inter alia*, intravenously), among many others. Thus, the following discussion describes various formulations for practicing the methods of the invention comprising administration of any anti-CTLA4 antibody in combination with any therapeutic agent, but the invention is not limited to these formulations, but comprises any formulation as can be readily determined by one skilled in the art once armed with the teachings provided herein for use in the methods of the invention.

In certain embodiments, the antibodies may be present in a neutral form (including zwitter ionic forms) or as a positively or negatively-charged species. In some embodiments, the antibodies may be complexed with a counterion to form a pharmaceutically acceptable salt.

The terms "pharmaceutically acceptable salt" refer to a complex comprising one or more antibodies and one or more counterions, where the counterions are derived from pharmaceutically acceptable inorganic and organic acids and bases.

Pharmaceutically acceptable inorganic bases include metallic ions. More preferred metallic ions include, but are not limited to, appropriate alkali metal salts, alkaline earth metal salts and other physiological acceptable metal ions. Salts derived from inorganic bases include aluminum, ammonium, calcium, cobalt, nickel, molybdenum, vanadium, manganese, chromium, selenium, tin, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, rubidium, sodium, and zinc, and in their usual valences.

Pharmaceutically acceptable acid addition salts of the antibodies of the present invention can be prepared from the following acids, including, without limitation formic, acetic,

acetamidobenzoic, adipic, ascorbic, boric, propionic, benzoic, camphoric, carbonic, cyclamic, dehydrocholic, malonic, edetic, ethylsulfuric, fendizoic, metaphosphoric, succinic, glycolic, gluconic, lactic, malic, tartaric, tannic, citric, nitric, ascorbic, glucuronic, maleic, folic, fumaric, propionic, pyruvic, aspartic, glutamic, benzoic, hydrochloric, hydrobromic, hydroiodic, lysine, isocitric, trifluoroacetic, pamoic, propionic, anthranilic, mesylic, orotic, oxalic, oxalacetic, oleic, stearic, salicylic, aminosalicylic, silicate, p-hydroxybenzoic, nicotinic, phenylacetic, mandelic, embonic, sulfonic, methanesulfonic, phosphoric, phosphonic, ethanesulfonic, ethanedisulfonic, ammonium, benzenesulfonic, pantothenic, naphthalenesulfonic, toluenesulfonic, 2-hydroxyethanesulfonic, sulfanilic, sulfuric, nitric, nitrous, sulfuric acid monomethyl ester, cyclohexylaminosulfonic, β-hydroxybutyric, glycine, glycylglycine, glutamic, cacodylate, diaminohexanoic, camphorsulfonic, gluconic, thiocyanic, oxoglutaric, pyridoxal 5-phosphate, chlorophenoxyacetic, undecanoic, N-acetyl-L-aspartic, galactaric and galacturonic acids.

Pharmaceutically acceptable organic bases include trimethylamine, diethylamine, N, N'-dibenzylethylenediamine, chloroprocaine, choline, dibenzylamine, diethanolamine, ethylenediamine, meglumine (N-methylglucamine), procaine, cyclic amines, quaternary ammonium cations, arginine, betaine, caffeine, clemizole, 2-ethylaminoethanol, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanediamine, butylamine, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, ethylglucamine, glucamine, glucosamine, histidine, hydrabamine, imidazole, isopropylamine, methylglucamine, morpholine, piperazine, pyridine, pyridoxine, neodymium, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, tripropylamine, triethanolamine, tromethamine, methylamine, taurine, cholate, 6-amino-2-methyl-2-heptanol, 2-amino-2-methyl-1,3-propanediol, 2-amino-2-methyl-1-propanol, aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids, strontium, tricine, hydrazine, phenylcyclohexylamine, 2-(N-morpholino)ethanesulfonic acid, bis(2-hydroxyethyl)amino-tris(hydroxymethyl)methane, *N*-(2-acetamido)-2-aminoethanesulfonic acid, 1,4-piperazinediethanesulfonic acid, 3-morpholino-2-hydroxypropanesulfonic acid, 1,3-bis[tris(hydroxymethyl)methylamino]propane, 4-morpholinepropanesulfonic acid, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid, 2-[(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulfonic acid, N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid, 4-(N-morpholino)butanesulfonic acid, 3-(*N,N*-bis[2-hydroxyethyl]amino)-2-hydroxypropanesulfonic acid, 2-hydroxy-3-[tris(hydroxymethyl)methylamino]-1-propanesulfonic acid, 4-(2-hydroxyethyl)piperazine-1-(2-hydroxypropanesulfonic acid), piperazine-1,4-bis(2-hydroxypropanesulfonic acid) dihydrate, 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid, *N,N*-bis(2-hydroxyethyl)glycine, N-(2-