UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 9,856,320 B2
APPLICATION NO. : 14/400667
DATED : January 2, 2018
INVENTOR(S) : Cogswell et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claims

Claim 8, Column 126, Lines 57-58, replace:
"colorectal cancer, colon cancer, colorectal cancer,"
With:
--colorectal cancer, colon cancer,--

Claim 10, Column 127, Lines 20-21, replace:
"wherein the cancer is the cancer is"
With:
--wherein the cancer is--

Claim 11, Column 127, Line 24, replace:
"combined with one of more of"
With:
--combined with one or more of--

Claim 19, Column 128, Line 30, replace:
"colorectal cancer, colon cancer, colorectal cancer,"
With:
--colorectal cancer, colon cancer,--

Claim 22, Column 128, Line 52, replace:
"combined with one of more of"
With:
--combined with one or more of--

Signed and Sealed this
Sixteenth Day of October, 2018

Andrei Iancu
Director of the United States Patent and Trademark Office

Amgen Exhibit 1002
Amgen v. BMS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

U.S. Patent No.: 9,856,320	Confirmation No.: 8185
Date of Patent: January 2, 2018	Art Unit: 1644
Inventors: COGSWELL *et al.*	Atty. Docket: 3338.0630002/ELE/C-K
Applicant: Bristol-Myers Squibb Company	

Title: **CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING**

Request for Certificate of Correction Under 37 C.F.R. § 1.322

Attn: Certificate of Correction Branch

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

Commissioner:

It is hereby requested that a Certificate of Correction under 37 C.F.R. § 1.322 be issued for the above-captioned United States Patent. This Certificate of Correction is being requested due to mistakes which appear in the printed patent. These mistakes were made by both the U.S. Patent and Trademark Office and by Applicant. The mistakes made by Applicant are of a clerical or typographical nature, or of a minor character. Patentee submits that correction of these errors does not introduce new matter.

Specifically, the printed patent contains the following errors for which a Certificate of Correction is respectfully requested:

In Claim 8, at column 126, lines 57-58, please replace "colorectal cancer, colon cancer, colorectal cancer," with –colorectal cancer, colon cancer,–. Support for this correction can be found throughout the specification as filed, including, *inter alia*, at page 5, lines 22-29.

In Claim 10, at column 127, lines 20-21, please replace "wherein the cancer is the cancer is" with –wherein the cancer is–. Support for this correction can be found in the Amendment and Reply, filed on August 2, 2017, at page 6, line 3 (claim 34).

In Claim 11, at column 127, line 24, please replace "combined with one of more of" with –combined with one or more of–. Support for this correction can be found throughout the specification as filed, including, *inter alia*, at page 53, lines 20-31.

In Claim 19, at column 128, line 30, please replace "colorectal cancer, colon cancer, colorectal cancer," with –colorectal cancer, colon cancer,–. Support for this correction can be found throughout the specification as filed, including, *inter alia*, at page 5, lines 22-29.

In Claim 22, at column 128, line 52, please replace "combined with one of more of" with –combined with one or more of–. Support for this correction can be found throughout the specification as filed, including, *inter alia*, at page 53, lines 20-31.

Remarks

The above-noted corrections do not involve such changes in the patent as would constitute new matter or would require reexamination.

A completed Form PTO/SB/44 accompanies this request, with the above-noted corrections printed thereon. Accordingly, a Certificate of Correction is believed proper and issuance thereof is respectfully requested.

The Commissioner 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 19, 2018

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

9942037_1

PTO/SB/44 (09-07)
Approved for use through 08/13/2013. OMB 0651-0033
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
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(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 9,856,320

APPLICATION NO. : 14/400,667

ISSUE DATE : January 2, 2018

INVENTOR(S) : COGSWELL *et al.*

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In Claim 8, at column 126, lines 57-58, please replace

"colorectal cancer, colon cancer, colorectal cancer,"

with

--colorectal cancer, colon cancer,--

In Claim 10, at column 127, lines 20-21, please replace

"wherein the cancer is the cancer is"

with

--wherein the cancer is--

MAILING ADDRESS OF SENDER (Please do not use customer number below):
Sterne, Kessler, Goldstein & Fox P.L.L.C.
1100 New York Avenue, NW
Washington DC 20005-3934

Atty. Dkt. No. 3338.0630002/ELE/C-K

This collection of information is required by 37 CFR 1.322, 1.323 and 1.324. 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 1.0 hour 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 are required 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: Attention Certificate of Corrections Branch, 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/44 (09-07)
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INVENTOR(S) : COGSWELL *et al.*

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In Claim 11, at column 127, line 24, please replace

"combined with one of more of"

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--combined with one or more of--

In Claim 19, at column 128, line 30, please replace

"colorectal cancer, colon cancer, colorectal cancer,"

with

--colorectal cancer, colon cancer,--

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Washington DC 20005-3934

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ISSUE DATE : January 2, 2018

INVENTOR(S) : COGSWELL *et al.*

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In Claim 22, at column 128, line 52, please replace

"combined with one of more of"

with

--combined with one or more of--

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Electronic Patent Application Fee Transmittal

Application Number:	14400667
Filing Date:	12-Nov-2014
Title of Invention:	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
First Named Inventor/Applicant Name:	John P. Cogswell
Filer:	Ji Eun Kim/Jeanette Yando
Attorney Docket Number:	3338.0630002/ELE/C-K/AHB

Filed as Large Entity

Filing Fees for U.S. National Stage under 35 USC 371

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:				
CERTIFICATE OF CORRECTION	1811	1	150	150

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**150**

Electronic Acknowledgement Receipt	
EFS ID:	33770167
Application Number:	14400667
International Application Number:	
Confirmation Number:	8185
Title of Invention:	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
First Named Inventor/Applicant Name:	John P. Cogswell
Customer Number:	115018
Filer:	Ji Eun Kim/Jeanette Yando
Filer Authorized By:	Ji Eun Kim
Attorney Docket Number:	3338.0630002/ELE/C-K/AHB
Receipt Date:	19-SEP-2018
Filing Date:	12-NOV-2014
Time Stamp:	17:06:21
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$150
RAM confirmation Number	092018INTEFSW17073700
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	Request for Certificate of Correction	2018-09-19-RequestforCOC.pdf	265499 3ffb10f1347ddfeab6ee290cfc4be09711fa9460	no	6
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30536 04db7b79b1e305bfe55070cec08400f83d51c82a	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	296035		

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.

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.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/400,667	01/02/2018	9856320	3338.0630002/ELE/C-K/AHB	8185

115018 7590 12/13/2017

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 359 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):

John P. Cogswell, Yardley, PA;
Bristol-Myers Squibb Company, Princeton, NJ;
Stacie M. Goldberg, Potomac, MD;
Ashok Kumar Gupta, Clarksburg, MD;
Maria Jure-Kunkel, Plainsboro, NJ;
Xi-Tao Wang, Wellesley, MA;
Jon M. Wigginton, Rockville, MD;

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United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/400,667	11/12/2014	John P. Cogswell	3338.0630002/ELE/C-K/AHB	8185

115018 7590 12/01/2017
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	

MAIL DATE	DELIVERY MODE
12/01/2017	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.

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.
14/400,667	12 November, 2014	COGSWELL ET AL.	3338.0630002/ELE/C-K/AHB

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

EXAMINER	
ILIA OUSPENSKI	
ART UNIT	PAPER
1644	20171130

DATE MAILED:

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

Commissioner for Patents

Applicant's IDS filed on 11/22/2017 is acknowledged and has been considered.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ILIA OUSPENSKI whose telephone number is (571)272-2920. The examiner can normally be reached on Monday-Friday 9 - 5.
If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel E. Kolker can be reached on 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 OUSPENSKI/
Primary Examiner, Art Unit 1644

PTO-90C (Rev.04-03)

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Substitute for form 1449/PTO

FOURTH SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 2

Complete if Known	
Application Number	14/400,667
Int'l Filing Date	May 13, 2013
First Named Inventor	COGSWELL, John P.
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0630002/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
	US18	US-20030039653-A1	02-27-2003	Chen et al.	
	US19	US-20090215812-A1	08-27-2009	Bedrosian et al.	
	US20	US-20110269948-A1	11-03-2011	Sanjuan et al.	
	US21	US-20110206701-A1	08-25-2011	Afar et al.	
	US22	US-20100151447-A1	06-17-2010	Ely; Scott	
	US23	US-20090217401-A1	08-27-2009	Korman et al.	
	US24	US-20110008369-A1	01-13-2011	Finnefrock 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]
	NPL5	National Institutes of Health, "Dose-escalation Study of Combination BMS-936558 (MDX-1106) and Ipilimumab in Subjects With Unresectable Stage III or Stage IV Malignant Melanoma," ClinicalTrials.gov Identifier NCT01024231, accessed at https://web.archive.org/web/20111023232455/https://clinicaltrials.gov/ct2/show/NCT01024231, accessed on September 22, 2017, 3 pages.	
	NPL6	SHIBAYAMA, S. and YOSHIDA, T., "Development of ONO-4538, fully human anti-PD-1 antibody for malignant tumors," Medical Science Digest 36(12):1120-1123, New Science Co., Japan (2010).	
	NPL7	GAO, Q., et al., "Overexpression of PD-L1 Significantly Associates with Tumor Aggressiveness and Postoperative Recurrence in Human Hepatocellular Carcinoma," Clinical Cancer Research 15(3);971-979, American Association for Cancer Research, United States (2009)	

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

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Sheet 2 of 2

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Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0630002/ELE/C-K/BMD

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.	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	
	NPL8	GHEBEH, H., et al., "The B7-H1 (PD-L1) T Lymphocyte-Inhibitory molecule Is Expressed in Breast cancer Patients with Infiltrating Ductal Carcinoma: Correlation with Important high-Risk Prognostic Factors Neoplasia 8(3):190-198, Elsevier, Netherlands (2006)				
	NPL9	ROTH, T., et al., "B7-H3 Ligand Expression by Prostate Cancer: A Novel Marker of Prognosis and Potential Target for Therapy," Cancer Res 67(16):7893-7900, American Association for Cancer, United States (2007)				
	NPL10	Office Action mailed November 9, 2017, in United States Patent Application No. 14/950,748, inventors Cogswell et al., filed November 24, 2015.				

8449453_1

Examiner Signature	/ILIA I OUSPENSKI/	Date Considered	11/30/2017

*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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(Use as many sheets as necessary)

Sheet 1 of 2

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Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0630002/ELE/C-K/BMD

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	US19	US-20090215812-A1	08-27-2009	Bedrosian et al.	
	US20	US-20110269948-A1	11-03-2011	Sanjuan et al.	
	US21	US-20110206701-A1	08-25-2011	Afar et al.	
	US22	US-20100151447-A1	06-17-2010	Ely; Scott	
	US23	US-20090217401-A1	08-27-2009	Korman et al.	
	US24	US-20110008369-A1	01-13-2011	Finnefrock 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]
	NPL5	National Institutes of Health, "Dose-escalation Study of Combination BMS-936558 (MDX-1106) and Ipilimumab in Subjects With Unresectable Stage III or Stage IV Malignant Melanoma," ClinicalTrials.gov Identifier NCT01024231, accessed at https://web.archive.org/web/20111023232455/https://clinicaltrials.gov/ct2/show/NCT01024231, accessed on September 22, 2017, 3 pages.	
	NPL6	SHIBAYAMA, S. and YOSHIDA, T., "Development of ONO-4538, fully human anti-PD-1 antibody for malignant tumors," Medical Science Digest 36(12):1120-1123, New Science Co., Japan (2010).	
	NPL7	GAO, Q., et al., "Overexpression of PD-L1 Significantly Associates with Tumor Aggressiveness and Postoperative Recurrence in Human Hepatocellular Carcinoma," Clinical Cancer Research 15(3);971-979, American Association for Cancer Research, United States (2009)	

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.

Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Substitute for form 1449/PTO

FOURTH SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 2 of 2

Complete if Known	
Application Number	14/400,667
Int'l Filing Date	May 13, 2013
First Named Inventor	COGSWELL, John P.
Art Unit	1644
Examiner Name	OUSPENSKI, Ilia I.
Attorney Docket Number	3338.0630002/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	
	NPL8	GHEBEH, H., et al., "The B7-H1 (PD-L1) T Lymphocyte-Inhibitory molecule Is Expressed in Breast cancer Patients with Infiltrating Ductal Carcinoma: Correlation with Important high-Risk Prognostic Factors Neoplasia 8(3):190-198, Elsevier, Netherlands (2006)				
	NPL9	ROTH, T., et al., "B7-H3 Ligand Expression by Prostate Cancer: A Novel Marker of Prognosis and Potential Target for Therapy," Cancer Res 67(16):7893-7900, American Association for Cancer, United States (2007)				
	NPL10	Office Action mailed November 9, 2017, in United States Patent Application No. 14/950,748, inventors Cogswell et al., filed November 24, 2015.				

8449453_1

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.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (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 08/24/2017
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 facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/400,667	11/12/2014	John P. Cogswell	3338.0630002/ELE/C-K/AHB	8185

TITLE OF INVENTION: CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	11/24/2017

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 03-02 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 has been filed for recordation as set forth in 37 CFR 3.11. 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, New Jersey

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

4a. The following fee(s) are submitted:
- ☒ Issue Fee
- ☐ Publication Fee (No small entity discount permitted)
- ☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ☐ A check is enclosed.
- ☒ Payment by credit card. Form PTO-2038 is attached.
- ☒ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number 19-0036 (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ☐ Applicant certifying micro entity status. See 37 CFR 1.29
  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.
- ☐ Applicant asserting small entity status. See 37 CFR 1.27
  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.
- ☐ Applicant changing to regular undiscounted fee 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 Nov. 21, 2017

Typed or printed name Ji-Eun Kim Registration No. 68,504

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Electronic Patent Application Fee Transmittal

Application Number:	14400667
Filing Date:	12-Nov-2014
Title of Invention:	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
First Named Inventor/Applicant Name:	John P. Cogswell
Filer:	Ji Eun Kim/Jeanette Yando
Attorney Docket Number:	3338.0630002/ELE/C-K/AHB

Filed as Large Entity

Filing Fees for U.S. National Stage under 35 USC 371

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	960	960

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**1140**

Electronic Acknowledgement Receipt	
EFS ID:	31010680
Application Number:	14400667
International Application Number:	
Confirmation Number:	8185
Title of Invention:	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
First Named Inventor/Applicant Name:	John P. Cogswell
Customer Number:	115018
Filer:	Ji Eun Kim/Jeanette Yando
Filer Authorized By:	Ji Eun Kim
Attorney Docket Number:	3338.0630002/ELE/C-K/AHB
Receipt Date:	22-NOV-2017
Filing Date:	12-NOV-2014
Time Stamp:	10:58:16
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$1140
RAM confirmation Number	112217INTEFSW10591100
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	NPL5_ClinicalTrials_NCT01024231.pdf	301530 01bb8bda0c2b2a14b26f68988a91297c5bc85d82	no	3
Warnings:					
Information:					
2	Non Patent Literature	NPL6_Shibayama.pdf	2640958 2e671976e7f16e685db45a0ae0d1b6358e2391e5	no	6
Warnings:					
Information:					
3	Non Patent Literature	NPL7_Gao.pdf	637169 e9966b253ee9deb6cbef04c2e37b80b22a64a7c2	no	10
Warnings:					
Information:					
4	Non Patent Literature	NPL8_Ghebeh.pdf	552986 7f0722678a2f4889f9d80a4b48549d35eabc7163	no	9
Warnings:					
Information:					
5	Non Patent Literature	NPL9_Roth.pdf	635751 f835c4b5c22d5eda00752764aaf70306e6e9fe28	no	9
Warnings:					
Information:					
6		33380630002-IDS.pdf	713992 18ccc694a642045d0b3e9368ae12f15b7cbdd4ac	yes	5

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**

	Transmittal Letter		1		3
	Information Disclosure Statement (IDS) Form (SB08)		4		5
Warnings:					
Information:					
7	Issue Fee Payment (PTO-85B)	33380630002-IssueFee.pdf	1280103 81f49dd8d52db69ba8cec51bf211f3aabb73ffbe	no	1
Warnings:					
Information:					
8	Non Patent Literature	NPL10_NFOA_14950748.PDF	308970 b79112656e2c1ec695c5f6b7aa3b914f57e7be7c	no	11
Warnings:					
Information:					
9	Fee Worksheet (SB06)	fee-info.pdf	32567 b8c0afb5307b4071ae83d78cbda08840a5e7fb6b	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	7104026		

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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

Inventors: COGSWELL *et al.*	Confirmation No.: 8185
Applicant: Bristol-Myers Squibb Company	Art Unit: 1644
Application No.: 14/400,667	Examiner: OUSPENSKI, Ilia I.
Int'l Filing Date: May 13, 2013	Atty. Docket: 3338.0630002/ELE/C-K/BMD

Title: **CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING**

Fourth Supplemental Information Disclosure Statement

Mail Stop Issue Fee

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

Commissioner:

Listed on the accompanying IDS Forms PTO/SB/08a equivalent and PTO/SB/08b equivalent are documents 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 Fourth Supplemental Information Disclosure Statement is a continuation of the numbering in Applicant's Third Supplemental Information Disclosure Statement filed on **August 2, 2017**, in connection with the above-captioned application.

Applicant has listed the publication date on the attached IDS Forms based on information presently available to the undersigned. However, the listed publication date 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 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. 37 C.F.R. § 1.97(d). The required fee is provided through online credit card payment authorization in the amount of $180.00. 37 C.F.R. § 1.17(p).

In addition, I hereby state that (1) each item of information contained in this Information Disclosure Statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this Information Disclosure Statement; and/or (2) 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)(1)-(2).

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 **NPL6**, SHIBAYAMA (2010), is in a foreign language, and was cited in a corresponding foreign office action. Document **NPL6** was cited by the foreign Examiner as a reference for providing a known technique. The Examiner contends that Document **NPL6** discloses that BMS-936558 (MDSX-1106) corresponds to an anti-PD-1 antibody.

Copies of documents **NPL5-NPL10** 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 **US18-US24** 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 an Office Action from the co-pending U.S. Patent Application No. 14/950,748, filed November 24, 2015, as document **NPL10**.

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 document has 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: Nov. 21, 2017

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

8449662_1

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United States Patent and Trademark Office
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115018 7590 08/24/2017
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: 08/24/2017

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/400,667	11/12/2014	John P. Cogswell	3338.0630002/ELE/C-K/AHB	8185

TITLE OF INVENTION: CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	11/24/2017

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), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (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 08/24/2017
Sterne, Kessler, Goldstein & Fox P.L.L.C.
1100 New York Avenue NW
Washington, DC 20005

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(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/400,667	11/12/2014	John P. Cogswell	3338.0630002/ELE/C-K/AHB	8185

TITLE OF INVENTION: CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	11/24/2017

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 03-02 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 has been filed for recordation as set forth in 37 CFR 3.11. 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. The following fee(s) are submitted:
- ❑ Issue Fee
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- ❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ❑ A check is enclosed.
- ❑ Payment by credit card. Form PTO-2038 is attached.
- ❑ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ❑ Applicant certifying micro entity status. See 37 CFR 1.29
  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.
- ❑ Applicant asserting small entity status. See 37 CFR 1.27
  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.
- ❑ Applicant changing to regular undiscounted fee 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. _____

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/400,667	11/12/2014	John P. Cogswell	3338.0630002/ELE/C-K/AHB	8185

115018 7590 08/24/2017

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: 08/24/2017

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 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, 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. 14/400,667	Applicant(s) COGSWELL ET AL.	
	Examiner ILIA OUSPENSKI	Art Unit 1644	AIA (First Inventor to File) Status No

-- 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 *08/02/2017*.
   ☐ 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 *14, 22, 23, 26, 28 and 30-46*. 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 a reply 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 OUSPENSKI/
Primary Examiner, Art Unit 1644

Examiner's Comment

1. Claims 14, 22, 23, 26, 28 and 30-46 are potentially subject to a provisional rejection on the ground of nonstatutory double patenting over claims 22-33 of copending Application USSN 15/122,827, published as US 2017/0088626 (of record), for the reasons set forth in the previous office action. This would be a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Given that this provisional nonstatutory double patenting rejection over copending USSN 15/122,827 is the only rejection remaining in the instant application, and that USSN 15/122,827 is a later-filed application relative to the instant application, the rejection is withdrawn to permit the instant application to issue first. See MPEP 804.

2. Any inquiry concerning this communication or earlier communications from the examiner should be directed to ILIA OUSPENSKI whose telephone number is (571)272-2920. The examiner can normally be reached on Monday-Friday 9 - 5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel E. Kolker can be reached on 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 OUSPENSKI/
ILIA OUSPENSKI, Ph.D.
Primary Examiner
Art Unit 1644

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

THIRD SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	1

Complete if Known	
Application Number	14/400,667
§ 371(c) Date	November 12, 2014
First Named Inventor	COGSWELL, John P.
Art Unit	1644
Examiner Name	OUSPENSKI, ILIA I
Attorney Docket Number	3338.0630002/ELE/C-K/BGC

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]
/I.I.O/	NPL4	National Institutes of Health Clinical Center, Clinical Trial Identifier NCT01024231 entitled "Dose-escalation Study of Combination BMS-936558 (MDX-1106) and Ipilimumab in Subjects With Unresectable Stage III or Stage IV Malignant Melanoma," ClinicalTrials.gov, available at https://clinicaltrials.gov/archive/NCT01024231/2012_04_30, last accessed on July 18, 2017, 4 pages (same clinical trial accessed on April 27, 2017 by Examiner in same application).	

5977022_1

Examiner Signature	/ILIA I OUSPENSKI/	Date Considered	08/23/2017

*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.

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BIB DATA SHEET

CONFIRMATION NO. 8185

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
14/400,667	11/12/2014 RULE	424	1644	3338.0630002/ELE/C-K/AHB

APPLICANTS

Bristol-Myers Squibb Company, Princeton, NJ;

INVENTORS

John P. Cogswell, Yardley, PA;
Stacie M. Goldberg, Potomac, MD;
Ashok Kumar Gupta, Clarksburg, MD;
Maria Jure-Kunkel, Plainsboro, NJ;
Xi-Tao Wang, Wellesley, MA;
Jon M. Wigginton, Rockville, MD;

**** CONTINUING DATA ***************************

This application is a 371 of PCT/US2013/040764 05/13/2013
which claims benefit of 61/647,442 05/15/2012
and claims benefit of 61/790,747 03/15/2013

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

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****

01/22/2015

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
		PA	34	22	5

ADDRESS

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

TITLE

CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING

FILING FEE RECEIVED	FEES: Authority has been given in Paper	☐ All Fees
3700	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

WEST Search History

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DATE: Wednesday, August 23, 2017

Hide?	Set Name	Query	Hit Count
Interference			
		DB=PGPB,USPT; THES=ASSIGNEE; PLUR=NO; OP=ADJ	
☐	L18	L16 and @ pd > 20170428	4
☐	L17	L9 and @ pd > 20170428	35
☐	L16	L15 and (treat $ or administ $).clm.	14
☐	L15	L14 and (cancer $ or tumor $ or tumour $ or malignan $ or neoplas $ or metasta $ or onco $).clm.	14
☐	L14	L13 and L6.clm. and L7.clm.	16
☐	L13	(L10 or L11 or L12)	27
☐	L12	L9 and bristol $.aanm.	26
☐	L11	L9 and bristol $.as.	16
☐	L10	L9 and (cogswell-j $ or goldberg-s $ or gupta-a $ or jure-kunkel-m $ or wang-x $ or wigginton-j $).in.	8
☐	L9	(L8 same (dosage $ or dosing $ or dose $ or regimen $ or schedul $ or once $ or twice $ or day $ or daily $ or week $ or month $ or frequen $ or "mg/kg"))	172
☐	L8	(L6 with L7 with (combin $ or together or concurren $ or simultaneous $))	574
☐	L7	((anti-ctla4 or anti-ctla-4 or anti-cd152 or ".alpha.ctla4" or ".alpha.ctla-4" or ".alpha.cd152" or ticilimumab or tremelimumab or ipilimumab) or ((CTLA4 or ctla-4 or cd152) near3 antibod $))	8721
☐	L6	((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 nivolumab or pembrolizumab) or ((pd-1 or pd1 or pdhd1 or cd279) near3 antibod $))	2748

END OF SEARCH HISTORY

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14400667	COGSWELL ET AL.
	Examiner	Art Unit
	ILIA 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 applications	4/30/2017	io
WEST: attached	4/30/2017	io
ProQuest: same as WEST	4/30/2017	io
Inventor search	4/30/2017	io
WEST and Inventor searches updated	8/23/2017	io

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
See search notes		8/23/2017	io

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14400667	COGSWELL ET AL.
	Examiner	**Art Unit**
	ILIA OUSPENSKI	1644

CPC						
Symbol					**Type**	**Version**
C07K		16	/	2818	F	2013-01-01
C07K		16	/	2827	I	2013-01-01
A61K		2039	/	505	A	2013-01-01
A61K		2039	/	507	A	2013-01-01
C07K		2317	/	76	A	2013-01-01
A61K		39	/	3955	I	2013-01-01
C07K		16	/	18	I	2013-01-01
C07K		16	/	2803	I	2013-01-01
G01N		33	/	57492	I	2013-01-01
G01N		2333	/	70596	A	2013-01-01
G01N		2800	/	52	A	2013-01-01
			/			
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			/			
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CPC Combination Sets							
Symbol					**Type**	**Set**	**Ranking**	**Version**
			/					
			/					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	22	
/ILIA OUSPENSKI/ Primary Examiner.Art Unit 1644	08/23/2017	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14400667	COGSWELL ET AL.
	Examiner	Art Unit
	ILIA OUSPENSKI	1644

US ORIGINAL CLASSIFICATION		INTERNATIONAL CLASSIFICATION										
CLASS	SUBCLASS	CLAIMED					NON-CLAIMED					
		C	0	7	K	16 / 28 (2006.01.01)						
CROSS REFERENCE(S)		A	6	1	K	39 / 395 (2006.01.01)						
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)											

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	22	
/ILIA OUSPENSKI/ Primary Examiner.Art Unit 1644	08/23/2017	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

U.S. Patent and Trademark Office

Part of Paper No. 20170823

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14400667	COGSWELL ET AL.
	Examiner	**Art Unit**
	ILIA OUSPENSKI	1644

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

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

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 22	
/ILIA OUSPENSKI/ Primary Examiner.Art Unit 1644 (Primary Examiner)	08/23/2017 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure NONE

U.S. Patent and Trademark Office Part of Paper No. 20170823

Substitute for form 1449/PTO

SECOND SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	14/400,667
§ 371(c) Date	November 12, 2014
First Named Inventor	COGSWELL, John P.
Art Unit	1644
Examiner Name	OUSPENSKI, ILIA I
Attorney Docket Number	3338.0630002/ELE/C-K/BGC

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
	US17	2017/0028040 A1	02-02-2017	Lan *et al.*	

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	CN-101215329-A	07-09-2008	FOURTH MILITARY MEDICAL UNIV		Abst.
	FP2	WO-2006133396-A2	12-14-2006	DANA-FARBER CANCER INSTITUTE		

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]
	NPL3	WANG, G.-Z. and LIU, C.-Q., "A therapeutic strategy of targeting PD1/PDL signal pathway," *Chinese Bulletin of Life Sciences 23(1)*:86-89, China Academic Journal Electronic Publishing House, China (2011).	Abst.

5483581_1

Examiner Signature	/ILIA I OUSPENSKI/	Date Considered	08/23/2017

*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/

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: COGSWELL *et al.*	Confirmation No.: 8185
Applicant: Bristol-Myers Squibb Company	Art Unit: 1644
Application No.: 14/400,667	Examiner: OUSPENSKI, Ilia I.
§ 371(c) Date: November 12, 2014	Atty. Docket: 3338.0630002/ELE/C-K/BGC

Title: **CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING**

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

Mail Stop Amendment

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

Sir:

In reply to the Office Action dated May 2, 2017, Applicant submits the following Amendments and Remarks.

Amendments to the Claims begin on page 2 of this paper.

Remarks begin on page 9 of this paper.

It is not believed that extensions of time or fees for net addition of claims 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 fees required therefor (including fees for net addition of claims) 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.

1-13. (Canceled)

14. (Currently Amended) A method of treating a human subject afflicted with a cancer comprising administering to the subject:

(a) an antibody or an antigen-binding portion thereof that specifically binds to and inhibits Programmed Death-1 (PD-1) ("anti-PD-1 antibody"); and

(b) an antibody or an antigen-binding portion thereof that specifically binds to and inhibits Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) ("anti-CTLA-4 antibody");

~~each antibody being administered at a dosage ranging from 0.1 mg/kg to 20.0 mg/kg body weight in a concurrent regimen~~ comprising:

(i) an induction dosing schedule comprising ~~combined~~ administration of the anti-PD-1 antibody and the anti-CTLA-4 antibody 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, 10, or 12 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, 10, or 12 doses; followed by

(ii) a maintenance dosing schedule comprising ~~combined~~ administration of the anti-PD-1 antibody and the anti-CTLA-4 antibody at a dosing frequency of ~~at least~~ once every 8, 12 or 16 weeks, or ~~at least~~ once a quarter, for ~~at least~~ 4, 6, 8, 10, 12 or 16 ~~doses.~~ doses; and wherein the anti-PD-1 antibody and the anti-

CTLA-4 antibody are administered in the induction dosing schedule at the following dosages:

(a) 0.1 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody;

(b) 5 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody;

(c) 10 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody;

(d) 0.1 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;

(e) 0.3 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;

(f) 1 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;

(g) 3 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;

(h) 5 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;

(i) 10 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;

(j) 0.1 mg/kg anti-PD-1 antibody and 0.1 mg/kg of anti-CTLA-4 antibody;

(k) 0.3 mg/kg anti-PD-1 antibody and 0.3 mg/kg of anti-CTLA-4 antibody;

(l) 0.5 mg/kg anti-PD-1 antibody and 0.5 mg/kg of anti-CTLA-4 antibody; or

(m) 5 mg/kg anti-PD-1 antibody and 5 mg/kg of anti-CTLA-4 antibody.

15-21. (Cancelled)

22. (Currently Amended) The method of ~~claim 15~~ claim 14, wherein the anti-PD-1 antibody is nivolumab.

23. (Currently Amended) The method of ~~claim 15~~ claim 14, wherein the anti-CTLA-4 antibody is ipilimumab.

24.-25. (Cancelled)

26. (Previously presented) The method of claim 14, wherein the anti-CTLA-4 antibody is administered during the induction dosing schedule once every 3 weeks for a total of 4 doses.

27. (Cancelled)

28. (Currently Amended) A method of treating a human subject afflicted with a cancer comprising administering to the subject 1 [[3]] mg/kg of an anti-PD-1 antibody and 3 [[1]] mg/kg of an anti-CTLA-4 antibody[[.]] every 3 weeks for 4 doses, followed by subsequently administering the anti-PD-1 antibody alone at a dosing frequency of once every 2 weeks.

29. (Cancelled)

30. (Currently Amended) The method of claim 14, wherein the ~~concurrent regimen~~ administering comprises:

(i) an induction dosing schedule comprising ~~combined~~ administration of the anti-PD-1 antibody and the anti-CTLA-4 antibody at a dosing frequency of once every 3 weeks for 4 doses, followed by administration of the anti-PD-1 antibody alone at a dosing frequency of once every 3 weeks for 4 doses; followed by

(ii) a maintenance dosing schedule comprising ~~combined~~ administration of the anti-PD-1 antibody and the anti-CTLA-4 antibody at a dosing frequency of once every 2 to 12 weeks for up to 8 doses.

31. (Previously presented) The method of claim 14, wherein the treatment provides a reduction in tumor size, reduction in tumor growth, elimination of the tumor, reduction in number of metastatic lesions over time, complete response, partial response, or stable disease.

32. (Previously presented) The method of claim 14, wherein the cancer is selected from liver cancer, hepatocellular carcinoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, breast cancer, lung cancer, squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, cutaneous or intraocular malignant melanoma, renal cancer, renal cell carcinoma, uterine cancer, ovarian cancer, colorectal cancer, colon cancer, colorectal cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, castration-resistant prostate 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, gastric cancer, 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 childhood, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the CNS, primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, asbestos-induced cancers, and a hematologic malignancy.

33. (Currently Amended) The method of claim 32, [[35,]] wherein the hematologic malignancy is selected from 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, lymphocytic lymphoma, 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.

34. (Currently Amended) The method of claim 14, wherein the cancer is ~~the cancer is~~ an advanced, recurring, metastatic, and/or refractory cancer.

35. (Previously presented) The method of claim 14, wherein the treatment is further combined with one of more of chemotherapy, radiation, surgery, hormone deprivation, angiogenesis inhibitors, and an anti-PD-L1 antibody or antigen-binding portion thereof.

36. (New) The method of claim 14, wherein the anti-PD-1 antibody and the anti-CTLA-4 antibody are administered in the maintenance dosing schedule at the following dosages:

(a) 0.1 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody;

(b) 5 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody;

(c) 10 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody;

(d) 0.1 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;

(e) 0.3 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;

(f) 1 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;

(g) 3 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;

(h) 5 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;

(i) 10 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;

(j) 0.1 mg/kg anti-PD-1 antibody and 0.1 mg/kg of anti-CTLA-4 antibody;

(k) 0.3 mg/kg anti-PD-1 antibody and 0.3 mg/kg of anti-CTLA-4 antibody;

(l) 0.5 mg/kg anti-PD-1 antibody and 0.5 mg/kg of anti-CTLA-4 antibody; or

(m) 5 mg/kg anti-PD-1 antibody and 5 mg/kg of anti-CTLA-4 antibody.

37. (New) The method of claim 28, wherein the anti-PD-1 antibody subsequently administered alone is administered at a dosage of 3 mg/kg.

38. (New) The method of claim 14, wherein 5 mg/kg of the anti-PD-1 antibody and 3 mg/kg of the anti-CTLA-4 antibody are administered to a human subject every 3 weeks for 4 doses in the induction dosing schedule.

39. (New) The method of claim 14, wherein 1 mg/kg of the anti-PD-1 antibody and 1 mg/kg of the anti-CTLA-4 antibody are administered to a human subject every 3 weeks for 4 doses in the induction dosing schedule.

40. (New) The method of claim 14, wherein 3 mg/kg of the anti-PD-1 antibody and 1 mg/kg of the anti-CTLA-4 antibody are administered to a human subject every 3 weeks for 4 doses in the induction dosing schedule.

41. (New) The method of claim 14, wherein 5 mg/kg of the anti-PD-1 antibody and 1 mg/kg of the anti-CTLA-4 antibody are administered to a human subject every 3 weeks for 4 doses in the induction dosing schedule.

42. (New) The method of claim 28, wherein the treatment provides a reduction in tumor size, reduction in tumor growth, elimination of the tumor, reduction in number of metastatic lesions over time, complete response, partial response, or stable disease.

43. (New) The method of claim 28, wherein the cancer is selected from liver cancer, hepatocellular carcinoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, breast cancer, lung cancer, squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, melanoma, renal cancer, renal cell carcinoma, uterine cancer, ovarian cancer, colorectal cancer, colon cancer, colorectal cancer, rectal cancer, cancer of the anal region, stomach cancer,

testicular cancer, castration-resistant prostate 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, gastric cancer, 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 childhood, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the CNS, primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, asbestos-induced cancers, and a hematologic malignancy.

44. (New) The method of claim 28, wherein the cancer is melanoma.

45. (New) The method of claim 28, wherein the cancer is an advanced, recurring, metastatic, and/or refractory cancer.

46. (New) The method of claim 28, wherein the treatment is further combined with one of more of chemotherapy, radiation, surgery, hormone deprivation, angiogenesis inhibitors, and an anti-PD-L1 antibody or antigen-binding portion thereof.

Remarks

I. *Status of the Claims*

Upon entry of the foregoing amendments, claims 14, 22, 23, 26, 28, and 30-46 are pending in the application, with claims 14 and 28 being the independent claims. Claims 14, 22, 23, 28, 33, and 34 are sought to be amended. New claims 36-46 are sought to be added. Support for these amendments and new claims can be found throughout the specification as originally filed. For example, support for claims 14, 28, and 36-41 can be found in U.S. 2015/0125463, which publication corresponds to the subject application, at paragraphs [0110]-[0114], [0162]-[0156], Examples 15-17, and original claim 15. For example, support for claims 42-45 can be found in U.S. 2015/0125463, at paragraph [0168]. For example, support for claim 46 can be found in U.S. 2015/0125463, at paragraphs [0118] and [0177]. Claims 1, 4, 10, 15, 16, 20, 21, 24, 25, 27, and 29 are sought to be cancelled. Claims 2-3, 5-9, 11-13, and 17-19 were previously canceled. These changes to the claims are believed to introduce no new matter, and their entry is respectfully requested.

II. *Rejections Under Pre-AIA 35 U.S.C. § 102(b)*

Previous claims 14 and 22-35 are rejected under 35 U.S.C. § 102(b) because the claimed invention is allegedly anticipated by the data in clinical trial NCT01024231 accessed by the Examiner on April 27, 2017 as evidenced by Ascierto *et al., Seminars in Oncology*, October 2010, 37(5): 508-516 ("Ascierto").

Applicant first points out that the version of clinical trial NCT01024231 accessed by the Examiner on April 27, 2017 is not the version that was available as of the priority date (May 15,

2012) of this application. Therefore, the clinical trial NCT01024231 data accessed on April 27, 2017 is not prior art under 35 U.S.C. § 102(b).

According to clinicaltrials.gov/archive/, however, a version of NCT01024231 was available on April 30, 2012—before the earliest priority date of the present application. Applicant submits this reference in an accompanying supplemental IDS. Applicant has listed the publication date on the IDS Form based on information presently available to the undersigned. However, the listed publication date should not be construed as an admission that the information was actually published on the date indicated or that the reference is prior art.

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

Not in acquiescence to the propriety of the rejection but rather solely to advance prosecution, Applicant has amended claims 14 and 28 and canceled claims 24, 25, 27, and 29. Insofar as the rejection applies to the amended claims, Applicant respectfully traverses.

Anticipation requires that all the elements and limitations of the claims are found, either explicitly or inherently, within a single reference, arranged in the same way as the claim. *In re Bond*, 910 F.2d 831, 832-33 (Fed. Cir. 1990); *Net MoneyIN, Inc. v. VeriSign, Inc.*, 545 F.3d 1359, 1370 (Fed. Cir. 2008). The absence of any claimed element from the reference negates anticipation. *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 1574 (Fed. Cir. 1984).

The version of NCT01024231 available on April 30, 2012 fails to disclose the specific doses of the anti-PD-1 antibody and the anti-CTLA-4 antibody recited in claim 14 and the specific dosing schedule recited in claim 28. Ascierto was cited by the Examiner as evidence of the NCT01024231 trial but also fails to disclose the specific doses of the anti-PD-1 antibody and the anti-CTLA-4 antibody recited in claim 14 and the specific dosing schedule recited in claim 28. Therefore, neither NCT01024231 available on April 30, 2012, nor Ascierto anticipates amended claim 14 or 28. Claims 22, 23, 26, and 30-35 depend directly or indirectly from claim 14 or 28 and thus incorporate every element of the independent claims. Therefore, the dependent claims are also not anticipated by NCT01024231 available on April 30, 2012 or Ascierto. Accordingly, Applicant respectfully requests that the rejections of amended claims 14, 22, 23, 26, 28, and 30-35 under 35 U.S.C. § 102(b) be reconsidered and withdrawn.

III. *Nonstatutory Double Patenting Rejection*

The Examiner provisionally rejected previous claims 14, 15, and 22-35 on the ground of nonstatutory double patenting as being unpatentable over claims 22-33 of co-pending Application No. 15/122,827. Office Action, p. 6. Applicant disagrees.

Nonetheless, Applicant notes that U.S. Application No. 15/122,827 is a co-pending Application with a later filing date than the present Application. Applicant respectfully requests that this provisional rejection be held in abeyance until subject matter that is otherwise patentable is identified.

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.

Respectfully submitted,

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

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

Date: August 2, 2017

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

5447374_1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: COGSWELL *et al.*	Confirmation No.: 8185
Applicant: Bristol-Myers Squibb Company	Art Unit: 1644
Application No.: 14/400,667	Examiner: OUSPENSKI, Ilia I.
§ 371(c) Date: November 12, 2014	Atty. Docket: 3338.0630002/ELE/C-K/BGC

Title: **CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING**

Third Supplemental Information Disclosure Statement

Mail Stop Amendment

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

Commissioner:

Listed on the accompanying IDS Form PTO/SB/08b equivalent is a document that is 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 July 17, 2017, in connection with the above-captioned application.

Applicant has listed the publication date on the attached IDS Form based on information presently available to the undersigned. However, the listed publication date 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 $180.00 in payment of the fee under 37 C.F.R. § 1.17(p).

A copy of document **NPL4** is 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 document has 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: Aug. 2, 2017

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

5977064_1

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	14/400,667
§ 371(c) Date	November 12, 2014
First Named Inventor	COGSWELL, John P.
Art Unit	1644
Examiner Name	OUSPENSKI, ILIA I
Attorney Docket Number	3338.0630002/ELE/C-K/BGC

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]
	NPL4	National Institutes of Health Clinical Center, Clinical Trial Identifier NCT01024231 entitled "Dose-escalation Study of Combination BMS-936558 (MDX-1106) and Ipilimumab in Subjects With Unresectable Stage III or Stage IV Malignant Melanoma," ClinicalTrials.gov, available at https://clinicaltrials.gov/archive/NCT01024231/2012_04_30, last accessed on July 18, 2017, 4 pages (same clinical trial accessed on April 27, 2017 by Examiner in same application).	

5977022_1

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:	14400667
Filing Date:	12-Nov-2014
Title of Invention:	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
First Named Inventor/Applicant Name:	John P. Cogswell
Filer:	Ji Eun Kim/Shanova Banks
Attorney Docket Number:	3338.0630002/ELE/C-K/AHB

Filed as Large Entity

Filing Fees for U.S. National Stage under 35 USC 371

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($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**180**

Electronic Acknowledgement Receipt	
EFS ID:	29964655
Application Number:	14400667
International Application Number:	
Confirmation Number:	8185
Title of Invention:	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
First Named Inventor/Applicant Name:	John P. Cogswell
Customer Number:	115018
Filer:	Ji Eun Kim/Kisha Cox
Filer Authorized By:	Ji Eun Kim
Attorney Docket Number:	3338.0630002/ELE/C-K/AHB
Receipt Date:	02-AUG-2017
Filing Date:	12-NOV-2014
Time Stamp:	17:15:50
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$180
RAM confirmation Number	080317INTEFSW17163100
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	Miscellaneous Incoming Letter	33380630002_skgfcl.pdf	458740 039ea561bd4c745506bc91be3baf2d856feb76fb	no	1
Warnings:					
Information:					
2		33380630002_am111.pdf	398110 79101155f0a599aa37428a721d9cb40031d16e73	yes	12

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment/Req. Reconsideration-After Non-Final Reject	1	1
	Claims	2	8
	Applicant Arguments/Remarks Made in an Amendment	9	12

Warnings:					
Information:					
3	Transmittal Letter	33380630002_3rdsuppids.pdf	401217 59b8d909edc7ec1f8bbc6d87d62cd7ae933319dd	no	2
Warnings:					
Information:					
4	Information Disclosure Statement (IDS) Form (SB08)	33380630002_sb08.pdf	94631 1353e0be6be18b325c745e5d2757b467ddddb0940	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					

5	Non Patent Literature	33380630002_NPL4.pdf	101972 3b710d9307eeea2d434ecac2bcc0dec5a17df136	no	4
Warnings:					
Information:					
6	Fee Worksheet (SB06)	fee-info.pdf	30887 e3eefa47de6c64de65f2851d1cc0b7523d410772	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1485557		

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.

JI-EUN KIM
DIRECTOR
(202) 772-8619
CKIM@SKGF.COM

Sterne Kessler Goldstein Fox
ATTORNEYS AT LAW

August 2, 2017

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

Confirmation No. 8185
Art Unit 1644
Attn: Mail Stop Amendment

Re: U.S. Utility Patent Application
Appl. No. 14/400,667; § 371(c) Date: November 12, 2014
For: **CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING**
Inventors: COGSWELL *et al.*
Our Ref: 3338.0630002/ELE/C-K/BGC

Commissioner:

Transmitted herewith for appropriate action are the following documents:

1. Online Credit Card Payment Authorization in the amount of **$180.00** to cover the fee for filing an Information Disclosure Statement;
2. Amendment and Reply Under 37 C.F.R. § 1.111;
3. Third Supplemental Information Disclosure Statement;
4. Form PTO/SB/08b (1 sheet) listing 1 document (NPL4); and
5. A copy of the cited document (NPL4).

In the event that extensions of time are necessary to prevent abandonment of this patent application, then such extensions of time are hereby petitioned.

Fee payment is provided through online credit card payment. 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

C-K/BGC:dbj
Enclosure

5952228_1.docx

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
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.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/400,667	Filing Date 11/12/2014	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)		
FOR	NUMBER FILED	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 $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ 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)		
	08/02/2017	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
AMENDMENT	Total (37 CFR 1.16(i))	* 22	Minus	** 21	= 1	X $80 =	80
	Independent (37 CFR 1.16(h))	* 2	Minus	***5	= 0	X $420 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	**80**

		(Column 1)		(Column 2)	(Column 3)		
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
AMENDMENT	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ 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.
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*** 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.

LIE
ROSS W. BROWN

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SECOND SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
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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
	US17	2017/0028040 A1	02-02-2017	Lan *et al.*	

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	CN-101215329-A	07-09-2008	FOURTH MILITARY MEDICAL UNIV		Abst.
	FP2	WO-2006133396-A2	12-14-2006	DANA-FARBER CANCER INSTITUTE		

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]
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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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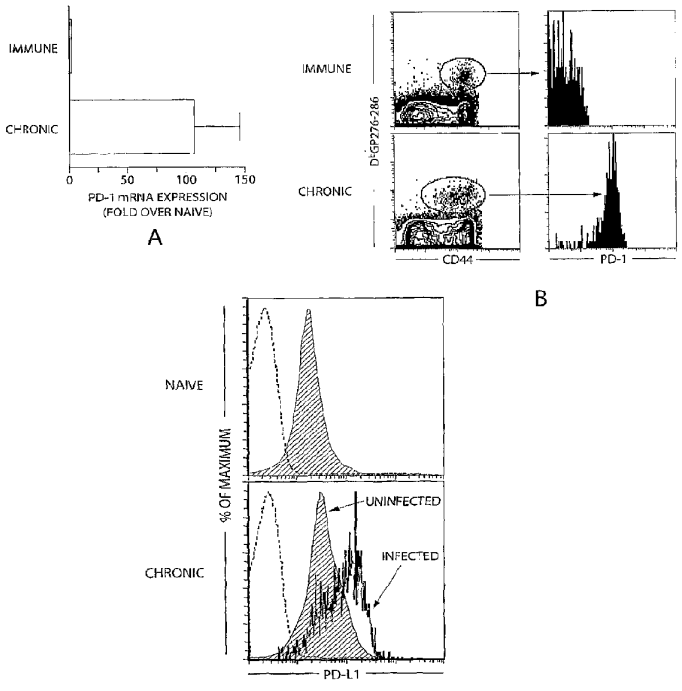
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(54) Title: METHODS AND COMPOSITIONS FOR THE TREATMENT OF PERSISTENT INFECTIONS



(57) Abstract: The present invention provides methods and compositions for the treatment, prevention, or reduction of persistent infections, such as chronic infections, latent infections, and slow infections and cancer. The methods and compositions of the invention are also useful for the alleviation of one or more symptoms associated with such infections and cancer.

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METHODS AND COMPOSITIONS FOR THE TREATMENT OF PERSISTENT INFECTIONS

Statement As To Federally Sponsored Research

This invention was made with U.S. government support under NIH grants AI39671 and CA84500. The government has certain rights in the invention.

Field of the Invention

In general, the present invention relates to methods and compositions for the treatment of persistent infections and cancer.

Background of the Invention

Although the development of preventative vaccines has significantly reduced the mortality rate of viral infections, the use of such vaccines against viruses that cause persistent infections (e.g., hepatitis C virus) has been met with limited success. In contrast to viruses that cause acute and self-limited infections, the immune response that is mounted against persistent infection-causing microbes is often transient and insufficient to clear the infection. As a result, the infectious microbe remains within the infected subject for extended periods of time, without necessarily causing constant host damage.

A major impediment in the eradication of persistent infection-causing microbes is the ability of such microbes to evade the immune system of the host organism. For example, certain viruses and parasites down-regulate the expression of host molecules necessary for efficient T cell recognition of infected cells. Persistent infections also cause the functional impairment of antigen specific CD8+ T cells, which are vital to the control and eradication of viral infections. Although the combination of therapeutic vaccines with cytokine adjuvants has been encouraging, the resulting immune responses have not successfully eradicated the pathogen.

Thus, better methods are needed to treat, prevent, or alleviate persistent infections.

Summary of the Invention

The present invention provides methods and compositions for the treatment, prevention, or reduction of, or alternatively the alleviation of one or more symptoms of, a persistent infection or cancer. The invention is based on the discovery that antigen specific CD8+ T cells become functionally tolerant ('exhausted') to the infectious agent following the induction of the Programmed Death-1 polypeptide (PD-1). Accordingly, by reducing the expression or activity of PD-1, PD-L1 or PDL2, the proliferation of functionally tolerant CD8+ T cells, the production of cytokines, and the rate of an infectious agent (e.g., viral, bacterial, fungal, parasite, mycoplasm or cancer) clearance is increased such that the immune response specific to the infectious agent is enhanced.

Accordingly, the invention provides a method of alleviating or preventing a symptom of a persistent infection (e.g., a viral infection, a bacterial infection, a fungal infection, a mycoplasm infection and a parasitic infection) or cancer by administering to a subject in need thereof (e.g., a human) a compound that reduces the activity or expression of a member of the CD28-like family (e.g., PD-1, CTLA-4, BTLA and a functional fragment or variant thereof) or CD28-like family ligands (e.g., PD-L1 or PD-L2). Alternatively, the subject is administered an antigen-specific T cell or B cell that has been contacted with an compound that reduces the expression or activity of a PD-1 polypeptide in the cell. For example, the antigen specific T cell or B cell is specific to a viral antigen. The T cell or B cell is derived from an autologous source or is derived from another subject of the same or different species as the subject being treated.

In addition, the invention features a method of increasing the cytotoxic activity of a T cell (e.g., anergic T cell or T cell having increased tolerance to antigens) by contacting the T cell with a compound that reduces the activity or expression of a PD-1 polypeptide.

In all foregoing aspects of the invention, persistent viral infections result from infections such as a hepatitis virus, a human immunodeficiency virus (HIV), a human T-lymphotrophic virus (HTLV), a herpes virus, an Epstein-Barr virus, or a human papilloma virus. Persistent viral infections may also include infections caused by a latent virus. Cancers include lymphoproliferative disorders such as angioimmunoblastic lymphoma and nodular lymphocyte

Hodgkin lymphoma. Desirably, the compound of the invention increases an antigen specific immune response by increasing the cytotoxic T-cell activity (e.g., an increase in cytotoxic cytokine production such as IFNγ, TNFα, or IL-2, an increase in T cell proliferation, or an increase in viral clearance) in the subject being treated. For example, the compound reduces the expression or activity of a PD ligand 1 (PD-L1) or a PD ligand 2 (PD-L2) or reduces the interaction between PD-1 and PD-L1 or the interaction between PD-1 and PD-L2. Exemplary compounds include antibodies (e.g., an anti-PD-1 antibody, an anti-PD-L1 antibody, and an anti-PD-L2 antibody), RNAi molecules (e.g., anti-PD-1 RNAi molecules, anti-PD-L1 RNAi, and an anti-PD-L2 RNAi), antisense molecules (e.g., an anti-PD-1 antisense RNA, an anti-PD-L1 antisense RNA, and an anti-PD-L2 antisense RNA), dominant negative proteins (e.g., a dominant negative PD-1 protein, a dominant negative PD-L1 protein, and a dominant negative PD-L2 protein), and small molecule inhibitors. Antibodies include monoclonal antibodies, humanized antibodies, deimmunized antibodies, and Ig fusion proteins. An exemplary anti-PD-L1 antibody includes clone EH12.

In addition to the compound that reduces PD-1 expression or activity, the subject being treated may also be administered a vaccine that may or may not include an adjuvant or a prime booster shot. Optionally, the subject is administered a second compound, such as an antiviral compound (e.g., vidarabine, acyclovir, gancyclovir, valgancyclovir, nucleoside-analog reverse transcriptase inhibitor (NRTI) such as AZT (Zidovudine), ddI (Didanosine), ddC (Zalcitabine), d4T (Stavudine), or 3TC (Lamivudine), non-nucleoside reverse transcriptase inhibitor (NNRTI) such as nevirapine or delavirdine, protease inhibitor such as saquinavir, ritonavir, indinavir, or nelfinavir, ribavirin, and interferon), an antibacterial compound, an antifungal compound, an antiparasitic compound, an anti-inflammatory compound, anti-neoplastic compounds or an analgesic. The second compound may also be a compound that reduces the expression or activity of cytotoxic T lymphocyte antigen 4 (CTLA-4) or B and T lymphocyte attenuator (BTLA). Other exemplary compounds that may be administered to the subject are anti-CTLA-4 antibodies, anti-BTLA antibodies, anti-CD28 antibodies, anti-ICOS antibodies, anti-ICOS-L antibodies, anti-B7-1 antibodies, anti-B7-2 antibodies, anti-B7-H3 antibodies, and anti-B7-H4 antibodies.

The present invention further provides a method for identifying a candidate compound that modulates the activity or expression of a PD-1 polypeptide that includes the steps of: (a)

contacting a cell expressing a PD-1 gene (e.g., PD-1 fusion gene) with a candidate compound; (b) measuring the expression or activity of PD-1 in the cell (e.g., by measuring the expression of PD-1 mRNA or protein); and (c) comparing the expression or activity of PD-1 in the cell compared to such expression or activity in a control cell not contacted with the compound. An increase or decrease in the expression or activity of PD-1 indicates the candidate compound as being useful for modulating the activity or expression of a PD-1 polypeptide.

Alternatively, the screening method may involve the steps of: (a) contacting a T cell that overexpresses a PD-1 gene with a candidate compound; and (b) determining the cytotoxic activity of the T cell; (c) comparing the cytotoxic activity of the T cell relative to such activity in a control cell not contacted with the compound. An increase or decrease in such activity identifies the candidate compound as being useful for modulating the activity or expression of a PD-1 polypeptide. Cytotoxic activity includes cytokine production, T cell proliferation, and viral clearance.

The invention further provides a screening method involving the steps of: (a) contacting a PD-1 polypeptide with a candidate compound; (b) determining whether the candidate compound interacts with the PD-1 polypeptide; and (c) identifying a candidate compound as useful for modulating PD-1 expression or activity. Desirably, the identified candidate compound interacts with the PD-1 polypeptide and reduces its activity.

The candidate compound identified by the screening methods described herein may reduce the interaction between PD-1 and PD-L1 or the interaction between PD-1 and PD-L2. The cell employed in any of the screening methods described herein include mammalian cells such as rodent cells or human cell. The cell is an immune cell, such as a T cell. Desirably, the PD-1 polypeptide used in such screening methods is a human PD-1 polypeptide.

Also provided herein is a method of diagnosing a subject as having or at risk of having a persistent infection or cancer involving the steps of: (a) providing a sample containing immune cells (e.g., T cell or B cell) from a subject, and (b) measuring the expression or activity of PD-1 in the sample. An increase in the expression or activity of PD-1 compared to such expression or activity in a control sample identifies the subject as having or at risk of having a persistent infection or cancer. Desirably, step (b) involves identifying antigen-specific immune cells, such as a viral antigen, bacterial antigen, parasitic antigen, or fungal antigen.

A method of selecting a treatment for a subject having or at risk of having a persistent infection or cancer is also described. This method involves the steps of: (a) providing a sample containing immune cells (e.g., T cell or B cell) from a subject; and (b) measuring the expression or activity of PD-1 in the immune cells, such that an increase in expression or activity of PD-1 compared to such expression or activity in a control sample identifies the subject as having or at risk of having a persistent infection or cancer; and (c) selecting a treatment for the subject diagnosed as having or at risk a persistent infection or cancer, such that the treatment includes a compound that reduces the expression or activity of PD-1. Desirably, step (b) involves identifying antigen-specific immune cells, such as a viral antigen, bacterial antigen, parasitic antigen, or fungal antigen.

Samples derived from subjects include blood samples, tissue biopsies, and bone marrow samples. Furthermore, control cells may be derived from a subject that does not have or at risk of having a persistent infection.

The invention further provides a composition that contains: (a) a compound that reduces the level or activity of PD-1; and (b) a second compound, such as an antiviral compound, an antibacterial compound, an antifungal compound, an antiparasitic compound, an anti-inflammatory compound, an analgesic, an anti-CTLA-4 antibody, an anti-BTLA antibody, an anti-CD28 antibody, an anti-ICOS antibody, an anti-ICOS-L antibody, an anti-B7-1 antibody, an anti-B7-2 antibody, an anti-B7-H3 antibody, or an anti-B7-H4 antibody.

The invention also provides a kit that contains (a) a compound that reduces the level or activity of PD-1; and (b) instructions for delivery of the compound to a subject. Alternatively, the kit contains (a) a first compound that reduces the level or activity of PD-1; (b) a second compound such as an antiviral compound, an antibacterial compound, an antifungal compound, an antiparasitic compound, an anti-inflammatory compound, an analgesic, an anti-CTLA-4 antibody, an anti-BTLA antibody, an anti-CD28 antibody, an anti-ICOS antibody, an anti-ICOS-L antibody, an anti-B7-1 antibody, an anti-B7-2 antibody, an anti-B7-H3 antibody, or an anti-B7-H4 antibody; and (c) instructions for delivery of the first compound and the second compound to a subject.

The present invention provides significant advantages over standard therapies for treatment, prevention, and reduction, or alternatively, the alleviation of one or more symptoms of persistent infections. Administration of the therapeutic agent that reduces the level or activity of

PD-1 increases CD8+ T cell cytotoxicity, in turn increasing the immune response to the infectious agent having the ability to establish a persistent infection. In addition, the candidate compound screening methods provided by this invention allow for the identification of novel therapeutics that modify the injury process, rather than merely mitigating the symptoms.

Unless otherwise defined, 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 invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

Figure 1A is a bar graph showing the levels of PD-1 mRNA in D^bGP33-41 and/or D^bGP276-286 specific T cells from naïve transgenic mice, lymphocytic choriomeningitis virus (LCMV) Armstrong immune (approximately 30 days post-infection) infected mice, or CD4-depleted LCMV-Cl-13 infected mice (approximately 30 days post-infection), as measured by gene array analysis.

Figure 1B is a series of images of a flow cytometry experiment showing PD-1 surface expression on CD8+ tetramer+ T cells in LCMV Armstrong immune and CD4 depleted LCMV-Cl-13 infected mice approximately 60 days post-infection. Anergic CD8+ T cells express high levels of PD-1 polypeptide on the cell surface approximately 60 days after chronic infection with LCMV-Cl-13 virus (labeled "chronic"), but virus-specific CD8+ T cells do not express PD-1 polypeptide after clearance of an acute LCMV Armstrong infection (labeled "immune").

Figure 1C is a series of images of a flow cytometry experiment demonstrating the presence of PD-L1 on splenocytes from chronically infected and uninfected mice. It demonstrates that PD-L1 expression is the highest on the splenocytes that are infected by the virus.

Figure 2A is a series of scatter plots showing that when Cl-13 infected mice are treated from day 23 to 37 post-infection there was approximately a 3 fold increase in the number of DbNP396-404 specific and DbGP33-41 specific CD8 T cells compared to the untreated controls. In order to determine any changes in function IFN-γ and TNF-α production was measured in response to 8 different LCMV epitopes.

Figure 2B is a scatter plot showing that when all the known CD8 T cell specificities are measured there is a 2.3 fold increase in total number of LCMV specific CD8 T cells.

Figure 2C is a series of flow cytometry graphs showing IFN-γ and TNF-α production in response to eight different LCMV epitopes.

Figure 2D is a scatter plot showing that more virus specific CD8 T cells in treated mice have the ability to produce TNF-α.

Figure 2E is a series of bar charts showing that PD-L1 blockade also resulted in increased viral control in the spleen liver lung and serum.

Figure 3A is a graph demonstrating the increase in DbGP33-41 and DbGP276-286 specific CD8+ T cells (labeled "GP33" and "GP276") in CD4-depleted C1-13 infected mice treated with anti-PD-L1 (labeled "αPD-L1") from day 46 to day 60 post-infection versus control (labeled "untx"), which demonstrates that mice treated with anti-PD-L1 contained approximately 7 fold more DbGP276-286 specific splenic CD8+ T cells and approximately 4 fold more DbGP33-41 specific splenic CD8+ T cells than untreated mice.

Figure 3B is a series of images demonstrating the increased frequency of DbGP33-41 and DbGP276-286 specific CD8+ T cells in the spleen of CD4-depleted C1-13 infected mice treated with anti-PD-L1 (labeled "αPD-L1 Tx") from day 46 to day 60 post-infection versus control (labeled "untx").

Figure 3C is a series of images demonstrating increased proliferation of DbGP276-286 specific CD8+ T cells in anti-PD-L1-treated mice, as measured by BrdU incorporation and Ki67 expression.

Figure 3D is a chart showing that mice having high levels of CD8+ T cell expansion demonstrate an appreciable response in peripheral blood mononuclear cells (PBMC), as shown by comparing DbGP276-286 specific CD8+ T cells in the PBMC as compared to DbGP276-286 specific CD8+ T cells in the spleen.

Figure 4A is a series of charts demonstrating the increase in IFN-γ producing DbGP276-286 and DbGP33-41 specific CD8+ T cells in anti-PD-L1-treated mice, as compared to controls. Higher frequencies of DbNP396-404, KbNP205-212, DbNP166-175, and DbGP92-101 specific CD8+ T cells were also detected in anti-PD-L1-treated mice.

Figure 4B is a chart demonstrating that in anti-PD-L1-treated mice, 50% of DbGP276-286 specific CD8+ T cells produce IFN-γ, as compared to 20% of DbGP276-286 specific CD8+ T cells in control mice.

Figure 4C is a series of images demonstrating that anti-PD-L1-treated chronically infected mice produce higher levels of TNF-α than untreated chronically infected mice, but still produce lower levels of TNF-α than immune mice infected with LCMV Armstrong virus.

Figure 4D is a chart demonstrating that treatment of LCMV-Cl-13 infected mice with anti-PD-L1 renews ex vivo lytic activity of the virus-specific T cells, as compared to untreated infected mice, measured using a ^{51}Cr release assay.

Figure 4E is a series of charts demonstrating the reduction of viral titers in various organs following treatment of LCMV-Cl-13 infected mice with α-PD-L1. Viral titers decreased approximately 3 fold in the spleen, 4 fold in the liver, 2 fold in the lung, and 2 fold in serum after 2 weeks of anti-PD-L1 treatment, as compared to untreated mice.

Figure 5A is a series of images of a flow cytometry experiment showing PD-1 surface expression using 10 HIV tetramers specific for dominant epitopes targeted in chronic clade C HIV infection. The percentages indicate the percentage of tetramer$^+$ cells that are PD-1$^+$.

Figure 5B is a series of charts demonstrating that the percentage and MFI of PD-1 is significantly upregulated on HIV-specific CD8 T cells compared to the total CD8 T cell population ($p<0.0001$) in antiretroviral therapy naïve individuals, and PD-1 is increased on the total CD8 T cell population in HIV-infected versus HIV-seronegative controls ($p=0.0033$ and $p<0.0001$, respectively). 120 HIV tetramer stains from 65 HIV-infected individuals and 11 HIV seronegative controls were included in the analysis.

Figure 5C is a series of charts showing the median percentage and MFI of PD-1 expression on tetramer$^+$ cells by epitope specificity.

Figure 5D is a chart depicting the variation in the percentage of PD-1^+ cells on different epitope-specific populations within individuals with multiple detectable responses. Horizontal bars indicate the median percentage of PD-1^+ HIV tetramer$^+$ cells in each individual.

Figure 6A is a series of charts demonstrating that there is no correlation between the number of HIV-specific CD8 T cells, as measured by tetramer staining, and plasma viral load, whereas there is a positive correlation between both the percentage and MFI of PD-1 on tetramer$^+$ cells and plasma viral load ($p=0.0013$ and $p<0.0001$, respectively).

Figure 6B is a series of charts showing that there is no correlation between the number of HIV tetramer$^+$ cells and CD4 count, whereas there is an inverse correlation between the percentage and MFI of PD-1 on HIV tetramer$^+$ cells and CD4 count ($p=0.0046$ and $p=0.0150$, respectively).

Figure 6C is a series of charts demonstrating that the percentage and MFI of PD-1 on the total CD8 T cell population positively correlate with plasma viral load ($p=0.0021$ and $p<0.0001$, respectively).

Figure 6D is a series of charts depicting the percentage and MFI of PD-1 expression on the total CD8 T cell population is inversely correlated with CD4 count ($p=0.0049$ and $p=0.0006$, respectively).

Figure 7A is a series of images of a flow cytometry experiment showing representative phenotypic staining of B*4201 TL9-specific CD8 T cells from subject SK222 in whom 98% of B*4201 TL9-specific CD8 T cells are PD-1^+.

Figure 7B is a chart illustrating a summary of phenotypic data from persons in whom >95% of HIV-specific CD8 T cells are PD-1^+. 7 to 19 samples were analyzed for each of the indicated phenotypic markers. The horizontal bar indicates median percentage of tetramer$^+$ PD-1^+ cells that were positive for the indicated marker.

Figure 8A is a series of images of a flow cytometry experiment showing the representative proliferation assay data from a B*4201 positive subject. After a 6-day stimulation with peptide, the percentage of B*4201 TL9-specific CD8 T cells increased from 5.7% to 12.4% in the presence of anti-PD-L1 blocking antibody.

Figure 8B is a line graph depicting the summary proliferation assay data indicating a significant increase in proliferation of HIV-specific CD8 T cells in the presence of anti-PD-L1 blocking antibody ($n=28$, $p=0.0006$, paired *t*-test).

Figure 8C is a bar graph showing the differential effects of PD-1/PD-L1 blockade on proliferation of HIV-specific CD8 T cells on an individual patient basis. White bars indicate fold increase of tetramer$^+$ cells in the presence of peptide alone, black bars indicate the fold increase of tetramer$^+$ cells in the presence of peptide plus anti-PD-L1 blocking antibody. Individuals in whom CFSE assays were performed for more than one epitope are indicated by asterisk, square, or triangle symbols.

Detailed Description

The use of antibiotics and vaccines in recent decades has significantly reduced the mortality rate due to microbial infections. The success of antimicrobial treatment modalities, however, has been limited by the ability of certain infectious agents to evade the immune system of the host organism and in turn, establish a persistent infection. For example, the immune response that is mounted against viruses such as hepatitis and HIV is not sufficient to clear the infectious agent, which remains in the infected subject. In such infections, antigen specific CD8+ T cells become functionally tolerant to the infectious agent, in a state known as 'anergy' or 'exhaustion'. Anergic T cells lose their cytotoxic activity, i.e., their ability to produce cytokines, proliferate, and clear the infectious agent.

The present invention is based upon the surprising discovery that T cell anergy is concomitant with an induction in PD-1 expression and that PD-1 expression correlates with certain types of lymphoproliferative disorders. Accordingly, the invention provides methods of increasing T-cell cytoxicity by contacting a T-cell with an agent that reduces the expression or activity of PD-1, PD-1 ligand (PD-L1) or PD-1 ligand 2 (PD-L2). More specifically, the invention provides methods of treating or preventing a persistent infection or lymphoproliferative disorders (e.g., cancers such as angioimmunoblastic lymphoma and nodular lymphocyte predominant Hodgkin lymphoma by administering to a subject an agent that reduces the expression or activity of PD-1. Reduction of PD-1, PD-L1 or PD-L2 expression or activity results in an increase in cytotoxic T cell activity, increasing the specific immune response to the infectious agent. The results provided herein show that the administration of anti-programmed death ligand-1 (PD-L1) blocking antibodies to persistently infected mice increased the cytotoxic activity of anergic T cells. Specifically, disruption of PD-1 signaling induced the expansion of anergic CD8+ T cells, enhanced cytokine production, and increased viral clearance.

Furthermore, CD8+ T cells generated during persistent infections of CD4 depleted mice proliferated and regained much of their function upon anti-PD-L1 treatment.

In order for T cells to respond to foreign proteins, two signals must be provided by antigen-presenting cells (APCs) to resting T lymphocytes. The first signal, which confers specificity to the immune response, is transduced via the T cell receptor (TCR) following recognition of foreign antigenic peptide presented in the context of the major histocompatibility complex (MHC). The second signal, termed costimulation, induces T cells to proliferate and become functional. Costimulation is neither antigen-specific, nor MHC-restricted and is provided by one or more distinct cell surface polypeptides expressed by APCs. If T cells are only stimulated through the T cell receptor, without receiving an additional costimulatory signal, they become nonresponsive, anergic, or die, resulting in downmodulation of the immune response.

The CD80 (B7-1) and CD86 (B7-2) proteins, expressed on APCs, are critical costimulatory polypeptides. While B7-2 plays a predominant role during primary immune responses, B7-1 is upregulated later in the course of an immune response to prolong primary T cell responses or costimulating secondary T cell responses. B7 polypeptides are capable of providing costimulatory or inhibitory signals to immune cells to promote or inhibit immune cell responses. For example, when bound to a costimulatory receptor, PD-L1 (B7-4) induces costimulation of immune cells or inhibits immune cell costimulation when present in a soluble form. When bound to an inhibitory receptor, B7-4 molecules can transmit an inhibitory signal to an immune cell. Exemplary B7 family members include B7-1, B7-2, B7-3 (recognized by the antibody BB-1), B7h (PD-L1), and B7-4 and soluble fragments or derivatives thereof. B7 family members bind to one or more receptors on an immune cell, such as CTLA4, CD28, ICOS, PD-1 and/or other receptors, and, depending on the receptor, have the ability to transmit an inhibitory signal or a costimulatory signal to an immune cell.

CD28 is a receptor that is constitutively expressed on resting T cells. After signaling through the T cell receptor, ligation of CD28 and transduction of a costimulatory signal induces T cells to proliferate and secrete IL-2. CTLA4 (CD152), a receptor homologous to CD28, is absent on resting T cells but its expression is induced following T cell activation. CTLA4 plays a role in negative regulation of T cell responses. ICOS, a polypeptide related to CD28 and CTLA4, is involved in IL-10 production. PD-1, the receptor to which PD-L1 and PD-L2 bind, is

also rapidly induced on the surface of T-cells. PD-1 is also expressed on the surface of B-cells (in response to anti-IgM) and on a subset of thymocytes and myeloid cells.

Engagement of PD-1 (for example by crosslinking or by aggregation), leads to the transmission of an inhibitory signal in an immune cell, resulting in a reduction of immune responses concomitant with an increase in immune cell anergy. PD-1 family members bind to one or more receptors, such as PD-L1 and PD-L2 on antigen presenting cells.

PD-L1 and PD-L2, both of which are human PD-1 ligand polypeptides, are members of the B7 family of polypeptides. Each PD-1 ligand contains a signal sequence, an IgV domain, an IgC domain, a transmembrane domain, and a short cytoplasmic tail. These ligands are expressed in placenta, spleen, lymph nodes, thymus, and heart. PD-L2 is also expressed in the pancreas, lung, and liver, while PD-L1 is expressed in fetal liver, activated T-cells and endothelial cells. Both PD-1 ligands are upregulated on activated monocytes and dendritic cells.

Definitions

As used herein, by "persistent infection" is meant an infection in which the infectious agent (e.g., virus, bacterium, parasite, mycoplasm, or fungus) is not cleared or eliminated from the infected host, even after the induction of an immune response. Persistent infections may be chronic infections, latent infections, or slow infections. While acute infections are relatively brief (lasting a few days to a few weeks) and resolved from the body by the immune system, persistent infections may last for months, years, or even a lifetime. These infections may also recur frequently over a long period of time, involving stages of silent and productive infection without cell killing or even producing excessive damage to the host cells. The causative infectious agents may also be detected in the host (e.g., inside specific cells of infected individuals) even after the immune response has resolved, using standard techniques. Mammals are diagnosed as having a persistent infection according to any standard method known in the art and described, for example, in U.S. Patent Nos. 6,368,832, 6,579,854, and 6,808,710 and U.S. Patent Application Publication Nos. 20040137577, 20030232323, 20030166531, 20030064380, 20030044768, 20030039653, 20020164600, 20020160000, 20020110836, 20020107363, and 20020106730, all of which are hereby incorporated by reference. For example, a subject may be diagnosed as having a persistent Chlamydial infection following the detection of Chlamydial species in a biological sample from this individual

using PCR analysis. Mammals need not have not been diagnosed with a persistent infection to be treated according to this invention. Microbial agents capable of establishing a persistent infection include viruses (e.g., papilloma virus, hepatitis virus, human immune deficiency virus, and herpes virus), bacteria (e.g., Eschericchia coli and Chlamydia spp.), parasites, (e.g., Plasmodium, Leishmania spp., Schistosoma spp., Trypanosoma spp., Toxoplasma spp.) and fungi.

By "alleviating a symptom of a persistent infection" is meant ameliorating any of the conditions or symptoms associated with the persistent infection before or after it has occurred. Alternatively, alleviating a symptom of a persistent infection may involve reducing the infectious microbial (e.g., viral, bacterial, fungal, mycoplasm, or parasitic) load in the subject relative to such load in an untreated control. As compared with an equivalent untreated control, such reduction or degree of prevention is at least 5%, 10%, 20%, 40%, 50%, 60%, 80%, 90%, 95%, or 100% as measured by any standard technique. Desirably, the persistent infection is completely cleared as detected by any standard method known in the art, in which case the persistent infection is considered to have been treated. A patient who is being treated for a persistent infection is one who a medical practitioner has diagnosed as having such a condition. Diagnosis may be by any suitable means. Diagnosis and monitoring may involve, for example, detecting the level of microbial load in a biological sample (e.g., tissue biopsy, blood test, or urine test), detecting the level of a surrogate marker of the microbial infection in a biological sample, detecting symptoms associated with persistent infections, or detecting immune cells involved in the immune response typical of persistent infections (e.g., detection of antigen specific T cells that are anergic) A patient in whom the development of a persistent infection is being prevented may or may not have received such a diagnosis. One in the art will understand that these patients may have been subjected to the same standard tests as described above or may have been identified, without examination, as one at high risk due to the presence of one or more risk factors (e.g., family history or exposure to infectious agent).

As used herein, by "PD-1" is meant a polypeptide that forms a complex with PD-L1 or PD-L2 proteins and is therefore involved in immune responses, such as the co-stimulation of T cells. The PD-1 proteins of the invention are substantially identical to the naturally occurring PD-1 (see, for example, Ishida et al. EMBO J. 11:3887-3895, 1992, Shinohara et al. Genomics 23:704-706, 1994; and U.S. Patent No. 5,698,520, incorporated by reference herein). PD-1

signaling may reduce, for example, CD8+ T cell cytoxicity by reducing T cell proliferation, cytokine production, or viral clearance. According to this invention, the PD-1 polypeptide reduces CD8+ T cell cytotoxic activity by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more than 100% below control levels as measured by any standard method.

By a "PD-1 gene" is meant a nucleic acid that encodes a PD-1 protein.

By "PD-1 fusion gene" is meant a PD-1 promoter and/or all or part of a PD-1 coding region operably linked to a second, heterologous nucleic acid sequence. In preferred embodiments, the second, heterologous nucleic acid sequence is a reporter gene, that is, a gene whose expression may be assayed; reporter genes include, without limitation, those encoding glucuronidase (GUS), luciferase, chloramphenicol transacetylase (CAT), green fluorescent protein (GFP), alkaline phosphatase, and .beta.-galactosidase.

By "reduce the expression or activity of PD-1" is meant to reduce the level or biological activity of PD-1 relative to the level or biological activity of PD-1 in an untreated control. According to this invention, such level or activity is reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or even greater than 100%, relative to an untreated control. For example, the biological activity of PD-1 is reduced if binding of PD-1 to PD-L1, PD-L2, or both is reduced, thereby resulting in a reduction in PD-1 signaling and therefore resulting in an increase in CD8+ T cell cytotoxicity. As used herein, the term "activity" with respect to a PD-1 polypeptide includes any activity which is inherent to the naturally occurring PD-1 protein, such as the ability to modulate an inhibitory signal in an activated immune cell, e.g., by engaging a natural ligand on an antigen presenting cell. Such modulation of an inhibitory signal in an immune cell results in modulation of proliferation of and/or cytokine secretion by an immune cell. PD-1 may also modulate a costimulatory signal by competing with a costimulatory receptor for binding of a B7 molecule. Thus, the term "PD-1 activity" includes the ability of a PD-1 polypeptide to bind its natural ligand(s), the ability to modulate immune cell costimulatory or inhibitory signals, and the ability to modulate the immune response. Accordingly, reducing PD-1 activity includes reducing the interaction of PD-1 to PD-L1 or PD-L2. This can be accomplished for example by blocking PD-L1 or PD-L2.

By "immune cell" is meant a cell of hematopoietic origin and that plays a role in the immune response. Immune cells include lymphocytes (e.g., B cells and T cells), natural killer

cells, and myeloid cells (e.g., monocytes, macrophages, eosinophils, mast cells, basophils, and granulocytes).

By "T cell" is meant a CD4+ T cell or a CD8+ T cell. The term T cell includes both TH1 cells and TH2 cells.

The term "T cell cytoxicity" includes any immune response that is mediated by CD8+ T cell activation. Exemplary immune responses include cytokine production, CD8+ T cell proliferation, granzyme or perforin production, and clearance of the infectious agent.

By "unresponsiveness" includes refractivity of immune cells to stimulation, e.g., stimulation via an activating receptor or a cytokine. Unresponsiveness can occur, e.g., because of exposure to immunosuppressants or exposure to high doses of antigen. As used herein, the term "anergy" or "tolerance" includes refractivity to activating receptor-mediated stimulation. Such refractivity is generally antigen-specific and persists after exposure to the tolerizing antigen has ceased. For example, anergy in T cells (as opposed to unresponsiveness) is characterized by lack of cytokine production, e.g., IL-2. T cell anergy occurs when T cells are exposed to antigen and receive a first signal (a T cell receptor or CD-3 mediated signal) in the absence of a second signal (a costimulatory signal). Under these conditions, re-exposure of the cells to the same antigen (even if reexposure occurs in the presence of a costimulatory molecule) results in failure to produce cytokines and, thus, failure to proliferate. Anergic T cells can, however, mount responses to unrelated antigens and can proliferate if cultured with cytokines (e.g., IL-2). For example, T cell anergy can also be observed by the lack of IL-2 production by T lymphocytes as measured by ELISA or by a proliferation assay using an indicator cell line. Alternatively, a reporter gene construct can be used. For example, anergic T cells fail to initiate IL-2 gene transcription induced by a heterologous promoter under the control of the 5' IL-2 gene enhancer or by a multimer of the AP1 sequence that can be found within the enhancer (Kang et al. Science 257:1134, 1992). Anergic antigen specific T cells may have a reduction of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or even 100% in cytotoxic activity relative a corresponding control antigen specific T cell.

By "purified antibody" is meant antibody which is at least 60%, by weight, free from proteins and naturally occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably 90%, and most preferably at least 99%, by weight, antibody, e.g., a PD-1, PD-L1, or PD-L2 specific antibody. A purified antibody

may be obtained, for example, by affinity chromatography using recombinantly-produced protein or conserved motif peptides and standard techniques.

By "specifically binds" is meant an antibody that recognizes and binds an antigen such as a PD-1, PD-L1, or PD-L2 polypeptide but that does not substantially recognize and bind other non-antigen molecules in a sample, e.g., a biological sample, that naturally includes protein. A preferred antibody binds to the PD-1, PD-L1, or PD-L2 polypeptides disclosed in U.S. Patent No. 6,808,710 and U.S. Patent Application Publication Nos. 20040137577, 20030232323, 20030166531, 20030064380, 20030044768, 20030039653, 20020164600, 20020160000, 20020110836, 20020107363, and 20020106730, all of which are hereby incorporated by reference.

By "neutralizing antibodies" is meant antibodies that interfere with any of the biological activities of a PD-1 polypeptide, particularly the ability of a PD-1 polypeptide to reduce an immune response such as the cytotoxicity of T cells. The neutralizing antibody may reduce the ability of a PD-1 polypeptide to reduce an immune response by, preferably 50%, more preferably by 70%, and most preferably by 90% or more. Any standard assay to measure immune responses, including those described herein, may be used to assess potentially neutralizing antibodies.

By "substantially identical," when referring to a protein or polypeptide, is meant a protein or polypeptide exhibiting at least 75%, but preferably 85%, more preferably 90%, most preferably 95%, or even 99% identity to a reference amino acid sequence. For proteins or polypeptides, the length of comparison sequences will generally be at least 20 amino acids, preferably at least 30 amino acids, more preferably at least 40 amino acids, and most preferably 50 amino acids or the full length protein or polypeptide. Nucleic acids that encode such "substantially identical" proteins or polypeptides constitute an example of "substantially identical" nucleic acids; it is recognized that the nucleic acids include any sequence, due to the degeneracy of the genetic code, that encodes those proteins or polypeptides. In addition, a "substantially identical" nucleic acid sequence also includes a polynucleotide that hybridizes to a reference nucleic acid molecule under high stringency conditions.

By "high stringency conditions" is meant any set of conditions that are characterized by high temperature and low ionic strength and allow hybridization comparable with those resulting from the use of a DNA probe of at least 40 nucleotides in length, in a buffer containing 0.5 M

$NaHPO_4$, pH 7.2, 7% SDS, 1 mM EDTA, and 1% BSA (Fraction V), at a temperature of 65 °C, or a buffer containing 48% formamide, 4.8XSSC, 0.2 M Tris-Cl, pH 7.6, 1X Denhardt's solution, 10% dextran sulfate, and 0.1% SDS, at a temperature of 42 °C. Other conditions for high stringency hybridization, such as for PCR, Northern, Southern, or in situ hybridization, DNA sequencing, etc., are well known by those skilled in the art of molecular biology. See, e.g., F. Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y., 1998, hereby incorporated by reference.

By "substantially pure" is meant a nucleic acid, polypeptide, or other molecule that has been separated from the components that naturally accompany it. Typically, the polypeptide is substantially pure when it is at least 60%, 70%, 80%, 90%, 95%, or even 99%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. For example, a substantially pure polypeptide may be obtained by extraction from a natural source, by expression of a recombinant nucleic acid in a cell that does not normally express that protein, or by chemical synthesis.

The term "isolated DNA" is meant DNA that is free of the genes which, in the naturally occurring genome of the organism from which the given DNA is derived, flank the DNA. Thus, the term "isolated DNA" encompasses, for example, cDNA, cloned genomic DNA, and synthetic DNA.

By "an effective amount" is meant an amount of a compound, alone or in a combination, required to reduce or prevent hypertension or to treat or prevent a chronic infection in a mammal. The effective amount of active compound(s) varies depending upon the route of administration, age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen.

By a "candidate compound" is meant a chemical, be it naturally-occurring or artificially-derived. Candidate compounds may include, for example, peptides, polypeptides, synthetic organic molecules, naturally occurring organic molecules, nucleic acid molecules, peptide nucleic acid molecules, and components and derivatives thereof. For example, a useful candidate compound according to the present invention reduces binding of PD-1 to PD-L1, PD-L2, or both.

The term "pharmaceutical composition" is meant any composition, which contains at least one therapeutically or biologically active agent and is suitable for administration to the patient. Any of these formulations can be prepared by well-known and accepted methods of the

art. See, for example, Remington: The Science and Practice of Pharmacy, 20.sup.th edition, (ed. A R Gennaro), Mack Publishing Co., Easton, Pa., 2000.

Methods of Treating

T-cell cytotoxicity is increased by contacting a T-cell with a compound that reduces the expression or activity of PD-1. The T-cell is a naive T-cell, memory T-cell or activated T-cell. Alternatively, the T-cell is an antigen specific T-cell. The antigen specific T cells is anergic or tolerant to the infectious agent. T-cell cytotoxicity is characterized by an increase in cell proliferation and cytokine release.

The methods are useful to alleviate the symptoms of a variety of infections and cancers. An infection or cancer is treated, prevented or a symptom is alleviated by administering to a subject a PD-1 inhibitor. The subject is a mammal such as human, a primate, mouse, rat, dog, cat, cow, horse, and pig. The subject is suffering from or at risk of developing infection. A subject suffering from or at risk of developing infection is by standard methods suitable for the particular infection.

The infection, e.g., bacterial, viral, fungal, mycoplasm, or parasitic is a persistent infection. Persistent infections, in contrast to acute infections are not effectively cleared by the induction of a host immune response. The infectious agent and the immune response reach equilibrium such that the infected subject remains infectious over a long period of time without necessarily expressing symptoms. Persistent infections include for example, latent, chronic and slow infections.

In a chronic infection, the infectious agent can be detected in the body at all times. However, the signs and symptoms of the disease may be present or absent for an extended period of time. Examples of chronic infection include hepatitis B (caused by HBV) and hepatitis C (caused by HCV) adenovirus, cytomegalovirus, Epstein-Barr virus, herpes simplex virus 1, herpes simplex virus 2, human herpesvirus 6, varicella-zoster virus, hepatitis B virus, hepatitis D virus, papilloma virus, parvovirus B19, polyomavirus BK, polyomavirus JC, measles virus, rubella virus, human immunodeficiency virus (HIV), human T cell leukemia virus I, and human T cell leukemia virus II. Parasitic

persistent infections may arise as a result of infection by Leishmania, Toxoplasma, Trypanosoma, Plasmodium, Schistosoma, and Encephalitozoon.

In a latent infection, the infectious agent (e.g., virus) is seemingly inactive and dormant such that the subject does always exhibit signs or symptoms. In a latent viral infection, the virus remains in equilibrium with the host for long periods of time before symptoms again appear; however, the actual viruses cannot be detected until reactivation of the disease occurs. Examples of latent infections include infections caused by HSV-1 (fever blisters), HSV-2 (genital herpes), and VZV (chickenpox-shingles).

In a slow infection, the infectious agents gradually increase in number over a very long period of time during which no significant signs or symptoms are observed. Examples of slow infections include AIDS (caused by HIV-1 and HIV-2), lentiviruses that cause tumors in animals, and prions.

In addition, persistent infections often arise as late complications of acute infections. For example, subacute sclerosing panencephalitis (SSPE) can occur following an acute measles infection or regrossive encephalitis can occur as a result of a rubella infection.

Cancers include for example angioimmunoblastic lymphoma or nodular lymphocyte predominant Hodgkin lymphoma.

Angioimmunoblastic lymphoma (AIL) is an aggressive (rapidly progressing) type of T-cell non-Hodgkin lymphoma marked by enlarged lymph nodes and hypergammaglobulinemia (increased antibodies in the blood). Other symptoms may include a skin rash, fever, weight loss, positive Coomb's test or night sweats. This malignancy usually occurs in adults. Patients are usually aged 40-90 years (median around 65) and are more often male. As AIL progresses, hepatosplenomegaly, hemolytic anemia, and polyclonal hypergammaglobulinemia may develop. The skin is involved in approximately 40-50% of patients.

Nodular lymphocyte predominant Hodgkin lymphoma is a B cell neoplasm that appears to be derived from germinal center B cells with mutated, non-functional immunoglobulin genes. Similar to angioimmunoblastic lymphoma, neoplastic cells are associated with a meshwork of follicular dendritic cells. PD-1 expression is seen in T cells closely associated with neoplastic CD20+ cell in nodular lymphocyte predominant Hodgkin lymphoma, in a pattern similar to that seen for CD57+ T cells. CD57 has been identified as another marker of germinal center-

associated T cells, along with CXCR5, findings which support the conclusion that the neoplastic cells in nodular lymphocyte predominant Hodgkin lymphoma have a close association with germinal center-associated T cells.

An inhibitor of PD-1 is any agent having the ability to reduce the expression or the activity of PD-1, PD-L1 or PD-2 in a cell. PD-1 expression or activity is reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% compared to such expression or activity in a control cell. The control cell is a cell that has not been treated with the PD-1 inhibitor. PD-1 expression or activity is determined by any standard method in the art, including those described herein. Optionally, the PD-1 inhibitor inhibits or reduces binding of PD-1 to PD-L1, PD-L2, or both. PD-1 inhibitors include polypeptides, polynucleotides, small molecule antagonists, or siRNA.

A PD-1 inhibitor polypeptide includes, for example, an antibody or fragment thereof that reduces PD-1 expression or signaling. Exemplary antibodies include anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-PD-L2 antibodies, anti-CTLA-4 antibodies, anti-BTLA antibodies, anti-CD28 antibodies, anti-ICOS antibodies, anti-ICOS-L antibodies, an anti-B7-1 antibody, an anti-B7-2 antibody, anti-B7-H3 antibodies, or anti-B7-H4 antibodies.

Alternatively, the PD-1 inhibitor is a dominant negative protein or a nucleic acid encoding a dominant negative protein that interferes with the biological activity of PD-1 (i.e. binding of PD-1 to PD-L1, PD-L2, or both). A dominant negative protein is any amino acid molecule having a sequence that has at least 50%, 70%, 80%, 90%, 95%, or even 99% sequence identity to at least 10, 20, 35, 50, 100, or more than 150 amino acids of the wild type protein to which the dominant negative protein corresponds. For example, a dominant-negative PD-1 has mutation such that it no longer able to binds PD-L1.

The dominant negative protein may be administered as an expression vector. The expression vector may be a non-viral vector or a viral vector (e.g., retrovirus, recombinant adeno-associated virus, or a recombinant adenoviral vector). Alternatively, the dominant negative protein may be directly administered as a recombinant protein systemically or to the infected area using, for example, microinjection techniques.

Small molecules includes, but are not limited to, peptides, peptidomimetics (e.g., peptoids), amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic and inorganic compounds (including heterorganic and

organomettallic compounds) having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 2,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

The PD-1 inhibitor is an antisense molecule, an RNA interference (siRNA) molecule, or a small molecule antagonist that targets PD-1 expression or activity. By the term "siRNA" is meant a double stranded RNA molecule which prevents translation of a target mRNA. Standard techniques of introducing siRNA into a cell are used, including those in which DNA is a template from which an siRNA RNA is transcribed. The siRNA includes a sense PD-1, PD-L1 or PD-L2 nucleic acid sequence, an anti-sense PD-1, PD-L1 or PD-L2 nucleic acid sequence or both. Optionally, the siRNA is constructed such that a single transcript has both the sense and complementary antisense sequences from the target gene, *e.g.,* a hairpin. Binding of the siRNA to an PD-1, PD-L1 or PD-L2 transcript in the target cell results in a reduction in PD-1, PD-L1 or PD-L2production by the cell. The length of the oligonucleotide is at least 10 nucleotides and may be as long as the naturally-occurring PD-1, PD-L1 or PD-L2 transcript. Preferably, the oligonucleotide is 19-25 nucleotides in length. Most preferably, the oligonucleotide is less than 75, 50 , 25 nucleotides in length.

Other suitable PD-1 inhibitors are described in, for example, in U.S. Patent No. 6,808,710 and U.S. Patent Application Publication Nos. 20040137577, 20030232323, 20030166531, 20030064380, 20030044768, 20030039653, 20020164600, 20020160000, 20020110836, 20020107363, and 20020106730, all of which are hereby incorporated by reference.

The preferred dose of the PD-1 inhibitor is a biologically active dose. A biologically active dose is a dose that will induce an increase in CD8+ T cell cytotoxic activity the increase in the immune response specific to the infectious agent. Desirably, the PD-1 inhibitor has the ability to reduce the expression or activity of PD-1 in antigen specific immune cells (e.g., T cells such as CD8+ T cells) by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more than 100% below untreated control levels. The levels or activity of PD-1 in immune cells is measured by any method known in the art, including, for example, Western blot analysis, immunohistochemistry, ELISA, and Northern Blot analysis. Alternatively, the biological activity of PD-1 is measured by assessing binding of PD-1 to PD-L1, PD-L2, or both. The

biological activity of PD-1 is determined according to its ability to increase CD8+ T cell cytotoxicity including, for example, cytokine production, clearance of the infectious agent, and proliferation of antigen specific CD8+ T cells. Preferably, the agent that reduces the expression or activity of PD-1 can increase the immune response specific to the infectious agent by at least 5% 10% 20% 30% 40% 50% 60% 70% 80% 90% or more than 100% above untreated control levels. The agent of the present invention is therefore any agent having any one or more of these activities. Although the agent of the invention is preferably expressed in CD8+ T cells, it is understood that any cell that can influence the immune response to persistent infections is also amenable to the methods of the invention and include, for example, B cells.

Optionally, the subject is administered one or more additional therapeutic agents. Additional therapeutic agents include, for example, antiviral compounds (e.g., vidarabine, acyclovir, gancyclovir, valgancyclovir, nucleoside-analog reverse transcriptase inhibitor (NRTI) (e.g., AZT (Zidovudine), ddI (Didanosine), ddC (Zalcitabine), d4T (Stavudine), or 3TC (Lamivudine)), non-nucleoside reverse transcriptase inhibitor (NNRTI) (e.g., (nevirapine or delavirdine), protease inhibitor (saquinavir, ritonavir, indinavir, or nelfinavir), ribavirin, or interferon), antibacterial compounds, antifungal compounds, antiparasitic compounds, anti-inflammatory compounds, anti-neoplastic agent or analgesics.

The additional therapeutic agent is administered prior to, concomitantly, or subsequent to administration of the PD-1 inhibitor. For example, the PD-1 inhibitor and the additional agent are administered in separate formulations within at least 1, 2, 4, 6, 10, 12, 18, or more than 24 hours apart. Optionally, the additional agent is formulated together with the PD-1 inhibitor. When the additional agent is present in a different composition, different routes of administration may be used. The agent is administered at doses known to be effective for such agent for treating, reducing, or preventing an infection.

Concentrations of the PD-1 inhibitor and the additional agent depends upon different factors, including means of administration, target site, physiological state of the mammal, and other medication administered. Thus treatment dosages may be titrated to optimize safety and efficacy and is within the skill of an artisan. Determination of the proper dosage and administration regime for a particular situation is within the skill of the art.

Optionally, the subject is further administered a vaccine that elicits a protective immune response against the infectious agent that causes a persistent infection. For example, the subject

receives a vaccine that elicits an immune response against human immunodeficiency virus (HIV), tuberculosis, influenza, or hepatitis C. Exemplary vaccines are described, for example, in Berzofsky et al. (J. Clin. Invest. 114:456-462, 2004). If desired, the vaccine is administered with a prime-booster shot or with adjuvants.

PD-1 inhibitors are administered in an amount sufficient to increase T cell, e.g., CD8+T cell, cytotoxicity. An increase in T-cell cytotoxicity results in an increased immune response and a reduction in the persistent infection. An increased immune response is measured, for example, by an increase in immune cell proliferation, e.g., T-cell or B cell, an increase in cytokine production, and an increase in the clearance of an infectious agent. Such reduction includes the alleviation of one or more of symptoms associated with the persistent infection. Administration of the PD-1 inhibitor reduces the persistent infection or alleviates one or more symptoms associated with the persistent infection by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% as compared to an untreated subject.

Treatment is efficacious if the treatment leads to clinical benefit such as, a reduction of the load of the infectious agent in the subject. When treatment is applied prophylactically, "efficacious" means that the treatment retards or prevents an infection from forming. Efficacy may be determined using any known method for diagnosing or treating the particular infection.

Therapeutic Administration

The invention includes administering to a subject a composition that includes a compound that reduces PD-1 expression or activity (referred to herein as an "PD-1 inhibitor" or "therapeutic compound").

An effective amount of a therapeutic compound is preferably from about 0.1 mg/kg to about 150 mg/kg. Effective doses vary, as recognized by those skilled in the art, depending on route of administration, excipient usage, and coadministration with other therapeutic treatments including use of other anti-infection agents or therapeutic agents for treating, preventing or alleviating a symptom of a particular infection or cancer. A therapeutic regimen is carried out by identifying a mammal, e.g., a human patient suffering from (or at risk of developing) an infection or cancer, using standard methods.

The pharmaceutical compound is administered to such an individual using methods known in the art. Preferably, the compound is administered orally, rectally, nasally, topically or parenterally, e.g., subcutaneously, intraperitoneally, intramuscularly, and intravenously. The

compound is administered prophylactically, or after the detection of an infection. The compound is optionally formulated as a component of a cocktail of therapeutic drugs to treat infection. Examples of formulations suitable for parenteral administration include aqueous solutions of the active agent in an isotonic saline solution, a 5% glucose solution, or another standard pharmaceutically acceptable excipient. Standard solubilizing agents such as PVP or cyclodextrins are also utilized as pharmaceutical excipients for delivery of the therapeutic compounds.

The therapeutic compounds described herein are formulated into compositions for other routes of administration utilizing conventional methods. For example, PD-1 inhibitor is formulated in a capsule or a tablet for oral administration. Capsules may contain any standard pharmaceutically acceptable materials such as gelatin or cellulose. Tablets may be formulated in accordance with conventional procedures by compressing mixtures of a therapeutic compound with a solid carrier and a lubricant. Examples of solid carriers include starch and sugar bentonite. The compound is administered in the form of a hard shell tablet or a capsule containing a binder, e.g., lactose or mannitol, a conventional filler, and a tableting agent. Other formulations include an ointment, suppository, paste, spray, patch, cream, gel, resorbable sponge, or foam. Such formulations are produced using methods well known in the art.

Where the therapeutic compound is a nucleic acid encoding a protein, the Therapeutic nucleic acid is administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular (*e.g.,* by use of a retroviral vector, by direct injection, by use of microparticle bombardment, by coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (*See, e.g.,* Joliot, *et al.,* 1991. *Proc Natl Acad Sci USA* 88:1864-1868), and the like. Alternatively, a nucleic acid therapeutic is introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination or remain episomal.

For local administration of DNA, standard gene therapy vectors used. Such vectors include viral vectors, including those derived from replication-defective hepatitis viruses (e.g., HBV and HCV), retroviruses (see, e.g., WO 89/07136; Rosenberg et al., 1990, N. Eng. J. Med. 323(9):570-578), adenovirus (see, e.g., Morsey et al., 1993, J. Cell. Biochem., Supp. 17E,), adeno-associated virus (Kotin et al., 1990, Proc. Natl. Acad. Sci. USA 87:2211-2215,),

replication defective herpes simplex viruses (HSV; Lu et al., 1992, Abstract, page 66, Abstracts of the Meeting on Gene Therapy, Sept. 22-26, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York), and any modified versions of these vectors. The invention may utilize any other delivery system which accomplishes *in vivo* transfer of nucleic acids into eucaryotic cells. For example, the nucleic acids may be packaged into liposomes, e.g., cationic liposomes (Lipofectin), receptor-mediated delivery systems, non-viral nucleic acid-based vectors, erytbrocyte ghosts, or microspheres (e.g., microparticles; see, e.g., U.S. Patent No. 4,789,734; U.S. Patent No. 4,925,673; U.S. Patent No. 3,625,214; Gregoriadis, 1979, Drug Carriers in Biology and Medicine, pp. 287-341 (Academic Press,). Naked DNA may also be administered.

DNA for gene therapy can be administered to patients parenterally, e.g., intravenously, subcutaneously, intramuscularly, and intraperitoneally. DNA or an inducing agent is administered in a pharmaceutically acceptable carrier, i.e., a biologically compatible vehicle which is suitable for administration to an animal e.g., physiological saline. A therapeutically effective amount is an amount which is capable of producing a medically desirable result, e.g., an decrease of a PD-1 gene product in a treated animal. Such an amount can be determined by one of ordinary skill in the art. As is well known in the medical arts, dosage for any given patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Dosages may vary, but a preferred dosage for intravenous administration of DNA is approximately 10^6 to 10^{22} copies of the DNA molecule. Typically, plasmids are administered to a mammal in an amount of about 1 nanogram to about 5000 micrograms of DNA. Desirably, compositions contain about 5 nanograms to 1000 micrograms of DNA, 10 nanograms to 800 micrograms of DNA, 0.1 micrograms to 500 micrograms of DNA, 1 microgram to 350 micrograms of DNA, 25 micrograms to 250 micrograms of DNA, or 100 micrograms to 200 micrograms of DNA. Alternatively, administration of recombinant adenoviral vectors encoding the PD-1 inhibitor into a mammal may be administered at a concentration of at least 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, or 10^{11} plaque forming unit (pfu).

PD-1 gene products are administered to the patient intravenously in a pharmaceutically acceptable carrier such as physiological saline. Standard methods for intracellular delivery of peptides can be used, e.g. packaged in liposomes. Such methods are well known to those of

ordinary skill in the art. It is expected that an intravenous dosage of approximately 1 to 100 moles of the polypeptide of the invention would be administered per kg of body weight per day. The compositions of the invention are useful for parenteral administration, such as intravenous, subcutaneous, intramuscular, and intraperitoneal.

PD-1 inhibitors are effective upon direct contact of the compound with the affected tissue. Accordingly, the compound may be administered topically. Alternatively, PD-1 inhibitors may be administered systemically. Additionally, compounds may be administered by implanting (either directly into an organ (e.g., intestine or liver) or subcutaneously) a solid or resorbable matrix which slowly releases the compound into adjacent and surrounding tissues of the subject. For example, for the treatment of gastrointestinal infection, the compound may be administered systemically (e.g., intravenously, rectally or orally) or locally (e.g., directly into gastric tissue). Alternatively, a PD-1 inhibitor-impregnated wafer or resorbable sponge is placed in direct contact with gastric tissue. The PD-1 inhibitor is slowly released *in vivo* by diffusion of the drug from the wafer and erosion of the polymer matrix. As another example, infection of the liver (i.e., hepatitis) is treated by infusing into the liver vasculature a solution containing the PD-1 inhibitor.

For the treatment of neurological infections, the PD-1 inhibitor may be administered intravenously or intrathecally (i.e., by direct infusion into the cerebrospinal fluid). For local administration, a compound-impregnated wafer or resorbable sponge is placed in direct contact with CNS tissue. The compound or mixture of compounds is slowly released *in vivo* by diffusion of the drug from the wafer and erosion of the polymer matrix. Alternatively, the compound is infused into the brain or cerebrospinal fluid using standard methods. For example, a burr hole ring with a catheter for use as an injection port is positioned to engage the skull at a burr hole drilled into the skull. A fluid reservoir connected to the catheter is accessed by a needle or stylet inserted through a septum positioned over the top of the burr hole ring. A catheter assembly (described, for example, in U.S. Patent No. 5,954,687) provides a fluid flow path suitable for the transfer of fluids to or from selected location at, near or within the brain to allow administration of the drug over a period of time.

For cardiac infections, the compound may be delivered, for example, to the cardiac tissue (i.e., myocardium, pericardium, or endocardium) by direct intracoronary injection through the chest wall or using standard percutaneous catheter based methods under fluoroscopic guidance.

Thus, the inhibitor may be directly injected into tissue or may be infused from a stent or catheter which is inserted into a bodily lumen. Any variety of coronary catheter or perfusion catheter may be used to administer the compound. Alternatively, the compound is coated or impregnated on a stent that is placed in a coronary vessel.

Pulmonary infections may be treated, for example, by administering the compound by inhalation. The compounds are delivered in the form of an aerosol spray from a pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide or a nebulizer.

One in the art will understand that the patients treated according to the invention may have been subjected to the same tests to diagnose a persistently infected subject or may have been identified, without examination, as one at high risk due to the presence of one or more risk factors (e.g., exposure to infectious agent, exposure to infected subject, genetic predisposition, or having a pathological condition predisposing to secondary infections). Reduction of persistent infection symptoms or damage may also include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, and amelioration or palliation of the disease state. Treatment may occur at home with close supervision by the health care provider, or may occur in a health care facility.

Methods to Measure Immune Response

Methods for measuring the immune response following treatment according to the present invention are well known in the art. The activity of T cells may be assessed, for example, by assays that detect cytokine production, assays measuring T cell proliferation, assays that measure the clearance of the microbial agent, and assays that measure CD8+ T cell cytotoxicity. These assays are described, for example, in U.S. Patent No. 6,808,710 and U.S. Patent Application Publication Nos. 20040137577, 20030232323, 20030166531, 20030064380, 20030044768, 20030039653, 20020164600, 20020160000, 20020110836, 20020107363, and 20020106730, all of which are hereby incorporated by reference.

Optionally, the ability of a PD-1 inhibitor to increase CD8+ T cell cytotoxicity is assessed by assays that measure the proliferation of CD8+ T cells (e.g., thymidine incorporation, BrdU assays, and staining with cell cycle markers (e.g., Ki67 and CFSE), described, for example, by Dong et al (Nature 5:1365-1369, 1999). In one example, T-cell proliferation is

monitored by culturing the purified T-cells expressing PD-1 with a PD-1 inhibitor, a primary activation signal as described above, and ^{3}H-thymidine. The level of T-cell proliferation is determined by measuring thymidine incorporation.

CD8+ T cell cytotoxicity is also assessed by lysis assays (e.g., 51Cr release assays or assays detecting the release of perforin or granzyme), assays that detect caspase activation, or assays that measure the clearance of the microbial agent from the infected subject. For example, the viral load in a biological sample from the infected subject (e.g., serum, spleen, liver, lung, or the tissue to which the virus is tropic) may be measured before and after treatment.

The production of cytokines such as IFNγ, TNF-α, and IL-2 may also be measured. For example, purified T-cells are cultured in the presence of the PD-1 inhibitor protein and a primary activation signal. The level of various cytokines in the supernatant can be determined by sandwich enzyme-linked immunosorbent assays or other conventional assays described, for example, in Dong et al (Nature 5:1365-1369, 1999).

If desired, the efficacy of the PD-1 inhibitor is assessed by its ability to induce co-stimulation of T cells. For example, a method for in vitro T-cell co-stimulation involves providing purified T-cells that express PD-1 with a first or primary activation signal by anti-CD3 monoclonal antibody or phorbol ester, or by antigen in association with class II MHC. The ability of a candidate compound agent to reduce PD-1 expression or activity and therefore provide the secondary or co-stimulatory signal necessary to modulate immune function, to these T-cells can then be assayed by any one of the several conventional assays well known in the art.

A B cell response is assessed by an antigen specific ELISA(e.g., LCMV, HIV, tuberculosis, or malaria), plasma cell ELISPOT, memory B-cell assay, phenotyping of B cell, and analysis of germinal centers by immunohistochemistry.

Screening Assays

The present invention provides screening methods to identify compounds that can inhibit the expression or activity of PD-1. Useful compounds include any agent that inhibits the biological activity or reduces the cellular level of PD-1. For example, candidate compounds may reduce binding of PD-1 to PD-L1, PD-L2, or both. Using such agents as lead compounds, for example, the present screening methods also allow the identification of further novel, specific inhibitors of PD-1 that function to treat, reduce, or prevent persistent infections, or alternatively,

that alleviate one or more symptoms associated with such infections. The method of screening may involve high-throughput techniques.

By a "candidate compound" is meant a chemical, be it naturally-occurring or artificially-derived. Candidate compounds may include, for example, peptides, polypeptides, synthetic organic molecules, naturally occurring organic molecules, nucleic acid molecules, peptide nucleic acid molecules, and components and derivatives thereof. For example, a useful candidate compound according to the present invention reduces binding of PD-1 to PD-L1, PD-L2, or both.

A number of methods are available for carrying out such screening assays. According to one approach, candidate compounds are added at varying concentrations to the culture medium of cells expressing PD-1. By a "PD-1 gene" is meant a nucleic acid that encodes a PD-1 protein. By "PD-1 fusion gene" is meant a PD-1 promoter and/or all or part of a PD-1 coding region operably linked to a second, heterologous nucleic acid sequence. In preferred embodiments, the second, heterologous nucleic acid sequence is a reporter gene, that is, a gene whose expression may be assayed; reporter genes include, without limitation, those encoding glucuronidase (GUS), luciferase, chloramphenicol transacetylase (CAT), green fluorescent protein (GFP), alkaline phosphatase, and beta-galactosidase.

Gene expression of PD-1 is then measured, for example, by standard Northern blot analysis (Ausubel et al., supra), using any appropriate fragment prepared from the nucleic acid molecule of PD-1 as a hybridization probe or by real time PCR with appropriate primers. The level of gene expression in the presence of the candidate compound is compared to the level measured in a control culture medium lacking the candidate molecule. If desired, the effect of candidate compounds may, in the alternative, be measured at the level of PD-1 polypeptide using the same general approach and standard immunological techniques, such as Western blotting or immunoprecipitation with an antibody specific to PD-1 for example. For example, immunoassays may be used to detect or monitor the level of PD-1. Polyclonal or monoclonal antibodies which are capable of binding to PD-1 may be used in any standard immunoassay format (e.g., ELISA or RIA assay) to measure the levels of PD-1. PD-1 can also be measured using mass spectroscopy, high performance liquid chromatography, spectrophotometric or fluorometric techniques, or combinations thereof.

Alternatively, the screening methods of the invention may be used to identify candidate compounds that decrease the biological activity of PD-1 by reducing binding of PD-1 to PD-L1,

PD-L2, or both by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% relative to an untreated control. For example, a candidate compound may be tested for its ability to decrease PD-1 activity in cells that naturally express PD-1, after transfection with cDNA for PD-1, or in cell-free solutions containing PD-1, as described further below. The effect of a candidate compound on the binding or activation of PD-1 can be tested by radioactive and non-radiaoctive binding assays, competition assays, and receptor signaling assays.

As a specific example, mammalian cells (e.g., rodent cells) that express a nucleic acid encoding PD-1 are cultured in the presence of a candidate compound (e.g., a peptide, polypeptide, synthetic organic molecule, naturally occurring organic molecule, nucleic acid molecule, or component thereof). Cells may either endogenously express PD-1 or may alternatively be genetically engineered by any standard technique known in the art (e.g., transfection and viral infection) to overexpress PD-1. The expression level of PD-1 is measured in these cells by means of Western blot analysis and subsequently compared to the level of expression of the same protein in control cells that have not been contacted by the candidate compound. A compound which promotes a decrease in the level of PD-1 activity as a result of reducing its synthesis or biological activity is considered useful in the invention.

In one particular example, a compound that interferes with PD-1binding to PD-L1, PD-L2, or both (thereby reducing the biological activity of PD-1), leading to an increase in an immune response, is useful according to the present invention. Given its ability to decrease the biological activity of PD-1, such a molecule may be used, for example, as a therapeutic agent to treat, reduce, or prevent a persistent infection, or alternatively, to alleviate one or more symptoms associated with such infections. As a specific example, a candidate compound may be contacted with two proteins, the first protein being a polypeptide substantially identical to PD-1 and the second protein being either PD-L1 or PD-L2 (i.e., a protein that binds the PD-1 polypeptide under conditions that allow binding and that results in a reduced immune response). According to this particular screening method, the interaction between these two proteins is measured following the addition of a candidate compound. A decrease in the binding PD-1 to the second polypeptide following the addition of the candidate compound (relative to such binding in the absence of the compound) identifies the candidate compound as having the ability to inhibit the interaction between the two proteins. Ultimately, the screening assay of the invention may be carried out, for example, in a cell-free system or using a yeast two-hybrid

system. If desired, one of the proteins or the candidate compound may be immobilized on a support as described above or may have a detectable group.

Alternatively, or in addition, candidate compounds may be screened for those which specifically bind to and thereby inhibit PD-1. The efficacy of such a candidate compound is dependent upon its ability to interact with PD-1. Such an interaction can be readily assayed using any number of standard binding techniques and functional assays (e.g., those described in Ausubel et al., supra). For example, a candidate compound may be tested in vitro for interaction and binding with PD-1 and its ability to modulate immune responses may be assayed by any standard assays (e.g., those described herein).

For example, a candidate compound that binds to PD-1 may be identified using a chromatography-based technique. For example, a recombinant PD-1 may be purified by standard techniques from cells engineered to express PD-1 (e.g., those described above) and may be immobilized on a column. Alternatively, the naturally-occurring PD-1 may be immobilized on a column. A solution of candidate compounds is then passed through the column, and a compound specific for PD-1 is identified on the basis of its ability to bind to PD-1 and be immobilized on the column. To isolate the compound, the column is washed to remove non-specifically bound molecules, and the compound of interest is then released from the column and collected. Compounds isolated by this method (or any other appropriate method) may, if desired, be further purified (e.g., by high performance liquid chromatography).

Screening for new inhibitors and optimization of lead compounds may be assessed, for example, by assessing their ability to modulate cytotoxic T cell activity or the immune response using standard techniques. In addition, these candidate compounds may be tested for their ability to function as anti-microbial agents (e.g., as described herein). Compounds isolated by this approach may also be used, for example, as therapeutics to treat, reduce, or prevent persistent infections, or alternatively, to alleviate one or more symptoms associated with such infections. Compounds which are identified as binding to PD-1 with an affinity constant less than or equal to 10 mM are considered particularly useful in the invention.

Potential therapeutic agents include organic molecules, peptides, peptide mimetics, polypeptides, and antibodies that bind to a nucleic acid sequence or polypeptide that encodes PD-1 and thereby inhibit or extinguish their activity. Potential anti-microbial agents also include small molecules that bind to and occupy the binding site of such polypeptides thereby preventing

binding to cellular binding molecules, such that normal biological activity is prevented. Other potential anti-microbial agents include antisense molecules.

Diagnostic and Prognostic Methods

Cancer, e.g. angioimmunoblastic T cell lymphoma or Nodular Lymphocyte Predominant Hodgkin's Lymphoma are detected by examining the amount of a PD-1 polypeptide in a test sample (*i.e.*, a patient derived sample). A change in the level if the PD-1 polypeptide compared to a control sample is indicative of cancer in the subject. The change may be an increase or a decrease in the PD-1 polypeptide relative to a control sample. The control sample is prepared (i.e., fractionated) in a similar fashion as the test sample.

A sample is for example, blood, serum, acsites fluid, urine, or other bodily fluids. Preferably the sample is a T-cell or a B-cell

The amount of the PD-1 is determined in the test sample and compared to the expression of the normal control level. By normal control level is meant the expression level of a PD-1 polypeptide typically found in a subject not suffering from a cancer. A increase of the level in the patient derived sample of a PD-1 indicates that the subject is suffering from or is at risk of developing cancer. In contrast, when the methods are applied prophylacticly, a similar level or an decrease in the level in the patient derived sample of a PD-1 polypeptide indicates that the subject is not suffering from or is at risk of developing cancer. An increase of the level in the patient derived sample of a PD-1 polypeptide indicates that the subject is suffering from or is at risk of developing cancer.

The alteration in the amount of the PD-1 polypeptide is statistically significant. By statistically significant is meant that the alteration is greater than what might be expected to happen by change alone. Statistical significance is determined by method known in the art. For example statistical significance is determined by p-value. The p-values is a measure of probability that a difference between groups during an experiment happened by chance. ($P(z \geq z_{observed})$). For example, a p-value of 0.01 means that there is a 1 in 100 chance the result occurred by chance. The lower the p-value, the more likely it is that the difference between groups was caused by treatment. An alteration is statistically significant if the p-value is at least 0.05. Preferably, the p-value is 0.04, 0.03, 0.02, 0.01, 0.005, 0.001 or less.

The "diagnostic accuracy" of a test, assay, or method concerns the ability of the test, assay, or method to distinguish between patients having cancer or at risk for cancer is based on

whether the patients have a "clinically significant presence" of a PD-1 polypeptide. By "clinically significant presence" is meant that the presence of the PD-1 polypeptide in the patient (typically in a sample from the patient) is higher or lower than the predetermined cut-off point (or threshold value) for that PD-1 polypeptide and therefore indicates that the patient has cancer for which the sufficiently high presence of that protein is a marker.

The terms "high degree of diagnostic accuracy" and "very high degree of diagnostic accuracy" refer to the test or assay for that PD-1 polypeptide with the predetermined cut-off point correctly (accurately) indicating the presence or absence of the cancer. A perfect test would have perfect accuracy. Thus, for individuals who have diabetes, the test would indicate only positive test results and would not report any of those individuals as being "negative" (there would be no "false negatives"). In other words, the "sensitivity" of the test (the true positive rate) would be 100%. On the other hand, for individuals who did not have diabetes, the test would indicate only negative test results and would not report any of those individuals as being "positive" (there would be no "false positives"). In other words, the "specificity" (the true negative rate) would be 100%. See, e.g., O'Marcaigh AS, Jacobson RM, "Estimating The Predictive Value Of A Diagnostic Test, How To Prevent Misleading Or Confusing Results," Clin. Ped. 1993, 32(8): 485-491, which discusses specificity, sensitivity, and positive and negative predictive values of a test, e.g., a clinical diagnostic test.

Changing the cut point or threshold value of a test (or assay) usually changes the sensitivity and specificity but in a qualitatively inverse relationship. For example, if the cut point is lowered, more individuals in the population tested will typically have test results over the cut point or threshold value. Because individuals who have test results above the cut point are reported as having the disease, condition, or syndrome for which the test is being run, lowering the cut point will cause more individuals to be reported as having positive results (i.e., that they have cancer). Thus, a higher proportion of those who have cancer will be indicated by the test to have it. Accordingly, the sensitivity (true positive rate) of the test will be increased. However, at the same time, there will be more false positives because more people who do not have the disease, condition, or syndrome (i.e., people who are truly "negative") will be indicated by the test to have PD-1 polypeptide values above the cut point and therefore to be reported as positive (i.e., to have the disease, condition, or syndrome) rather than being correctly indicated by the test to be negative. Accordingly, the specificity (true negative rate) of the test will be decreased.

Similarly, raising the cut point will tend to decrease the sensitivity and increase the specificity. Therefore, in assessing the accuracy and usefulness of a proposed medical test, assay, or method for assessing a patient's condition, one should always take both sensitivity and specificity into account and be mindful of what the cut point is at which the sensitivity and specificity are being reported because sensitivity and specificity may vary significantly over the range of cut points.

There is, however, an indicator that allows representation of the sensitivity and specificity of a test, assay, or method over the entire range of cut points with just a single value. That indicator is derived from a Receiver Operating Characteristics ("ROC") curve for the test, assay, or method in question. See, e.g., Shultz, "Clinical Interpretation Of Laboratory Procedures," chapter 14 in Teitz, Fundamentals of Clinical Chemistry, Burtis and Ashwood (eds.), 4th edition 1996, W.B. Saunders Company, pages 192-199; and Zweig et al., "ROC Curve Analysis: An Example Showing The Relationships Among Serum Lipid And Apolipoprotein Concentrations In Identifying Patients With Coronory Artery Disease," Clin. Chem., 1992, 38(8): 1425-1428.

An ROC curve is an x-y plot of sensitivity on the y-axis, on a scale of zero to one (i.e., 100%), against a value equal to one minus specificity on the x-axis, on a scale of zero to one (i.e., 100%). In other words, it is a plot of the true positive rate against the false positive rate for that test, assay, or method. To construct the ROC curve for the test, assay, or method in question, patients are assessed using a perfectly accurate or "gold standard" method that is independent of the test, assay, or method in question to determine whether the patients are truly positive or negative for the disease, condition, or syndrome (for example, coronary angiography is a gold standard test for the presence of coronary atherosclerosis). The patients are also tested using the test, assay, or method in question, and for varying cut points, the patients are reported as being positive or negative according to the test, assay, or method. The sensitivity (true positive rate) and the value equal to one minus the specificity (which value equals the false positive rate) are determined for each cut point, and each pair of x-y values is plotted as a single point on the x-y diagram. The "curve" connecting those points is the ROC curve.

The area under the curve ("AUC") is the indicator that allows representation of the sensitivity and specificity of a test, assay, or method over the entire range of cut points with just a single value. The maximum AUC is one (a perfect test) and the minimum area is one half. The closer the AUC is to one, the better is the accuracy of the test.

By a "high degree of diagnostic accuracy" is meant a test or assay (such as the test of the invention for determining the clinically significant presence of PD-1 polypeptide, which thereby indicates the presence of diabetes) in which the AUC (area under the ROC curve for the test or assay) is at least 0.70, desirably at least 0.75, more desirably at least 0.80, preferably at least 0.85, more preferably at least 0.90, and most preferably at least 0.95.

By a "very high degree of diagnostic accuracy" is meant a test or assay in which the AUC (area under the ROC curve for the test or assay) is at least 0.875, desirably at least 0.90, more desirably at least 0.925, preferably at least 0.95, more preferably at least 0.975, and most preferably at least 0.98.

Optionally, expression of other known biomarkers for a particular cancer are also determined as further indication of whether or not the subject is carrying a cancer. For example, CD10, bcl-6, CD20, CD57 or CXCR5 is detected.

The PD-1 polypeptide and the additional biomarkers are detected in any suitable manner, but is typically detected by contacting a sample from the patient with an antibody which binds the PD-1 or biomarker and then detecting the presence or absence of a reaction product. The antibody may be monoclonal, polyclonal, chimeric, or a fragment of the foregoing, as discussed in detail above, and the step of detecting the reaction product may be carried out with any suitable immunoassay. The sample from the subject is typically a biological fluid as described above, and may be the same sample of biological fluid used to conduct the method described above.

Expression of a PD-1 polypeptide also allows for the course of treatment of cancer to be monitored. In this method, a biological sample is provided from a subject undergoing treatment, e.g., surgical, chemotherapeutic or hormonal treatment, for a cancer. If desired, biological samples are obtained from the subject at various time points before, during, or after treatment. Expression of a PD-1 is then determined and compared to a reference, e.g. control whose cancer state is known. The reference sample has been exposed to the treatment. Alternatively, the reference sample has not been exposed to the treatment. Optionally, such monitoring is carried out preliminary to a second look surgical surveillance procedures and subsequent surgical surveillance procedures. For example, samples may be collected from subjects who have received initial surgical treatment for cancer and subsequent treatment with anti-neoplastic agents for that cancer to monitor the progress of the treatment.

If the reference sample is from a subject that does not have cancer, a similarity or a decrease in the amount of the PD-1 polypeptide in the test sample and the reference sample indicates that the treatment is efficacious. However, an increase in the amount of the PD-1 polypeptide in the test sample and the reference sample indicates a less favorable clinical outcome or prognosis.

By "efficacious" is meant that the treatment leads to an decrease in the amount of a PD-1 polypeptide, or a decrease in size, prevalence, or metastatic potential of a tumor in a subject. Assessment of cancer is made using standard clinical protocols. Efficacy is determined in association with any known method for diagnosing or treating the particular tumor. Expression of a PD-1 polypeptide also allows the identification of patients who will be responsive to systemic, e.g., chemotherapeutic, hormonal or radiation therapy. In this method, a biological sample is provided from a subject prior to undergoing surgical treatment, for a cancer. Expression of a PD-1 polypeptide is then determined and compared to a biological sample obtained from the subject after surgical removal of the cancer. The patient will likely be responsive to systemic treatment if the amount of the PD-1 polypeptide decreases after surgical removal the cancer. In contrast a the patient will likely not be responsive to systemic treatment if the amount of the polypeptide remains constant or increase after surgical removal of the cancer.

Expression of the PD-1 polypeptide or other cancer biomarkers is determined at the protein or nucleic acid level using any method known in the art. For example, Northern hybridization analysis using probes which specifically recognize one or more of these sequences can be used to determine gene expression. Alternatively, expression is measured using reverse-transcription-based PCR assays, *e.g.*, using primers specific for the differentially expressed sequence of genes. Expression is also determined at the protein level, *i.e.*, by measuring the levels of peptides encoded by the gene products described herein, or activities thereof. Such methods are well known in the art and include, *e.g.*, immunoassays based on antibodies to proteins encoded by the genes. Any biological material can be used for the detection/quantification of the protein or it's activity. Alternatively, a suitable method can be selected to determine the activity of proteins encoded by the marker genes according to the activity of each protein analyzed.

The subject is preferably a mammal. The mammal is, *e.g.*, a human, non-human primate, mouse, rat, dog, cat, horse, or cow. Subjects are typically human females or human males The subject has been previously diagnosed as carrying a cancer, and possibly has already undergone treatment for the cancer. Alternatively, the subject has not been previously diagnosis as carrying a cancer. The present invention is useful with all patients at risk for a cancer. Although each type of cancer has their own set of risk factors, the risk of developing cancer increases as with aged, gender, race and personal and family medical history. Other risk factors are largely related to lifestyle choices, while certain infections, occupational exposures and some environmental factors can also be related to developing cancer.

Diagnosis of cancer is typically made through the identification of a mass on an examination, though it may also be through other means such as a radiological diagnosis, or ultrasound. Treatment is typically through cytoreductive surgery, followed by treatment with antineoplastic agents such as docetaxel , vinorelbine gemcitabine , capecitabine or a combinations of cyclophosphamide, methotrexate, and fluorouracil; cyclophosphamide, doxorubicin , and fluorouracil; doxorubicin and cyclophosphamide; doxorubicin and cyclophosphamide with paclitaxel; doxorubicin followed by CMF; or Cyclophosphamide, epirubicin and fluorouracil. In addition, many patients will require radiation therapy.

Immunoassays carried out in accordance with the present invention may be homogeneous assays or heterogeneous assays. In a homogeneous assay the immunological reaction usually involves the specific antibody (e.g., PD-1 polypeptide), a labeled analyte, and the sample of interest. The signal arising from the label is modified, directly or indirectly, upon the binding of the antibody to the labeled analyte. Both the immunological reaction and detection of the extent thereof are carried out in a homogeneous solution. Immunochemical labels which may be employed include free radicals, radioisotopes, fluorescent dyes, enzymes, bacteriophages, or coenzymes.

In a heterogeneous assay approach, the reagents are usually the sample, the antibody, and means for producing a detectable signal. Samples as described above may be used. The antibody is generally immobilized on a support, such as a bead, plate or slide, and contacted with the specimen suspected of containing the antigen in a liquid phase. The support is then separated from the liquid phase and either the support phase or the liquid phase is examined for a detectable signal employing means for producing such signal. The signal is related to the

presence of the analyte in the sample. Means for producing a detectable signal include the use of radioactive labels, fluorescent labels, or enzyme labels. For example, if the antigen to be detected contains a second binding site, an antibody which binds to that site can be conjugated to a detectable group and added to the liquid phase reaction solution before the separation step. The presence of the detectable group on the solid support indicates the presence of the antigen in the test sample. Examples of suitable immunoassays are radioimmunoassays, immunofluorescence methods, or enzyme-linked immunoassays.

Those skilled in the art will be familiar with numerous specific immunoassay formats and variations thereof, which may be useful for carrying out the method disclosed herein. See generally E. Maggio, Enzyme-Immunoassay, (1980) (CRC Press, Inc., Boca Raton, Fla.); see also U.S. Pat. No. 4,727,022 to Skold et al. titled "Methods for Modulating Ligand-Receptor Interactions and their Application," U.S. Pat. No. 4,659,678 to Forrest et al. titled "Immunoassay of Antigens," U.S. Pat. No. 4,376,110 to David et al., titled "Immunometric Assays Using Monoclonal Antibodies," U.S. Pat. No. 4,275,149 to Litman et al., titled "Macromolecular Environment Control in Specific Receptor Assays," U.S. Pat. No. 4,233,402 to Maggio et al., titled "Reagents and Method Employing Channeling," and U.S. Pat. No. 4,230,767 to Boguslaski et al., titled "Heterogeneous Specific Binding Assay Employing a Coenzyme as Label." Antibodies are conjugated to a solid support suitable for a diagnostic assay (e.g., beads, plates, slides or wells formed from materials such as latex or polystyrene) in accordance with known techniques, such as precipitation. Antibodies as described herein may likewise be conjugated to detectable groups such as radiolabels (e.g., 35 S, 125 I, 131 I), enzyme labels (e.g., horseradish peroxidase, alkaline phosphatase), and fluorescent labels (e.g., fluorescein) in accordance with known techniques.

Diagnostic kits for carrying out the methods described herein are produced in a number of ways. In one embodiment, the diagnostic kit comprises (a) an antibody (e.g., PD-1 polypeptide) conjugated to a solid support and (b) a second antibody of the invention conjugated to a detectable group. The reagents may also include ancillary agents such as buffering agents and protein stabilizing agents, e.g., polysaccharides and the like. The diagnostic kit may further include, where necessary, other members of the signal-producing system of which system the detectable group is a member (e.g., enzyme substrates), agents for reducing background interference in a test, control reagents, apparatus for conducting a test, and the like.

Alternatively, a test kit contains (a) an antibody , and (b) a specific binding partner for the antibody conjugated to a detectable group. Ancillary agents as described above may likewise be included. The test kit may be packaged in any suitable manner, typically with all elements in a single container along with a sheet of printed instructions for carrying out the test.

This invention is based in part on the experiments described in the following examples. These examples are provided to illustrate the invention and should not be construed as limiting.

Example 1: Inhibition of the PD-1 Pathway in Chronically-Infected Mice Using Anti-PD-L1 Antibodies

Mice infected with various strains of the lymphocytic choriomeningitis virus (LCMV) were used to study the effect of chronic viral infection on $CD8^+$ T cell function. The LCMV Armstrong strain causes an acute infection that is cleared within 8 days, leaving behind a long-lived population of highly functional, resting memory $CD8^+$ T cells. The LCMV Cl-13 strain, in contrast, establishes a persistent infection in the host, characterized by a viremia that lasts up to 3 months. The virus remains in some tissues indefinitely and antigen specific $CD8^+$ T cells become functionally impaired. D^bNP396-404 $CD8^+$ T cells are physically deleted, while D^bGP33-41 and D^bGP276-286 $CD8^+$ T cells persist but lose the ability to proliferate or secrete anti-viral cytokines, such as IFN-γ and TNF-α.

C57BL/6 mice were purchased from the National Cancer Institute (Frederick, MD). Mice were infected *i.v.* with $2x10^6$ pfu of LCMV-Cl-13. CD4 depletions were performed by injecting 500 μg of GK1.5 in PBS the day of infection and the day following the infection. LCMV immune mice are generated by infecting mice *i.p.* with $2x10^5$ pfu LCMV Armstrong.

Gene array analysis was performed on FACS-purified naïve D^bGP33-41 specific P14 transgenic $CD8^+$ T cells, D^bGP33-41 specific memory $CD8^+$ T cells derived from LCMV Armstrong immune mice, and D^bGP33-41 specific or D^bGP276-286 specific $CD8^+$ T cells derived from $CD4^+$ depleted LCMV Cl-13 infected mice. RNA isolation and gene array analysis were performed as described in Kaech et al., (Cell 111:837-51, 2002). PD-1 mRNA was highly expressed in exhausted $CD8^+$ T cells relative to memory $CD8^+$ T cells (Figure 1A). Furthermore, PD-1 was expressed on the surface of $CD8^+$ T cells in LCMV Cl-13 infected mice, but was not present on the surface of $CD8^+$ T cells after clearance of LCMV Armstrong (Figure 1B).

Chronically infected mice also expressed higher levels of one of the ligands of PD-1, PD-L1, on most lymphocytes and APC compared to uninfected mice. Thus, viral antigen persistence and $CD8^+$ T cell exhaustion are concomitant with an induction in PD-1 expression.

To test the hypothesis that blocking the PD-1/PD-L1 pathway may restore T cell function and enhance viral control during chronic LCMV infection, the PD-1/PD-L1 co-inhibitory pathway was disrupted during chronic LCMV infection using αPD-L1 blocking antibodies. A blocking monoclonal antibody against PD-L1 was administered *i.p.* every third day to mice infected with LCMV Cl-13 (200 μg of rat anti-mouse PD-L1 IgG2b monoclonal antibodies (clone 10F.5C5 or 10F.9G2)) from day 23 to day 37 post-infection. At day 37, there was approximately 2.5 fold more D^bNP396-404 specific $CD8^+$ T cells and 3 fold more D^bGP33-41 specific $CD8^+$ T cells in treated mice relative to the untreated controls (Figure 2A). The induction in proliferation was specific to $CD8^+$ T cells since the number of CD4+ T cells in the spleen were approximately the same in both treated mice and untreated mice (~$6x10^4$ IA^bGP61-80 of CD4+ T cells per spleen).

In addition to an increase in CD8+ T cell proliferation, the inhibition of PD-1 signaling also resulted in an increased production of anti-viral cytokines in virus-specific $CD8^+$ T cells. The production of IFN-γ and TNF-α by CD8+ T cells to eight different CTL epitopes was determined. The combined response was 2.3 fold higher in treated mice as compared to untreated mice (Figures 2B and 2C). A 2-fold increase in the frequency of TNF-α producing cells was also observed following treatment (Figure 2D). Viral clearance was also accelerated as the virus was cleared from the serum, spleen, and liver of treated mice. Reduced viral titers were observed in the lung and kidney (~10 fold) by day 37 post-infection (14 days following initiation of treatment) in treated mice. Untreated mice, however, displayed significant levels of virus in all these tissues (Figure 2E). Viral titers in serum and tissue homogenates were determined using Vero cells, as described in Ahmed et al. (J. Virol. 51:34-41, 1984). The results showing that a PD-1 inhibitor increases CD8+ T cell proliferation and viral clearance therefore indicate that the inhibition of PD-1 signaling restores CD8+ T cell function. Furthermore, inhibition of PD-1 signaling also enhanced B cell responses as the number of LCMV specific antibody secreting cells in the spleen was also increased (>10-fold) following treatment.

$CD4^+$ T cells play a key role in the generation and maintenance of CD8+ T cell responses. In this regard, CD8+ T cells primed in the absence of CD4+ T cell (so-called "helpless" CD8+ T cells) are incapable of mounting normal immune responses. Furthermore, chronic LCMV infection is more severe in the absence of CD4+ T cells. Accordingly, helpless T cells generated during LCMV-Cl-13 infection display an even more profound functional impairment than T cells generated in the presence of CD4+ T cells. $D^bNP396-404$ specific $CD8^+$ T cells are deleted to undetectable levels, and $D^bGP33-41$ and $D^bGP276-286$ $CD8^+$ T cells completely lose the ability to secrete IFN-γ and TNF-α.

$CD4^+$ T cells were depleted at the time of LCMV-Cl-13 infection and mice were treated with anti-PD-L1 antibodies treatment from day 46 to day 60 post-infection. LCMV-specific $CD4^+$ T cells were not detectable by intracellular IFN-γ staining before or after treatment. Following treatment, treated mice had approximately 7 fold more $D^bGP276-286$ $CD8^+$ T cells and 4 fold more $D^bGP33-41$ $CD8^+$ T cells in their spleen than untreated control mice (Figure 3A). The number of virus-specific $CD8^+$ T cells in the spleen was also increased (Figure 3B). This increase in virus-specific $CD8^+$ T cells in treated mice was attributed to an increase in proliferation, as detected by BrdU incorporation. 43% of $D^bGP276-286$ $CD8^+$ T cells incorporated intermediate levels of BrdU and 2% incorporated high levels of BrdU in untreated mice, while 50% $D^bGP276-286$ $CD8^+$ T cells incorporated intermediate levels of BrdU and 37% incorporated high levels of BrdU in treated mice. BrdU analysis was performed by introducing 1mg/ml BrdU in the drinking water during treatment and staining was performed according to the manufacturer's protocol (BD Biosciences, San Diego, CA). Moreover, treated mice contained a higher percentage of $CD8^+$ T cells expressing the cell cycle-associated protein Ki67 (60% versus 19% in untreated mice, Figure 3C). Response to treatment in $CD8^+$ T cells in the PBMC was restricted to mice having high levels of $CD8^+$ T cell expansion.

PD-1 inhibition also increased anti-viral cytokine production in helpless, exhausted virus-specific $CD8^+$ T cells. Following treatment, the number of $D^bGP33-41$ and $D^bGP276-286$ $CD8^+$ T cells that produce IFN-γ was markedly increased (Figure 4A), though higher numbers of $D^bNP396-404$, $K^bNP205-212$, $D^bNP166-175$, and $D^bGP92-101$ specific $CD8^+$ T cells were also detected in treated mice (Figure 4A). 50% of $D^bGP276-286$ specific $CD8^+$ T cells from treated mice can produce IFN-γ compared to the 20% of $D^bGP276-286$ specific $CD8^+$ T cells in control

untreated mice. (Figure 4B). Levels of IFN-γ and TNF-α produced by $D^bGP276-286$ specific $CD8^+$ T cells from treated mice, however, were lower than fully functional $D^bGP276-286$ specific memory cells. (Figure 4C).

PD-1 inhibition also increased the lytic activity of helpless, exhausted virus-specific $CD8^+$ T cells. *Ex vivo* lytic activity of virus-specific $CD8^+$ T cells was detected following treatment, using a ^{51}Cr release assay (Wherry et al., 2003. J. Virol. 77:4911-27). Viral titers were reduced by approximately 3 fold in the spleen, 4 fold in the liver, 2 fold in the lung, and 2 fold in serum after 2 weeks of treatment relative to untreated mice. (Figure 4E).

These results therefore demonstrate that blocking the PD-1 pathway breaks CTL peripheral tolerance to a chronic viral infection, and that exhausted $CD8^+$ T cells deprived of $CD4^+$ T cell help are not irreversibly inactivated.

Example 2: Administration of anti-viral vaccine and PD-1 inhibitor

One approach for boosting T cell responses during a persistent infection is therapeutic vaccination. The rationale for this approach is that endogenous antigens may not be presented in an optimal or immunogenic manner during chronic viral infection and that providing antigen in the form of a vaccine may provide a more effective stimulus for virus-specific T and B cells. Using the chronic LCMV model, mice were administered a recombinant vaccinia virus expressing the LCMV GP33 epitope as a therapeutic vaccine (VVGP33), which resulted in a modest enhancement of CD8+ T cell responses in some chronically infected mice Four out of the nine chronically infected mice that received the therapeutic vaccine showed a positive response while none of the control mice had a significant increase in the immune response against GP33. When this therapeutic vaccination was combined with a PD-L1 inhibitor, LCMV specific T cell responses were boosted to a greater level than compared to either treatment alone and the effect of combined treatment was more than additive.

Example 3: Inhibition of the PD-1 Pathway in Chronically-Infected Mice Using PD-1 RNAi

RNA interference (RNAi) is capable of silencing gene expression in mammalian cells. Long double stranded RNAS (dsRNAs) are introduced into cells and are next processed into smaller, silencing RNAs (siRNAs) that target specific mRNA molecules or a small group of mRNAs. This technology is particularly useful in situations where antibodies are not functional.

For example, RNAi may be employed in a situation in which unique splice variants produce soluble forms of PD-1 and CTLA-4.

PD-1 silencer RNAs are inserted into a commercially available siRNA expression vector, such as p*Silencer*™ expression vectors or adenoviral vectors (Ambion, Austin, TX). These vectors are then contacted with target exhausted T cells *in vivo* or *ex vivo* (See, Example 4).

Example 4: *Ex vivo* Rejuvenation of Exhausted T Cells

Virus-specific exhausted $CD8^+$ T cells are isolated from LCMV-Cl-13 chronically infected mice using magnetic beads or density centrifugation. Transfected $CD8^+$ T cells are contacted with a monoclonal antibody that targets PD-L1, PD-L2 or PD-1. As described in Example 1, inhibition of the PD-1 pathway results in the rejuvenation of the $CD8^+$ T cells. Accordingly, there is an increase in CD8+ T cell proliferation and cytokine production, for example. These rejuvenated $CD8^+$ T cells are reintroduced into the infected mice and viral load is measured as described in Example 1.

Example 5: *In vitro* Screening of Novel $CD8^+$ T Cell Rejuvenator Compounds

Compounds that modulate the PD-1 pathway can be identified in *in vivo* and *ex vivo* screening assays based on their ability to reverse $CD8^+$ T cell exhaustion resulting from chronic viral infection.

Exhausted $CD8^+$ T cells are derived from mice chronically infected with LCMV-Cl-13 and next contacted with a test compound. The amount of anti-viral cytokines (*e.g.*, IFN-γ or TNF-α) released from the contacted T cell is measured, for example, by ELISA or other quantitative method, and compared to the amount, if any, of the anti-viral cytokine released from the exhausted T cell not contacted with the test compound. An increase in the amount of anti-viral cytokine released by treated cells relative to such amount in untreated cells identifies the compound as a PD-1 inhibitor, useful to modulate T cell activity.

Example 6: *In vivo* Screening of Novel $CD8^+$ T Cell Rejuvenator Compounds

Exhausted $CD8^+$ T cells are derived from mice chronically infected with LCMV-Cl-13. A test compound is administered intravenously to the infected mice. The amount of anti-viral

cytokines (*e.g.*, IFN-γ or TNF-α) that is released into the serum of treated and untreated mice is measured, for example, by ELISA or other quantitative method, and compared. An increase in the amount of anti-viral cytokine found in the serum in treated mice relative to such amount in untreated mice identifies the test compound as a PD-1 inhibitor. Alternatively, the viral titer (*e.g.*, serum viral titer) can be determined prior and subsequent to treatment of the test compound.

Example 7: Chimpanzees as a Model for Immunotherapy of Persistent HCV Infection.

Chimpanzees provide a model of HCV persistence in humans. Defects in T cell immunity leading to life-long virus persistence both include a deficit in HCV-specific CD4+ T helper cells and impaired or altered CD8+ T effector cell activity. Persistently infected chimpanzees are treated with antibodies against CTLA-4, PD-1, or a combination of the two. The efficacy of blockade of the inhibitory pathways, combined with vaccination using recombinant structural and non-structural HCV proteins, and whether such strategies can enhance the frequency and longevity of virus-specific memory T cells are determined. The defect in T cell immunity is exclusively HCV-specific in persistently infected humans and chimpanzees. The blood and liver of infected chimpanzees are examined for expression of CTLA-4, PD-1, BTLA and their ligands and for the presence of Treg cells. Antiviral activity may then be restored by delivering to chimpanzees humanized monoclonal antibodies that block signaling through these molecules.

Persistently infected chimpanzees are treated with humanized αCTLA-4 antibodies (MDX-010, Medarex) or αPD-1 antibodies. The initial dose of MDX-010 is 0.3 mg/kg followed 2 weeks later by 1.0mg/kg and then 3, 10, 30 mg/kg at three week intervals. After treatment with antibodies to co-inhibitory molecules, the humoral and cellular immune responses as well as the HCV RNA load will be determined. Samples are collected at weeks 1, 2, 3, 5, and 8, and then at monthly intervals. Samples include: 1) serum for analysis of transaminases, autoantibodies, neutralizing antibodies to HCV, and cytokine responses, 2) plasma for viral load and genome evolution, 3) PBMC for in vitro measures of immunity, costimulatory/inhibitory receptor expression and function, 4) fresh (unfixed) liver for isolation of intrahepatic lymphocytes and RNA, and 5) fixed (formalin/paraffin embedded) liver for histology and immunohistochemical

analysis. Regional lymph nodes are also collected at 2 or 3 time points to assess expression of co-inhibitory molecules and splice variants by immunohistochemistry and molecular techniques. Assays to evaluate the efficacy and safety of these therapies safety will be performed as described herein.

To determine if vaccination with HCV antigens potentiates the therapeutic effect of antibodies to PD-1, chimpazees are treated as follows: 1) intramuscular immunization with recombinant envelope glycoproteins E1 and E2 (in MF59 adjuvant) and other proteins (core plus NS 3, 4, and 5 formulated with ISCOMS) at weeks 0, 4, and 24; 2) intramuscular immunization with the vaccine used in 1) but co-administered with αCTLA-4 antibodies (30 mg of each/Kg body weight, intravenously at weeks 0, 4, and 24 when vaccine is given); 3) identical to 2) except that αPD-1 (or BTLA) antibodies are substituted for the CTLA-4 antibodies; 4) identical to Groups 2 and 3 except that a combination of CTLA-4 and PD-1 (or BTLA) antibodies are used in addition to the vaccine. HCV-specific T and B cell responses are monitored at monthly intervals after immunization for a period of 1 year.

Markers examined on HCV-tetramer+ and total T cells in this analysis include markers of differentiation (e.g. CD45RA/RO, CD62L, CCR7, and CD27), activation (e.g. CD25, CD69, CD38, and HLA-DR), survival/proliferation (e.g. bcl-2 and Ki67), cytotoxic potential (e.g. granzymes and perforin), and cytokine receptors (CD122 and CD127). An interesting correlation exists between pre-therapy levels of the chemokine IP-10 and response to PEG IFN-γ/ribavirin. IP-10 levels are measured to investigate a potential correlation between negative regulatory pathways or HCV-specific T cell responses and IP-10 levels. Expression of inhibitory receptors and ligands on PBMC are performed by flow cytometry.

Example 8: PD-1 Immunostaining in Reactive Lymphoid Tissue

Materials

Case material was obtained from the Brigham & Women's Hospital, Boston, MA, in accordance with institutional policies. All diagnoses were based on the histologic and immunophenotypic features described in the World Health Organization Lymphoma Classification system (Jaffe ES, et al. 2001) and in all cases diagnostic material was reviewed by a hematopathologist.

Immunostaining

Immunostaining for PD-1 was performed on formalin-fixed paraffin embedded tissue sections following microwave antigen retrieval in 10 mM citrate buffer, pH 6.0 with a previously described anti-human PD-1 monoclonal antibody (2H7; 5), using a standard indirect avidin-biotin horseradish peroxidase method and diaminobenzidine color development, as previously described (Jones D, et al. 1999; Dorfman DM, et al. 2003). Cases were regarded as immunoreactive for PD-1 if at least 25% of neoplastic cells exhibited positive staining. PD-1 staining was compared with that of mouse IgG isotype control antibody diluted to identical protein concentration for all cases studied, to confirm staining specificity.

Monoclonal antibody 2H7 for PD-1 was used to stain formalin-fixed, paraffin-embedded specimens of reactive lymphoid tissue, thymus, and a range of cases of B cell and T cell lymphoproliferative disorders. In specimens of tonsil exhibiting reactive changes, including follicular hyperplasia, a subset of predominantly small lymphocytes in the germinal centers exhibited cytoplasmic staining for PD-1, with infrequent PD-1-positive cells seen in the interfollicular T cell zones. The PD-1 staining pattern in germinal centers was virtually identical to that seen with an antibody to CD3, a pan-T cell marker, whereas an antibody to CD20, a pan-B cell marker, stained the vast majority of germinal center B cells. Similar results were seen in histologic sections of reactive lymph node and spleen. No PD-1 staining was observed in adult thymus.

Example 9: PD-1 Immunostaining in Paraffin Embedded Tissue Sections of B Cell and T Cell Lymphoproliferative Disorders

A range of B cell and T cell lymphoproliferative disorders for PD-1 expression were studied; the results are summarized in Table 1. Forty-two cases of B cell lymphoproliferative disorders were examined for PD-1 expression, including representative cases of precursor B lymphoblastic leukemia/lymphoblastic lymphoma, as well as a range of lymphoproliferative disorders of mature B cells, including a number of B cell non-Hodgkin lymphomas of follicular origin, including 6 cases of follicular lymphoma and 7 cases of Burkitt lymphoma. None of the B cell lymphoproliferative disorders showed staining for PD-1. In some cases, non-neoplastic reactive lymphoid tissue was present, and showed a PD-1 staining pattern as seen in tonsil and other reactive lymphoid tissue noted above.

Similarly, in 25 cases of Hodgkin lymphoma, including 11 cases of classical Hodgkin lymphoma and 14 case of lymphocyte predominant Hodgkin lymphoma, the neoplastic cells did

not exhibit staining for PD-1. Interestingly, in all 14 cases of lymphocyte predominant Hodgkin lymphoma, the T cells surrounding neoplastic CD20-positive L&H cells were immunoreactive for PD-1, similar to the staining pattern noted for CD57+ T cells in lymphocyte predominant Hodgkin lymphoma. These PD-1-positive cells were a subset of the total CD3+ T cell population present.

A range of T cell lymphoproliferative disorders were studied for expression of PD-1; the results are summarized in Table 1. Cases of precursor T cell lymphoblastic leukemia/lymphoblastic lymphoma, a neoplasm of immature T cells of immature T cells, were negative for PD-1, as were neoplasms of peripheral, post-thymic T cells, including cases of T cell prolymphocytic leukemia, peripheral T cell lymphoma, unspecified, anaplastic large cell lymphoma, and adult T cell leukemia/lymphoma. In contrast, all 19 cases of angioimmunoblastic lymphoma contained foci of PD-1-positive cells that were also immunoreactive for pan-T cell markers such as CD3. PD-1-positive cells were consistently found at foci of expanded CD21+ follicular dendritic cells (FDC) networks, a characteristic feature of angioimmunoblastic lymphoma.

TABLE 1. PD-1 immunostaining in lymphoproliferative disorders.

	PD-1 immunostaining
B cell LPDs	0/42*
B-LL/LL	0/3
CLL	0/4
MCL	0/4
FL	0/6
MZL	0/3
HCL	0/3
DLBCL	0/6
BL	0/7
LPL	0/3
MM	0/3
Hodgkin lymphoma	0/25
Classical	0/11
Nodular lymphocyte predominant	0/14**
T cell LPDs	18/55

T-LL/LL	0/5
T-PLL	0/3
AIL	19/19
PTCL, unspecified	0/14
ALCL	0/12
ATLL	0/3

Abbreviations: B-LL/LL – precursor B cell lymphoblastic lymphoma/lymphoblastic leukemia; CLL – chronic lymphocytic leukemia; MCL – mantle cell lymphoma; FL – follicular lymphoma; MZL – marginal zone lymphoma; HCL – hairy cell leukemia; DLBCL – diffuse large B cell lymphoma; BL – Burkitt lymphoma; LPL – lymphoplasmacytic lymphoma; MM – multiple myeloma; T-LL/L – precursor T lymphoblastic leukemia/lymphoblastic lymphoma; T-PLL- T cell prolymphocytic leukemia; AIL – angioimmunoblastic lymphoma; PTCL – peripheral T cell lymphoma, unspecified; ALCL – anaplastic large cell lymphoma; ATLL- adult T cell leukemia/lymphoma.

* number of immunoreactive cases/total number of cases

** PD-1-positive cells form rosettes around neoplastic L&H cells in 14/14 cases

Example 10: General Methods for Studying PD-1 expression on HIV-Specific human CD8 T Cells

The following methods were used to perform the experiments detailed inn Examles 11-14.

Study subjects

Study participants with chronic clade C HIV-1 infection were recruited from outpatient clinics at McCord Hospital, Durban, South Africa, and St. Mary's Hospital, Mariannhill, South Africa. Peripheral blood was obtained from 65 subjects in this cohort, all of whom were antiretroviral therapy naïve at the time of analysis. Subjects were selected for inclusion based on their expressed HLA alleles matching the ten class I tetramers that were constructed (see below). The median viral load of the cohort was 42,800 HIV-1 RNA copies/ml plasma (range 163 – 750,000), and the median absolute CD4 count was 362 (range 129 – 1179). Information regarding duration of infection was not available. All subjects gave written informed consent for the study, which was approved by local institutional review boards.

Construction of PD-1 and PD-L1 antibodies

Monoclonal antibodies to human PD-L1 (29E.2A3, mouse IgG2b) and PD-1 (EH12, mouse IgG1) were prepared as previously described and have been shown to block the PD-1:PD-L1 interaction.

MHC class I tetramers

Ten HIV MHC Class I tetramers, synthesized as previously described (Altman JD, et al. 1996), were used for this study: A*0205 GL9 (p24, GAFDLSFFL; SEQ ID NO:1), A*3002 KIY9 (Integrase, KIQNFRVYY; SEQ ID NO:2), B*0801 DI8 (p24, DIYKRWII; SEQ ID NO:3), B*0801 FL8 (Nef, FLKEKGGL; SEQ ID NO:4), B*4201 RM9 (Nef, RPQVPLRPM; SEQ ID NO:5), B*4201 TL9 (p24, TPQDLNTML; SEQ ID NO:6), B*4201 TL10 (Nef, TPGPGVRYPL; SEQ ID NO:7), B*4201 YL9 (RT, YPGIKVKQL; SEQ ID NO:8), B*8101 TL9 (p24, TPQDLNTML; SEQ ID NO:9), and Cw0304 YL9 (p24, YVDRFFKTL; SEQ ID NO:10). The plasmid constructs expressing A*0205, A*3002, and Cw*0304 were kindly provided by Drs. Eugene Ravkov and John Altman, NIH Tetramer Core Facility, Atlanta, Georgia.

HLA class I tetramer staining and phenotypic analysis

Freshly isolated peripheral blood mononuclear cells (PBMC, 0.5 million) were stained with tetramer for 20 minutes at 37°C. The cells were then washed once with phosphate buffered saline (PBS), pelleted, and stained directly with fluorescein isothiosyanate (FITC)-conjugated anti-CD8 (Becton Dickinson), phycoerythrin-conjugated anti-PD-1 (clone EH12), and ViaProbe (Becton Dickinson). Cells were incubated for 20 minutes at room temperature, washed once in PBS, and resuspended in 200 µl PBS with 1% paraformaldehyde and acquired on a fluorescence-activated cell sorter (FACSCalibur, Becton Dickinson). A minimum of 100,000 events were acquired on the FACSCalibur.

CFSE proliferation assays

One million freshly isolated PBMC were washed twice in PBS, pelleted, and resuspended in 1 ml of 0.5 µM carboxy-fluorescein diacetate, succinimidyl ester (CFSE, Molecular Probes) for 7 minutes at 37°C. The cells were washed twice in PBS, resuspended in 1 ml R10 medium (RPMI 1640 supplemented with glutiathione, penicillin, streptomycin, and 10% fetal calf serum [FCS]), and plated into one well of a 24-well plate. Initial studies revealed that a final concentration of 0.2 µg/ml peptide yielded optimal proliferative responses, therefore this was the final peptide concentration in the well used for each assay. Negative control wells consisted of PBMC in medium alone, or PBMC in medium with purified anti-PD-L1 (10 µg/ml), and positive control wells were stimulated with 10 µg/ml of phytohemagluttinin (PHA). Following 6-day

incubation in a 37°C incubator, the cells were washed with 2 ml PBS and stained with PE-conjugated MHC Class I tetramers, ViaProbe (Becton Dickinson), and anti-CD8-APC antibodies. Cells were acquired on a FACSCalibur and analyzed by CellQuest® software (Becton Dickinson). Cells were gated on ViaProbe$^-$ CD8$^+$ lymphocytes. The fold increase in tetramer$^+$ cells was calculated by dividing the percentage of CD8$^+$ tetramer$^+$ cells in the presence of peptide by the percentage of CD8$^+$ tetramer$^+$ cells in the absence of peptide stimulation.

Statistical Analysis

Spearman correlation, Mann-Whitney test, and paired *t*-test analyses were performed using GraphPad Prism Version 4.0a. All tests were 2-tailed and p values of $p<0.05$ were considered significant.

Example 11: PD-1 Expression on HIV-Specific CD8 T Cells

A panel of 10 MHC Class I tetramers specific for dominant HIV-1 clade C virus CD8 T cell epitopes was synthesized, based on prevalent HLA alleles and frequently targeted epitopes in Gag, Nef, Integrase, and RT (Kiepiela P, et al. 2004), allowing direct visualization of surface PD-1 expression on these cells. High resolution HLA typing was performed on the entire cohort, and a subset of 65 antiretroviral therapy naive persons was selected for study based on expression of relevant HLA alleles. A total of 120 individual epitopes were examined, and representative ex vivo staining of PD-1 on HIV tetramer$^+$ cells is shown in Figure 5A. PD-1 expression was readily apparent on these tetramer$^+$ cells, and was significantly higher than in the total CD8 T cell population from the same individuals ($p<0.0001$); in turn, PD-1 expression on both tetramer$^+$ CD8 T cells and the total CD8 T cell population was significantly higher than in HIV-seronegative controls (Figure 5B). For eight of the ten tetramers tested at least one person was identified in whom the level of expression on antigen-specific CD8 cells was 100% (Figure 5C). PBMC from 3 to 25 individuals were stained for each HIV tetramer response, with median PD-1 expression levels ranging from 68% to 94% of tetramer$^+$ cells (Figure 5C). These findings were further confirmed by analysis of the mean fluorescence intensity (MFI) of PD-1 on both tetramer$^+$ cells and the total CD8 T cell population (Figure 5B,C).

It was next determined whether there was evidence for epitope-specific differences in terms of PD-1 expression levels in persons with multiple detectable responses. Of the 65 persons examined, 16 individuals had between 3 and 5 tetramer positive responses each. PD-1 expression was nearly identical and approaching 100% for each response analyzed for three of

the sixteen subjects; however, the other 13 individuals displayed different patterns of PD-1 expression depending on the epitope (Figure 5D). These data indicate that PD-1 expression may be differentially expressed on contemporaneous epitope-specific CD8 T cells from a single person, perhaps consistent with recent data indicating epitope-specific differences in antiviral efficacy (Tsomides TJ, et al. 1994; Yang O, et al. 1996; Loffredo JT, et al. 2005).

Example 12: The Relationship Between PD-1 Expression and HIV Disease Progression

The relationship was determined between PD-1 expression on HIV-specific CD8 T cells and plasma viral load and CD4 counts, both of which are predictors of HIV disease progression. Consistent with previous studies, the relationship between the number of tetramer positive cells and viral load or CD4 count failed to show any significant correlation (Figure 6A, B). In contrast, there were significant positive correlations with viral load and both the percentage and MFI of PD-1 expression on HIV tetramer positive cells ($p=0.0013$ and $p<0.0001$, respectively; Figure 6A). There were also inverse correlations between CD4 count and both the percentage and MFI of PD-1 on HIV tetramer positive cells ($p=0.0046$ and $p=0.0150$, respectively; Figure 6B). Since the tetramers tested likely represent only a fraction of the HIV-specific CD8 T cell population in these subjects, the relationship between PD-1 expression on all CD8 cells and these parameters was also examined. There were significant positive correlations between viral load and both the percentage and MFI of PD-1 expression on the total CD8 T cell population ($p=0.0021$ and $p<0.0001$, respectively; Figure 6C), and inverse correlations were also observed between CD4 count and both the percentage and MFI of PD-1 expression on the total CD8 T cell population ($p=0.0049$ and $p=0.0006$, respectively; Figure 6D). In this same group, PD-1 expression on CMV-specific CD8 T cells was tested in 5 subjects, and significantly less PD-1 was expressed on these cells compared to HIV-specific CD8 T cells (median 23% CMV tetramer$^+$ PD-1$^+$, $p=0.0036$, data not shown), and was not different than bulk CD8 T cells in these same individuals, indicating that high PD-1 expression is not a uniform feature of all virus-specific CD8 T cells. These data suggest increasing amounts of antigen in chronic HIV infection result in increased expression of PD-1 on CD8 T cells, and are consistent with murine data in chronic LCMV infection, in which PD-1 expression is associated with functional exhaustion of CD8 T cells (Barber DL, et al. 2005). Moreover, they provide the first clear association, in a

large study including analysis of multiple epitopes, between HIV-specific CD8 T cells and either viral load or CD4 count.

Example 13: The Relationship Between PD-1 Expression and CD8 T Cell Memory Status and Function

PD-1 expression was next analyzed in the context of a number of additional phenotypic markers associated with CD8 T cell memory status and function, including CD27, CD28, CD45RA, CD57, CD62L, CD127, CCR7, perforin, granzyme B, and Ki67 (Figure 7). Representative stainings for these markers on B*4201 TL9 tetramer$^+$ cells from one individual are shown in Figure 7A, and aggregate data for 13 subjects are shown in Figure 7B. These studies were limited to those tetramer responses that were greater than 95% PD-1 positive, as multiparameter flow cytometry of greater than 4 colors was not available in KwaZulu Natal. The HIV tetramer$^+$ PD-1$^+$ cells express high levels of CD27 and granzyme B, very low levels of CD28, CCR7, and intracellular Ki67, low levels of CD45RA and perforin, and intermediate levels of CD57 and CD62L (Figure 7B). These data indicate that HIV-specific PD-1$^+$ T cells display an effector/effector memory phenotype, and are consistent with previous reports of skewed maturation of HIV-specific CD8 T cells (Champagne P, et al. 2001; Appay V, et al. 2002; Hess C, et al. 2004). In addition, virus sequencing was performed to determine whether these cells were driving immune escape (Brown JA, et al. 2003). Of 45 of these tetramer-positive responses evaluated, the viral epitopes in only 5 were different from the South African clade C consensus sequence (data not shown), indicating these cells exert little selection pressure in vivo.

Previous experiments in mice using the LCMV model showed that in vivo blockade of PD-1/PD-L1 interaction by infusion of anti-PD-L1 blocking antibody results in enhanced functionality of LCMV-specific CD8 T cells as measured by cytokine production, killing capacity, proliferative capacity, and, most strikingly, reduction in viral load (Barber DL, et al. 2005).

Example 14: Effect of Blockading the PD-1/PD-L1 Pathway on Proliferation of HIV-Specific CD8 T Cells

Because HIV-specific CD8 T cells also exhibit impaired proliferative capacity (Migueles SA, et al. 2002; Lichterfeld M, et al. 2004), it was determined whether blockade of the PD-1/PD-L1 could enhance this function in vitro. Representative data from a B*4201-positive individual are shown in Figure 8A. Incubation of freshly isolated CFSE-labeled PBMC with medium alone,

or medium with anti-PD-L1 antibody, resulted in maintenance of a population of B*4201-TL9-specific CD8 T cells (1.2% of CD8 T cells) that remained $CFSE^{hi}$ after six days in culture. Simulation of CFSE-labeled PBMC for 6 days with TL9 peptide alone resulted in a 4.8-fold expansion of $CFSE^{lo}$ B*4201 TL9 $tetramer^+$ cells, whereas stimulation of CFSE-labeled PBMC with TL9 peptide in the presence of anti-PD-L1 blocking antibody further enhanced proliferation of TL9-specific cells, resulting in a 10.3-fold increase in $tetramer^+$ cells. CFSE proliferation assays were performed on 28 samples in the presence and absence of purified anti-human PD-L1 blocking antibody. A significant increase in the proliferation of HIV-specific $CD8^+$ T cells was observed in the presence of peptide plus anti-PD-L1 blocking antibody as compared to the amount of proliferation following stimulation with peptide alone (Figure 8B; p=0.0006, paired *t*-test). The fold increase of $tetramer^+$ cells in the presence of anti-PD-L1 blocking antibody varied by individual and by epitope within a given individual (Figure 8C), again suggesting epitope-specific differences in the degree of functional exhaustion of these responses.

OTHER EMBODIMENTS

While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

Claims

1. A method of alleviating or preventing a symptom of persistent infection or cancer comprising administering to a subject in need thereof a compound that reduces the activity or expression of a Programmed Cell Death-1 (PD-1) polypeptide in said subject.

2. The method of claim 1, wherein said persistent infection is a viral infection, a bacterial infection, a fungal infection, a mycoplama infection, or a parasitic infection.

3. The method of claim 2, wherein said viral infection is an infection with a hepatitis virus, a human immunodeficiency virus (HIV), a human T-lymphotrophic virus (HTLV), a herpes virus, an Epstein-Barr virus, or a human papilloma virus.

4. The method of claim 2, wherein said parasitic infection is malaria.

5. The method of claim 2, wherein said bacterial infection is tuberculosis

6. The method of claim 1, wherein said cancer is angioimmunoblastic lymphoma or nodular lymphocyte predominant Hodgkin lymphoma.

7. The method of claim 1, wherein said compound reduces the expression or activity of a PD-1 ligand (PD-L1) or a PD-1 ligand 2 (PD-L2).

8. The method of claim 1, wherein said compound reduces the interaction between PD-1 and PD-L1 or the interaction between PD-1 and PD-L2.

9. The method of claim 1, wherein said compound increases the cytotoxic T-cell activity in said subject.

10. The method of claim 9, wherein said cytotoxic T-cell activity is cytokine production, T cell proliferation, or infectious agent clearance.

11. The method of claim 10, wherein said infectious agent is a virus, a bacterium, a fungus, a mycoplasm, or a parasite.

12. The method of claim 9, wherein said T-cell is a cancer specific T-cell.

13. The method of claim 10, wherein said cytokine is IFNγ, TNFα, or IL-2.

14. The method of claim 1, wherein said compound is an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-PD-1 RNAi, an anti-PD-L1 RNAi, an anti-PD-L2 RNAi, an anti-PD-1 antisense RNA, an anti-PD-L1 antisense RNA, an anti-PD-L2 antisense RNA, a dominant negative PD-1 protein, a dominant negative PD-L1 protein, or a dominant negative PD-L2 protein.

15. The method of claim 12, wherein said antibody is a monoclonal antibody, a humanized antibody, a deimmunized antibody, or an Ig fusion protein.

16. The method of claim 1, wherein said compound is a small molecule.

17. The method of claim 1, further comprising administering to said subject a vaccine.

18. The method of claim 17, wherein said vaccine comprises an adjuvant or a prime booster shot.

19. The method of claim 1, further comprising administering to said subject a second compound.

20. The method of claim 19, wherein said second compound is an antiviral compound, an antibacterial compound, an antifungal compound, an antiparasitic compound, an anti-inflammatory compound, an antineoplastic compound or an analgesic.

21. The method of claim 19, wherein said second compound reduces the expression or activity of cytotoxic T lymphocyte antigen 4 (CTLA-4) or B and T lymphocyte attenuator (BTLA).

22. The method of claim 19, wherein said second compound reducing cancer cell volume.

23. The method of claim 19, wherein said second compound is an anti-CTLA-4 antibody, an anti-BTLA antibody, an anti-CD28 antibody, an anti-ICOS antibody, an anti-ICOS-L antibody, an anti-B7-1 antibody, an anti-B7-2 antibody, an anti-B7-H3 antibody, or an anti-B7-H4 antibody.

24. The method of claim 1, wherein said subject is a human.

25. A method of alleviating or preventing a symptom of a persistent infection or cancer comprising administering to a subject in need thereof a compound that reduces the activity or expression of a member of the CD28-like family, wherein said CD28-like family member is selected from the group consisting of PD-1, CTLA-4, BTLA, and a functional fragment or variant thereof.

26. The method of claim 25, wherein said persistent infection is a viral infection.

27. The method of claim 26, wherein said viral infection is an infection with a hepatitis virus, a human immunodeficiency virus (HIV), a human T-lymphotrophic virus (HTLV), a herpes virus, an Epstein-Barr virus, or a human papilloma virus.

28. The method of claim 25, wherein said compound increases the cytotoxic T cell activity in said subject.

29. The method of claim 28, wherein said cytotoxic T cell activity is cytokine production, T cell proliferation, or infectious agent clearance.

30. The method of claim 25, wherein said compound is an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-CTLA-4 antibody, an anti-BTLA antibody, an anti-CD28 antibody, an anti-ICOS antibody, an anti-ICOS-L antibody, an anti-B7-1 antibody, an anti-B7-2 antibody, an anti-B7-H3 antibody, or an anti-B7-H4 antibody.

31. The method of claim 25, further comprising administering to said subject a vaccine.

32. The method of claim 25, further comprising administering to said subject a second compound.

33. A method of alleviating or preventing a symptom of a persistent infection or cancer in a subject comprising administering to said subject an antigen-specific T cell that has been contacted with an compound that reduces the expression or activity of a PD-1 polypeptide in said cell.

34. The method of claim 33, wherein subject is further administered an antigen specific B cell that has been contacted with a compound that reduces the expression or activity of a PD-1 polypeptide in said cell.

35. The method of claim 33, wherein said T cell is from an autologous source.

36. The method of claim 33, wherein said T cell is derived from a subject of the same species as said subject.

37. The method of claim 33, wherein said T cell is derived from a subject of a different species than said subject being treated.

38. The method of claim 33, wherein said antigen-specific T cell is specific for a viral antigen, bacterial antigen, a fungal antigen or a parasitic antigen or a cancer antigen.

39. The method of claim 38, wherein said viral antigen is an antigen of a hepatitis virus, a human immunodeficiency virus (HIV), a human T-lymphotrophic virus (HTLV), a herpes virus, an Epstein-Barr virus, or a human papilloma virus.

40. A method of increasing the cytotoxic activity of a T cell comprising contacting said T cell with a compound that reduces the activity or expression of a PD-1 polypeptide.

41. The method of claim 40, wherein said biological activity is reduced by reducing the expression or activity of PD-1 ligand (PD-L1) or PD-1 ligand 2 (PD-2L).

42. The method of claim 40, wherein said compound reduces the interaction between PD-1 and PD-L1 or the interaction between PD-1 and PD-L2.

43. The method of claim 40, wherein said cytotoxic activity is cytokine production, T cell proliferation, or infectious agent clearance.

44. The method of claim 40, wherein said compound is an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-PD-1 RNAi, an anti-PD-L1 RNAi, an anti-PD-L2 RNAi, an anti-PD-1 antisense RNA, an anti-PD-L1 antisense RNA, an anti-PD-L2 antisense RNA, a dominant negative PD-1 protein, a dominant negative PD-L1 protein, or a dominant negative PD-L2 protein.

45. The method of claim 40, further comprising contacting said cell with an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-CTLA-4 antibody, an anti-BTLA antibody, an anti-CD28 antibody, an anti-ICOS antibody, an anti-ICOS-L antibody, an anti-B7-1 antibody, an anti-B7-2 antibody, an anti-B7-H3 antibody, or an anti-B7-H4 antibody.

46. A method of diagnosing a subject as having or at risk of having a persistent infection or cancer comprising:

(a) providing a sample from said subject, said sample comprising immune cells; and

(b) measuring the expression or activity of PD-1 in said sample from said subject, wherein an increase in expression or activity of PD-1 compared to such expression or activity in a control sample identifies said subject as having or at risk of having a persistent infection or cancer.

47. A method of selecting a treatment for a subject having or at risk of having a persistent infection or cancer comprising the steps of:

(a) providing a sample from said subject comprising immune cells;

(b) measuring the expression or activity of PD-1 in said immune cells, wherein an increase in expression or activity of PD-1 compared to such expression or activity in a control sample identifies said subject as having or at risk of having a persistent infection or cancer.;

(c) selecting a treatment for the subject diagnosed as having or at risk a persistent infection or cancer, wherein said treatment comprises a compound that reduces the expression or activity of PD-1.

48. The method of claim 46 or 47, wherein said step (b) further comprises identifying antigen-specific immune cells, wherein said antigen is a viral antigen, bacterial antigen, parasitic antigen, or fungal antigen.

49. The method of claim 46 or 47, wherein said sample is a blood sample, a tissue biopsy, or a bone marrow sample.

50. The method of claim 46 or 47, wherein said control cells are derived from a subject that does not have or at risk of having a persistent infection or cancer.

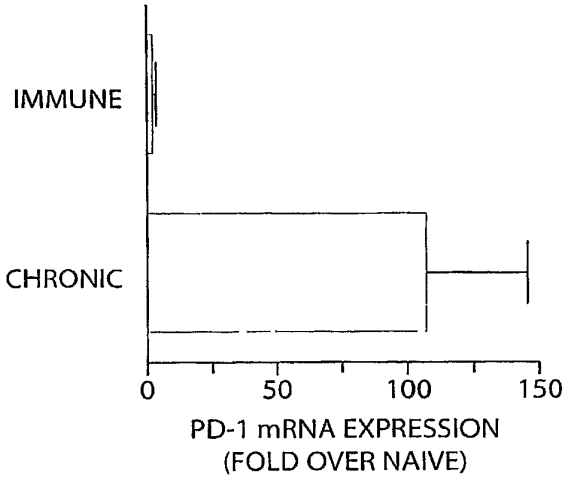

IMMUNE
CHRONIC
0
50
100
150
PD-1 mRNA EXPRESSION
(FOLD OVER NAIVE)

Fig. 1A

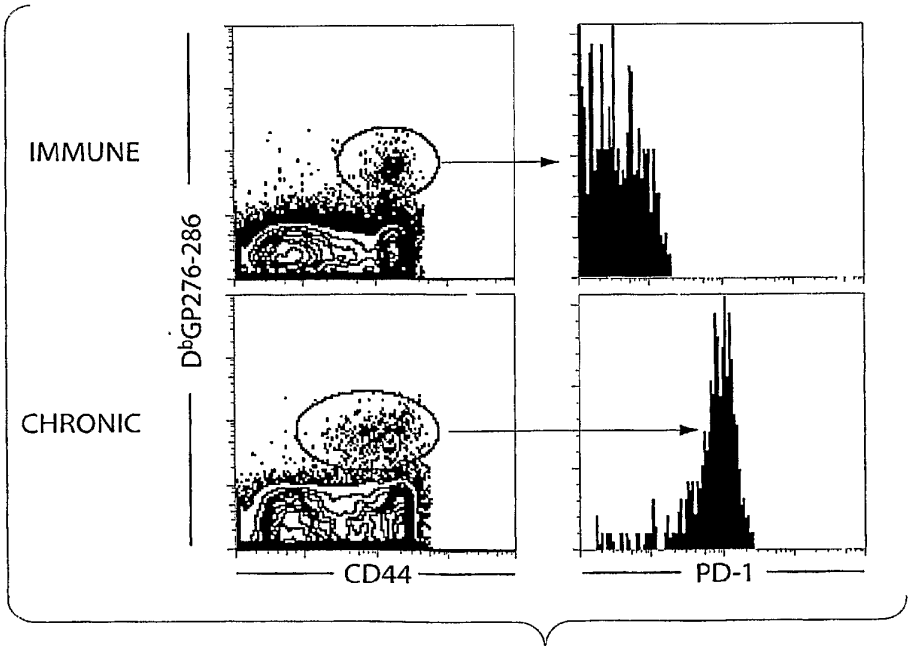

IMMUNE
CHRONIC
DbGP276-286
CD44
PD-1

Fig. 1B

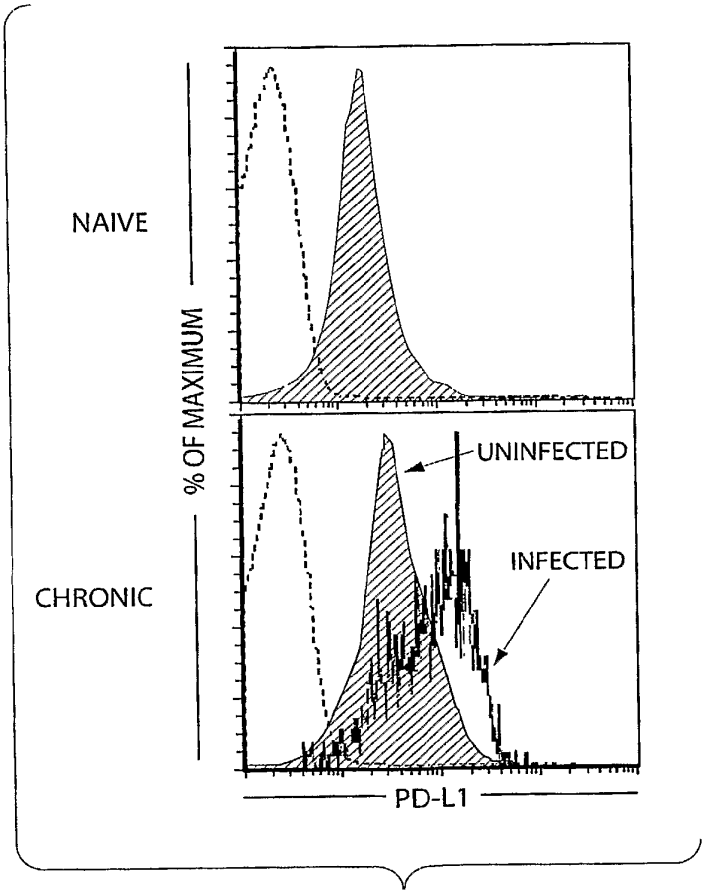


Fig. 1C

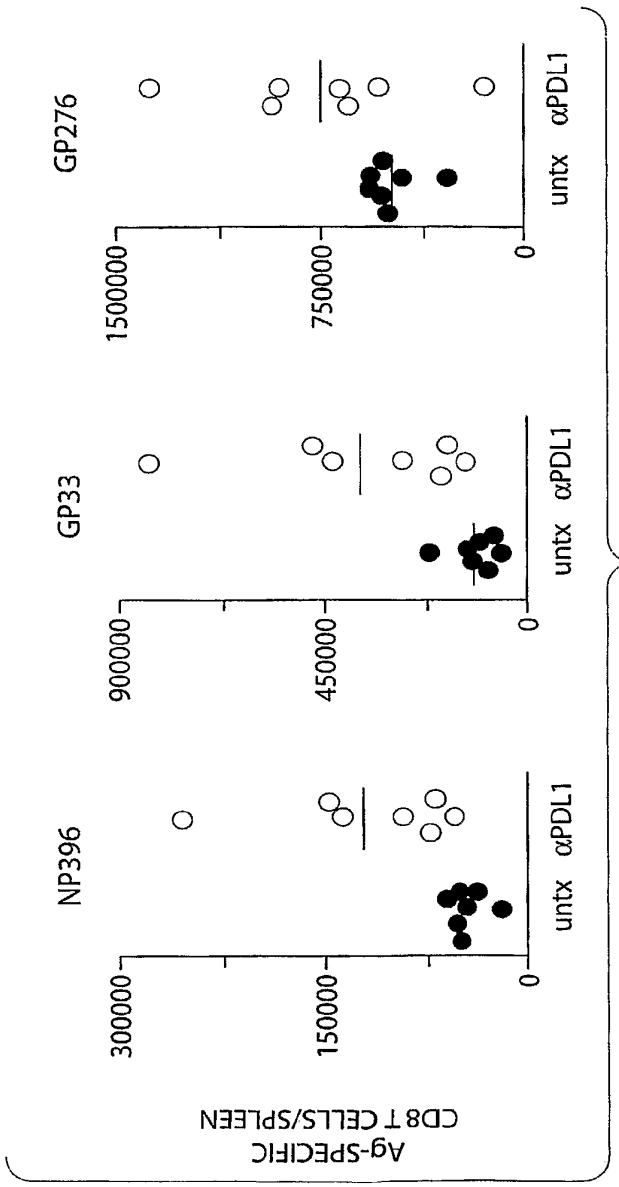

Ag-SPECIFIC
CD8 T CELLS/SPLEEN
NP396
GP33
GP276
300000
150000
0
900000
450000
0
1500000
750000
0
untx
αPDL1

Fig. 2A

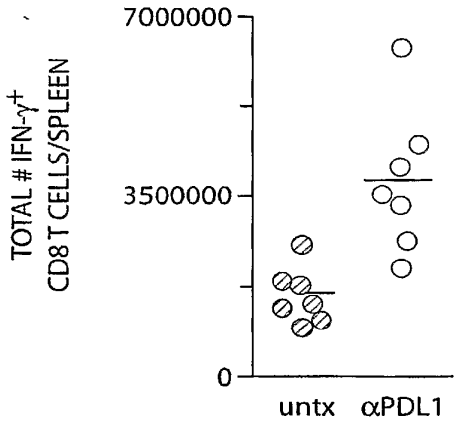


Fig. 2B

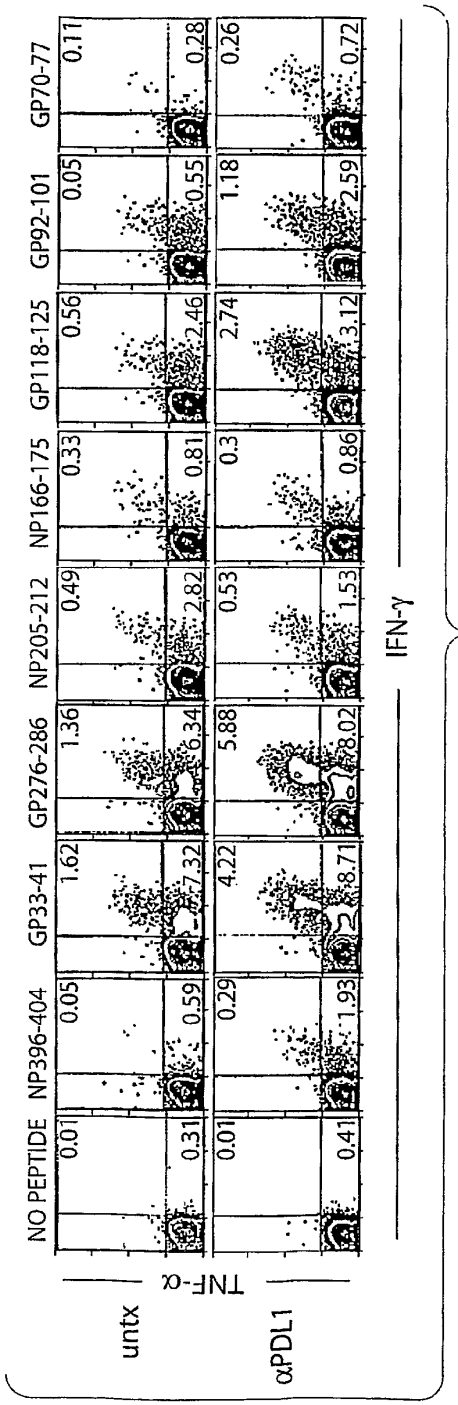


Fig. 2C

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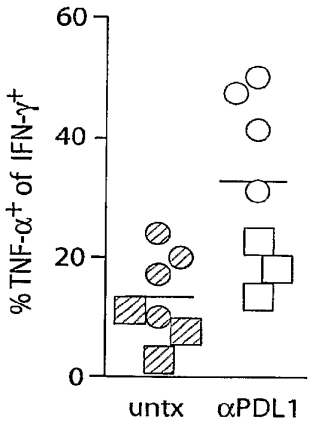


Fig. 2D

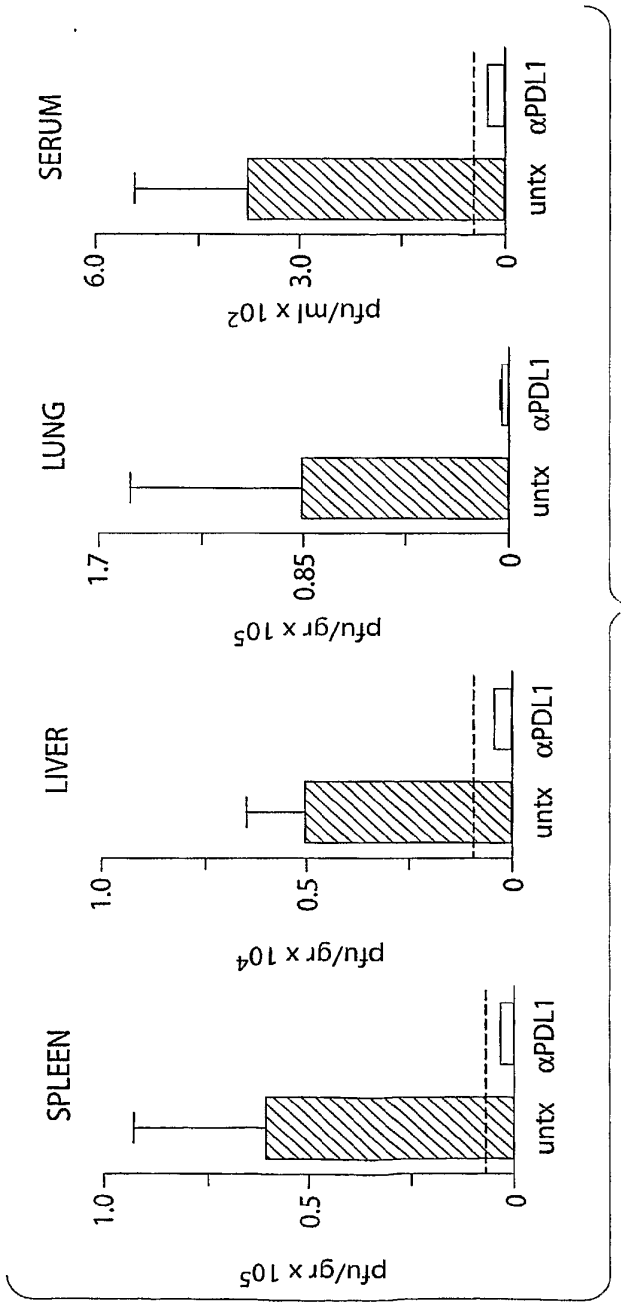

SERUM
pfu/ml x 10²
6.0
3.0
0
untx
αPDL1
LUNG
pfu/gr x 10⁵
1.7
0.85
0
untx
αPDL1
LIVER
pfu/gr x 10⁴
1.0
0.5
0
untx
αPDL1
SPLEEN
pfu/gr x 10⁵
1.0
0.5
0
untx
αPDL1

Fig. 2E

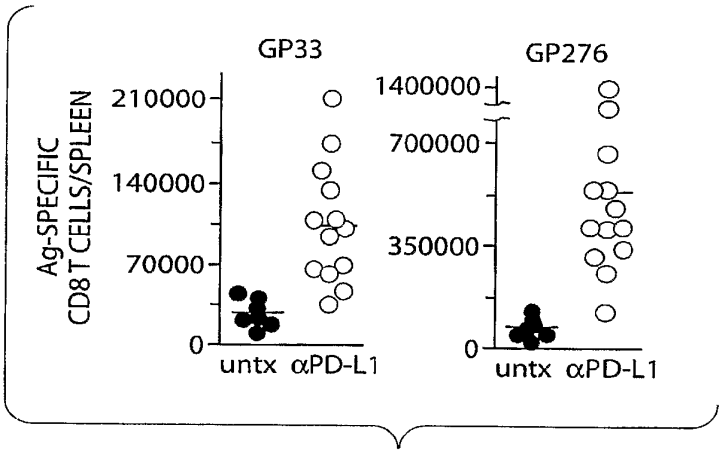

GP33
GP276
Ag-SPECIFIC CD8 T CELLS/SPLEEN
210000
140000
70000
0
1400000
700000
350000
0
untx
αPD-L1
untx
αPD-L1

Fig. 3A

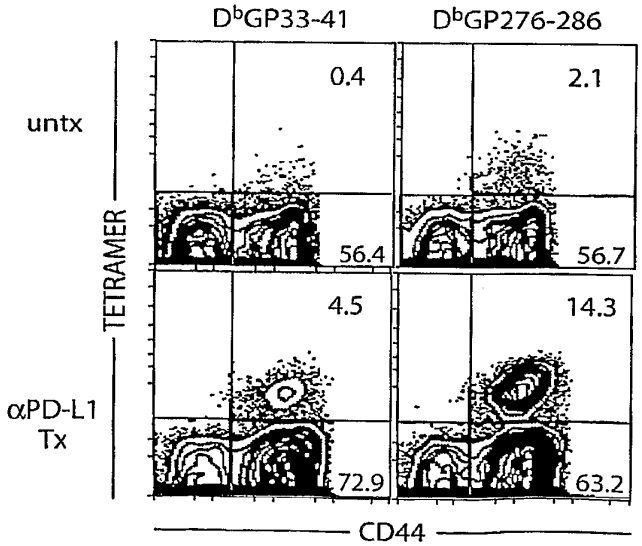

DbGP33-41
DbGP276-286
untx
αPD-L1 Tx
TETRAMER
0.4
2.1
56.4
56.7
4.5
14.3
72.9
63.2
CD44

Fig. 3B

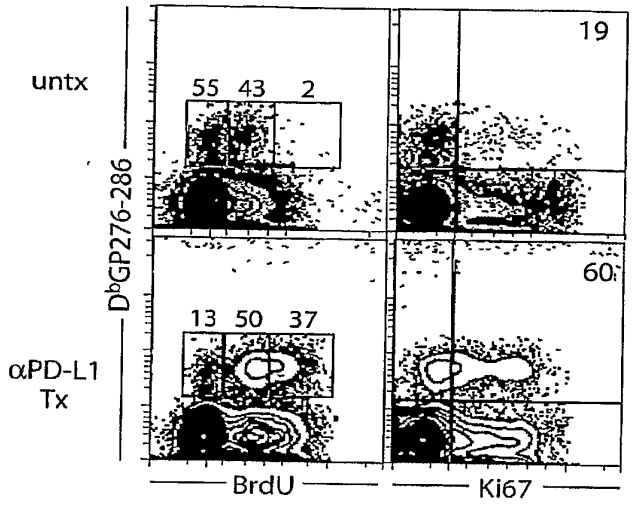

untx
αPD-L1
Tx
DbGP276-286
55 43 2
19
13 50 37
60
BrdU
Ki67

Fig. 3C

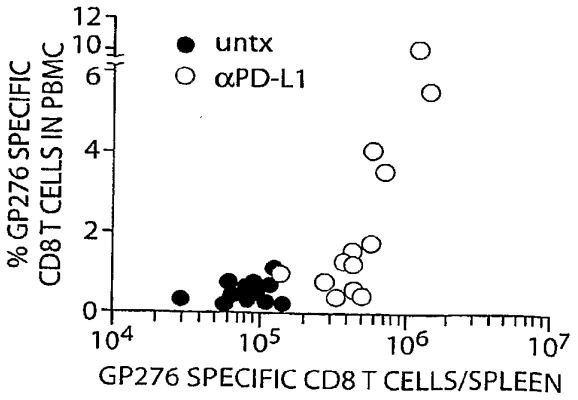

untx
αPD-L1
% GP276 SPECIFIC CD8 T CELLS IN PBMC
12
10
6
4
2
0
10^4
10^5
10^6
10^7
GP276 SPECIFIC CD8 T CELLS/SPLEEN

Fig. 3D

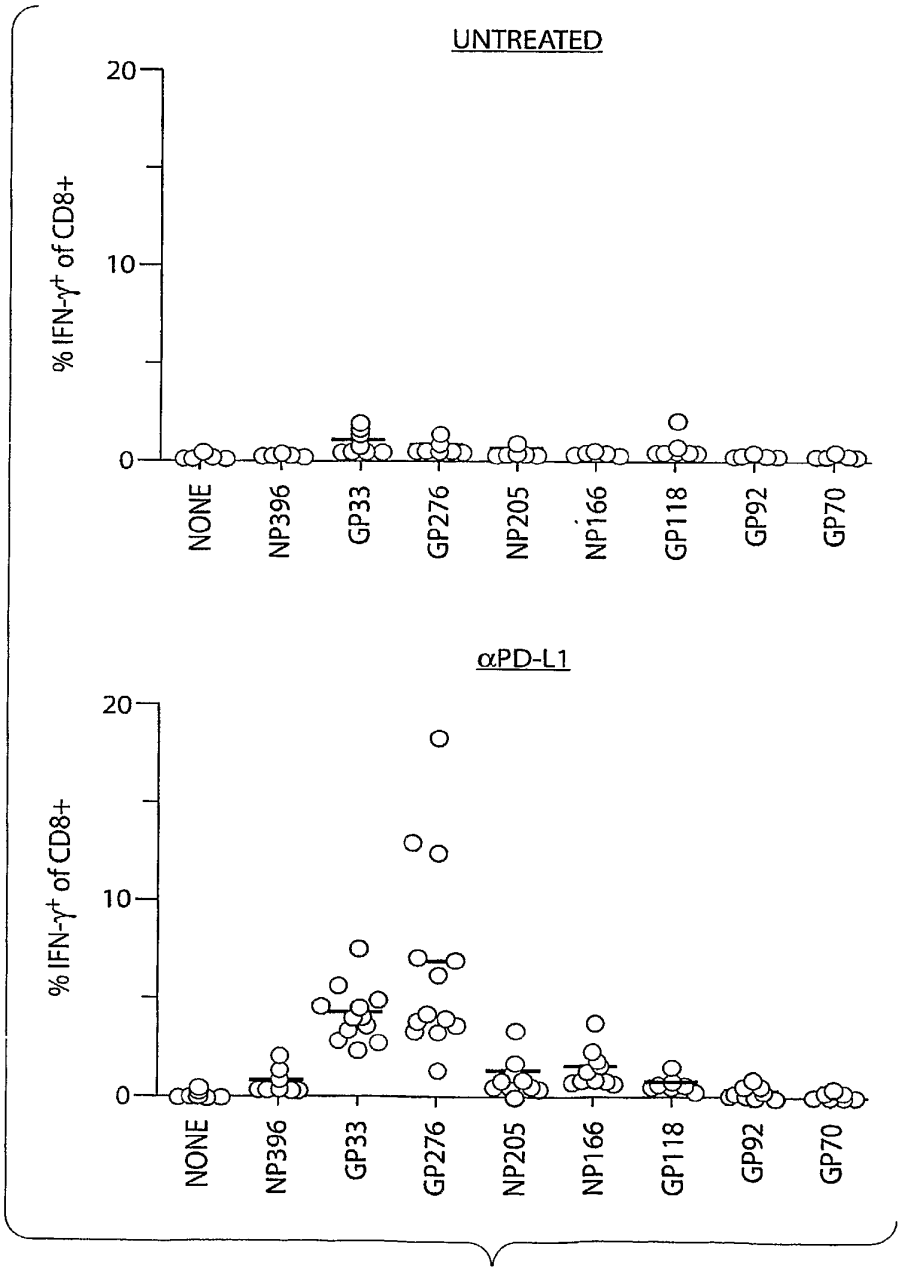

UNTREATED
% IFN-γ+ of CD8+
20
10
0
NONE
NP396
GP33
GP276
NP205
NP166
GP118
GP92
GP70
αPD-L1
% IFN-γ+ of CD8+
20
10
0
NONE
NP396
GP33
GP276
NP205
NP166
GP118
GP92
GP70

Fig. 4A

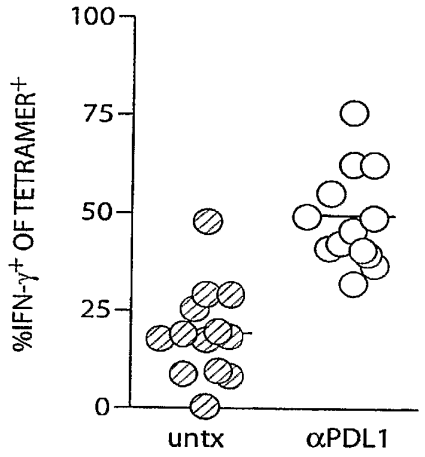

100
75
50
25
0
%IFN-γ+ OF TETRAMER+
untx
αPDL1

Fig. 4B

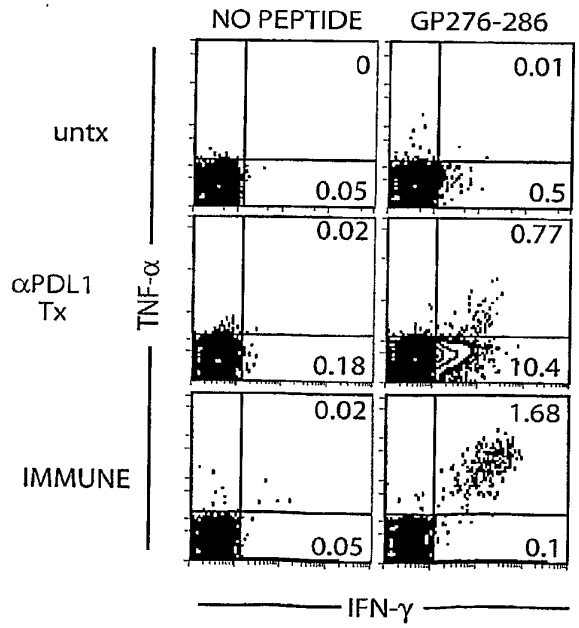

NO PEPTIDE
GP276-286
untx
αPDL1 Tx
IMMUNE
TNF-α
IFN-γ
0
0.01
0.05
0.5
0.02
0.77
0.18
10.4
0.02
1.68
0.05
0.1

Fig. 4C

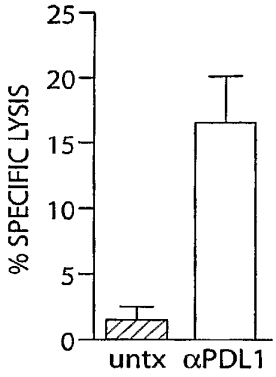

% SPECIFIC LYSIS
25
20
15
10
5
0
untx
αPDL1

Fig. 4D

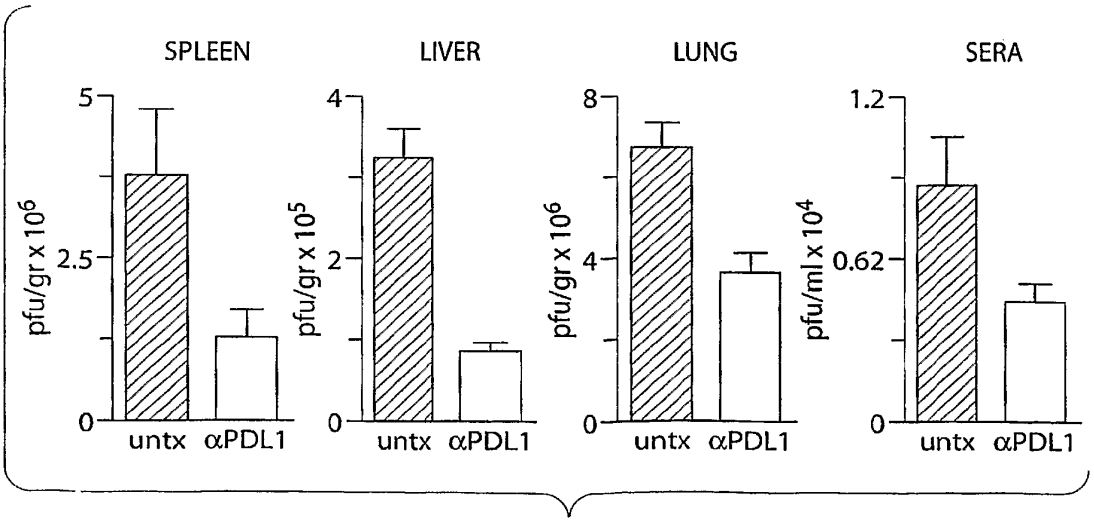

SPLEEN
LIVER
LUNG
SERA
pfu/gr x 10^6
pfu/gr x 10^5
pfu/gr x 10^6
pfu/ml x 10^4
5
2.5
0
4
2
0
8
4
0
1.2
0.62
0
untx
αPDL1

Fig. 4E

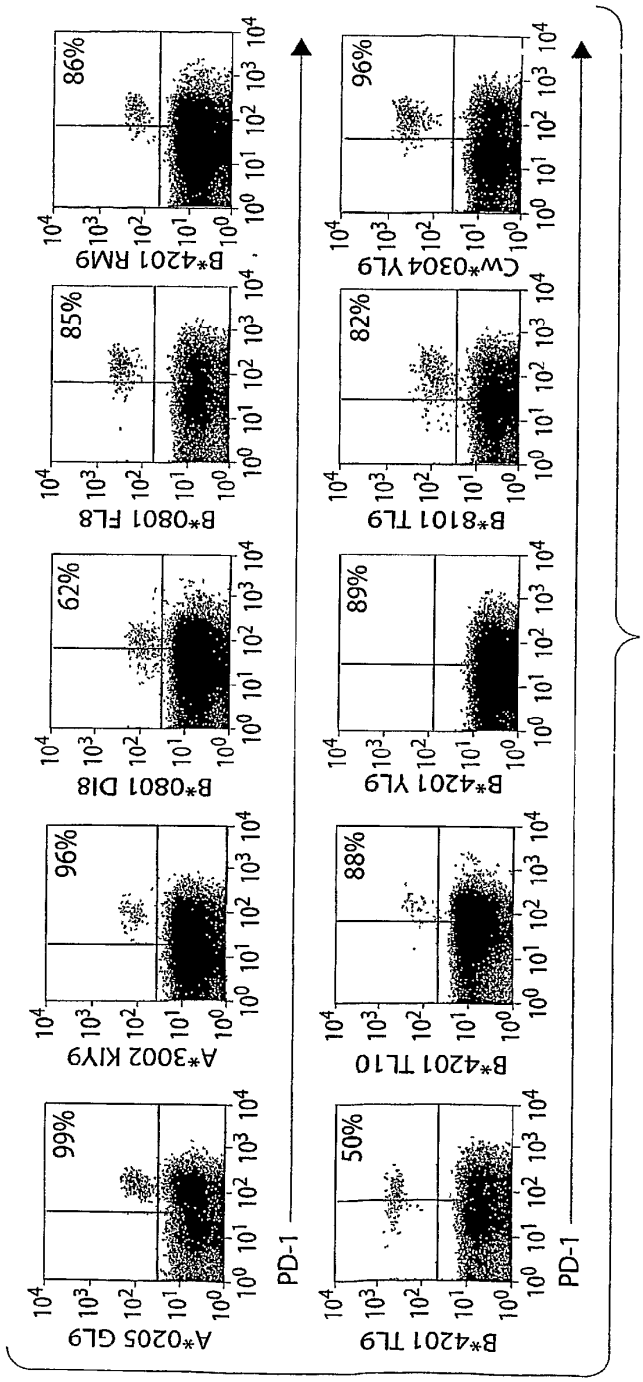


Fig. 5A

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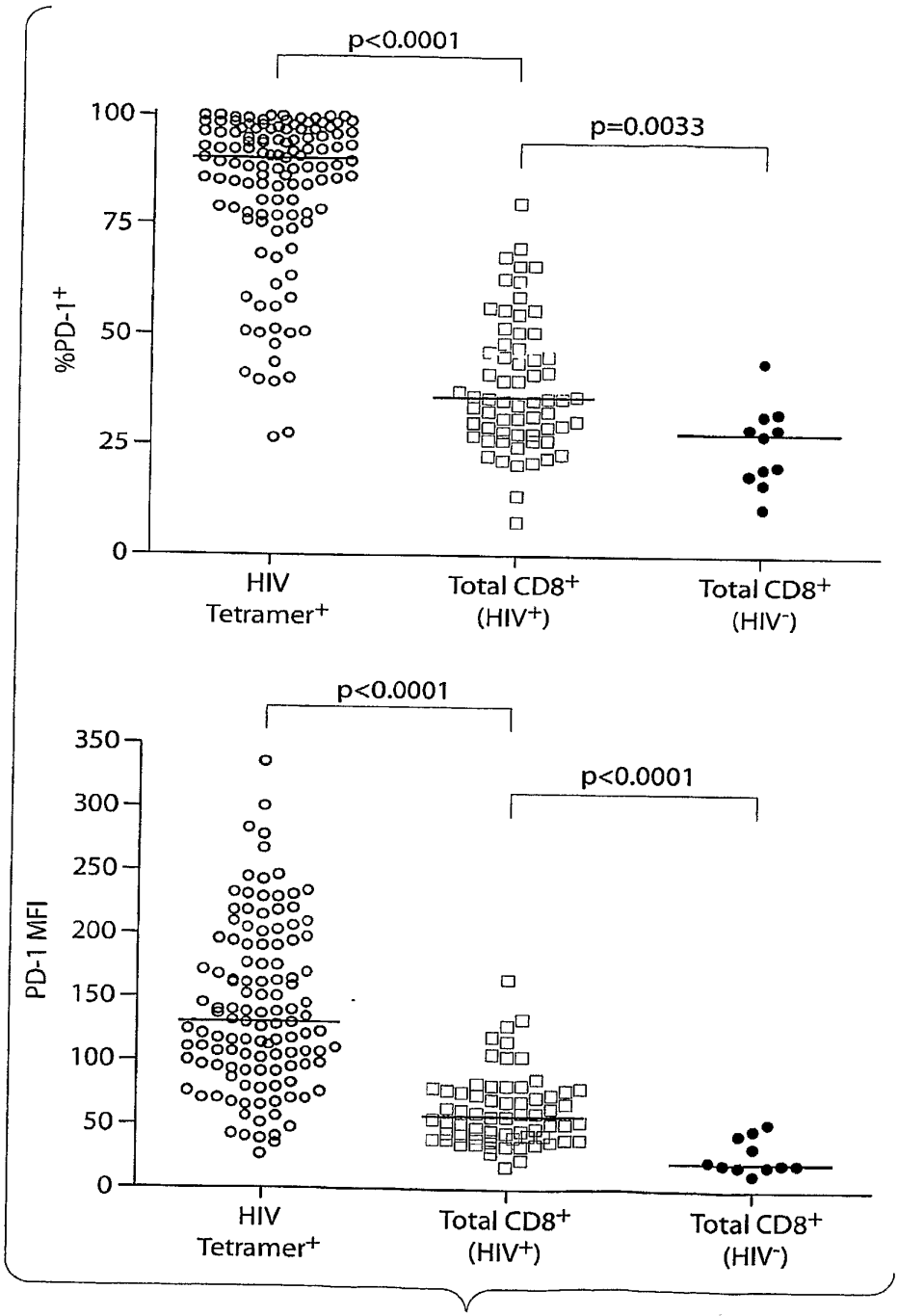

p<0.0001
p=0.0033
100
75
50
25
0
%PD-1+
HIV Tetramer+
Total CD8+ (HIV+)
Total CD8+ (HIV-)
p<0.0001
p<0.0001
350
300
250
200
150
100
50
0
PD-1 MFI
HIV Tetramer+
Total CD8+ (HIV+)
Total CD8+ (HIV-)

Fig. 5B

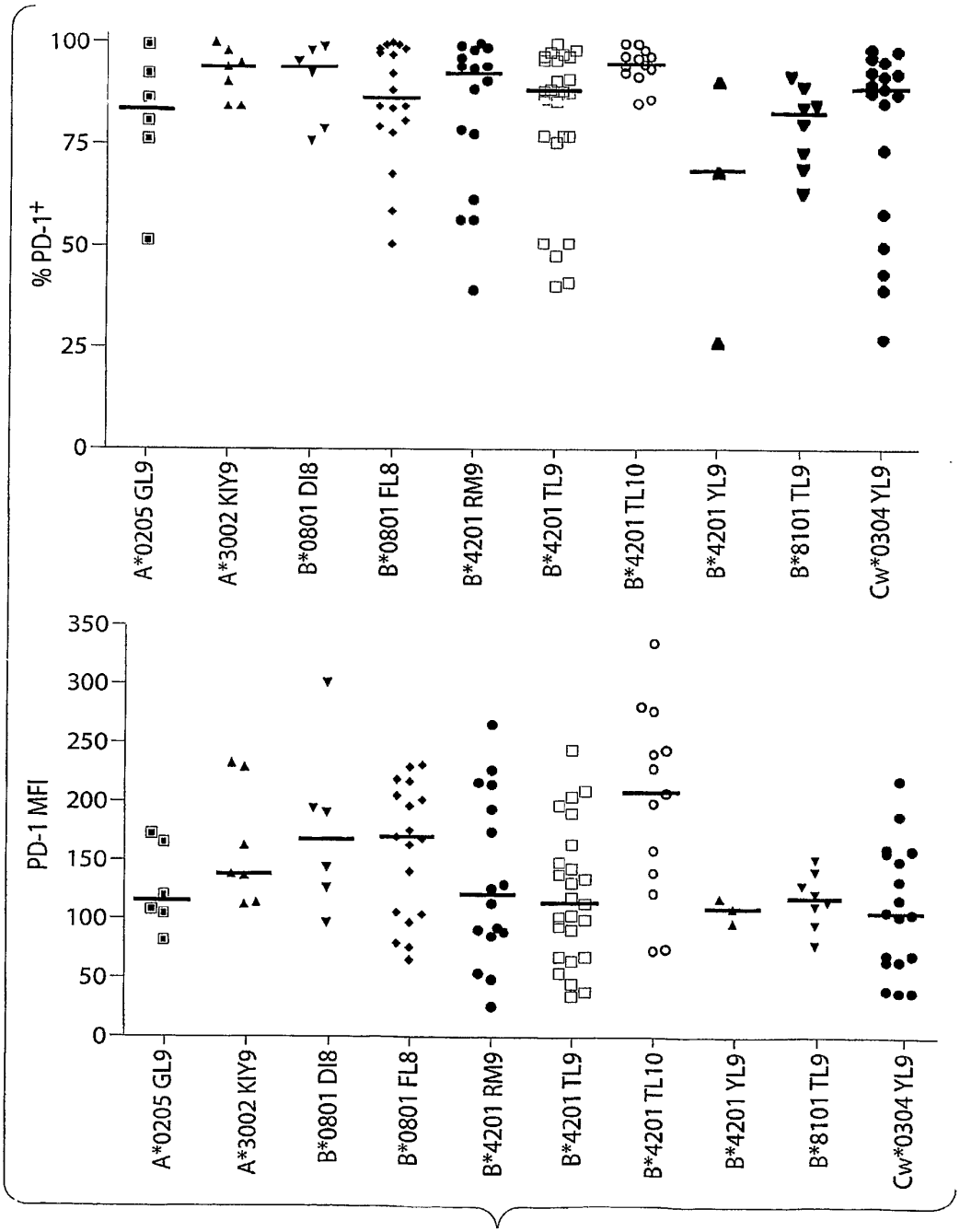


Fig. 5C

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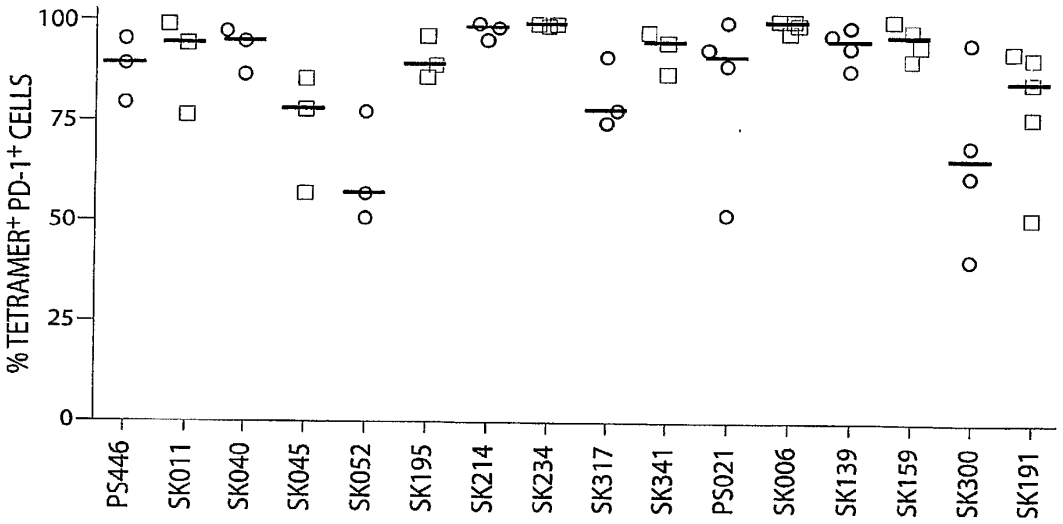


Fig. 5D

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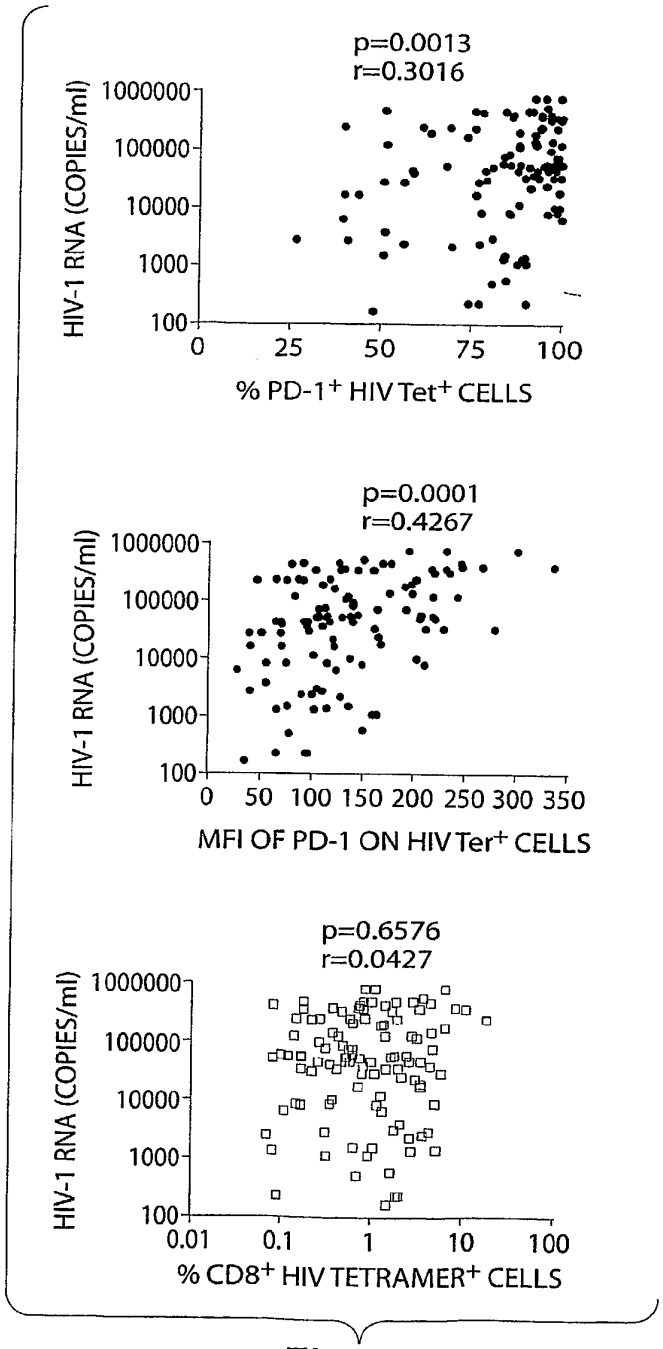

p=0.0013
r=0.3016
HIV-1 RNA (COPIES/ml)
1000000
100000
10000
1000
100
0
25
50
75
100
% PD-1+ HIV Tet+ CELLS
p=0.0001
r=0.4267
HIV-1 RNA (COPIES/ml)
1000000
100000
10000
1000
100
0
50
100
150
200
250
300
350
MFI OF PD-1 ON HIV Ter+ CELLS
p=0.6576
r=0.0427
HIV-1 RNA (COPIES/ml)
1000000
100000
10000
1000
100
0.01
0.1
1
10
100
% CD8+ HIV TETRAMER+ CELLS

Fig. 6A

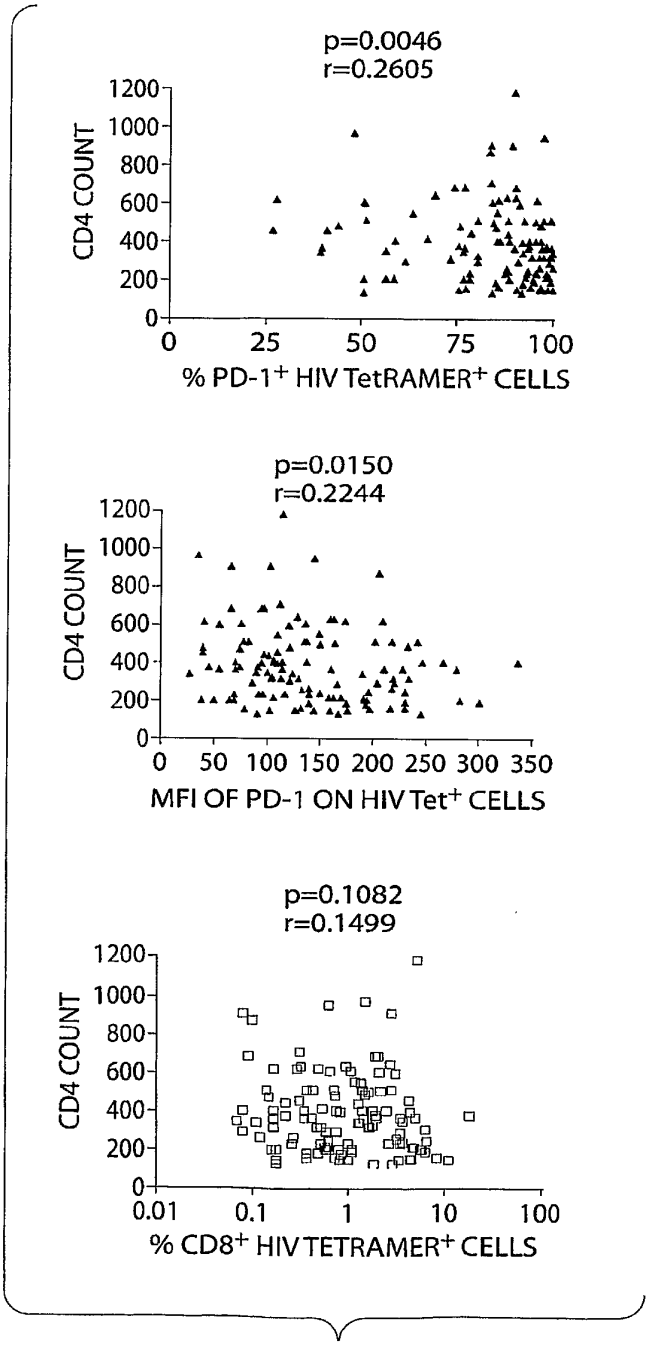

p=0.0046
r=0.2605
CD4 COUNT
1200
1000
800
600
400
200
0
0
25
50
75
100
% PD-1+ HIV TetRAMER+ CELLS
p=0.0150
r=0.2244
CD4 COUNT
0
50
100
150
200
250
300
350
MFI OF PD-1 ON HIV Tet+ CELLS
p=0.1082
r=0.1499
CD4 COUNT
0.01
0.1
1
10
100
% CD8+ HIV TETRAMER+ CELLS

Fig. 6B

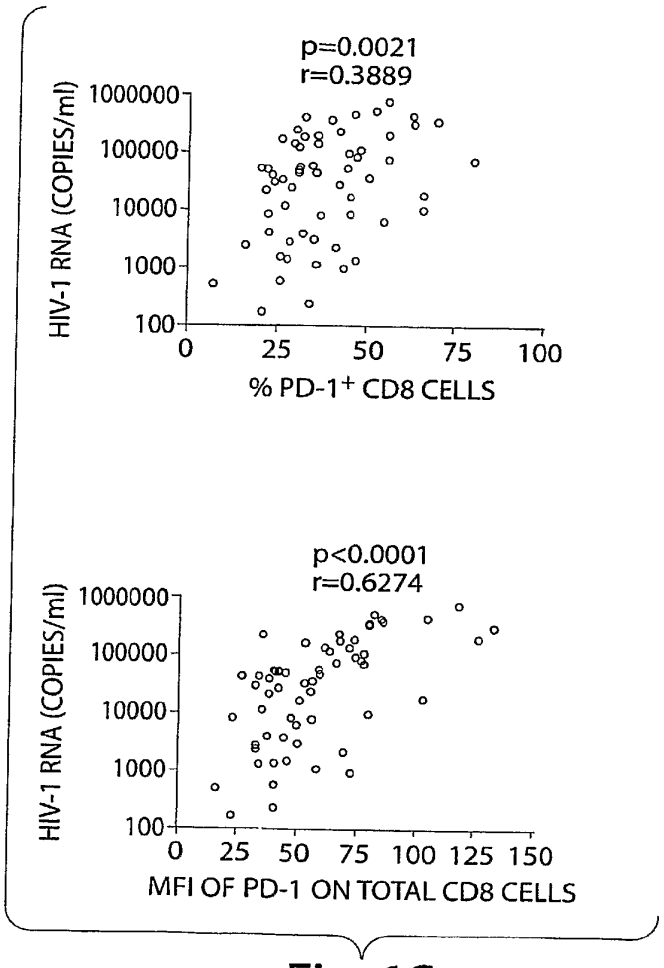

p=0.0021
r=0.3889
HIV-1 RNA (COPIES/ml)
1000000
100000
10000
1000
100
0
25
50
75
100
% PD-1+ CD8 CELLS
p<0.0001
r=0.6274
HIV-1 RNA (COPIES/ml)
1000000
100000
10000
1000
100
0
25
50
75
100
125
150
MFI OF PD-1 ON TOTAL CD8 CELLS

Fig. 6C

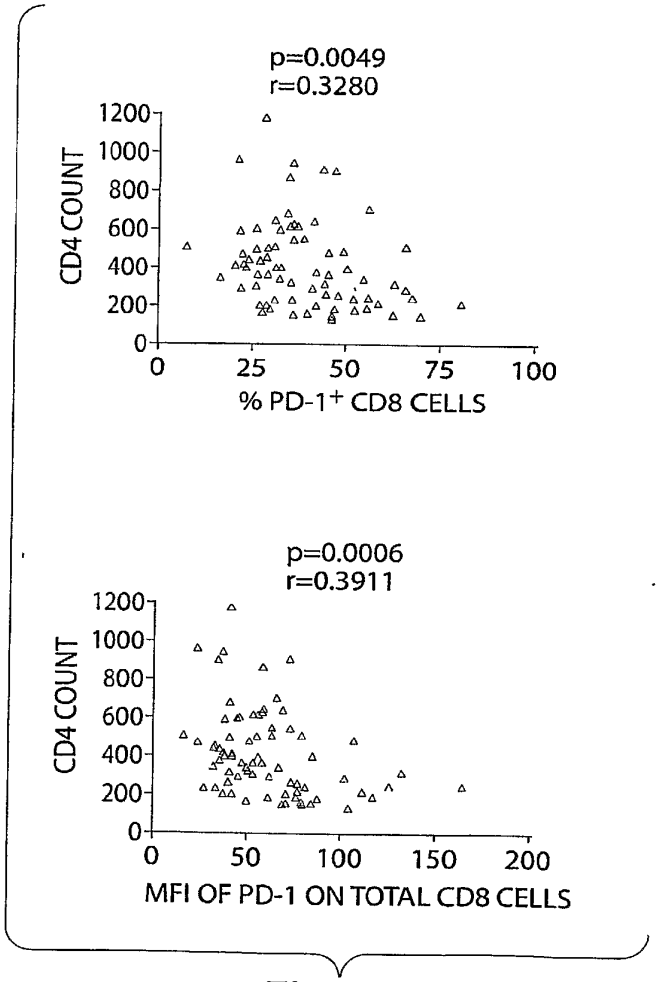

p=0.0049
r=0.3280
CD4 COUNT
1200
1000
800
600
400
200
0
0
25
50
75
100
% PD-1+ CD8 CELLS
p=0.0006
r=0.3911
CD4 COUNT
1200
1000
800
600
400
200
0
0
50
100
150
200
MFI OF PD-1 ON TOTAL CD8 CELLS

Fig. 6D

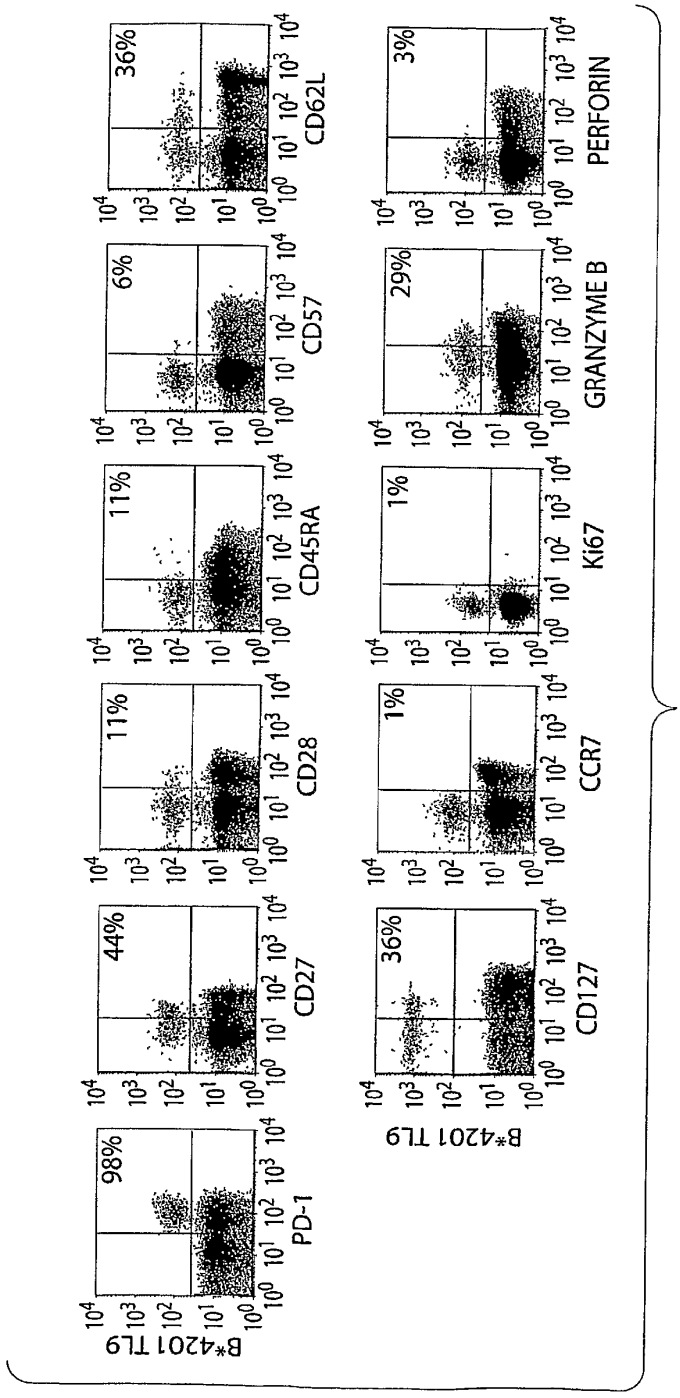


Fig. 7A

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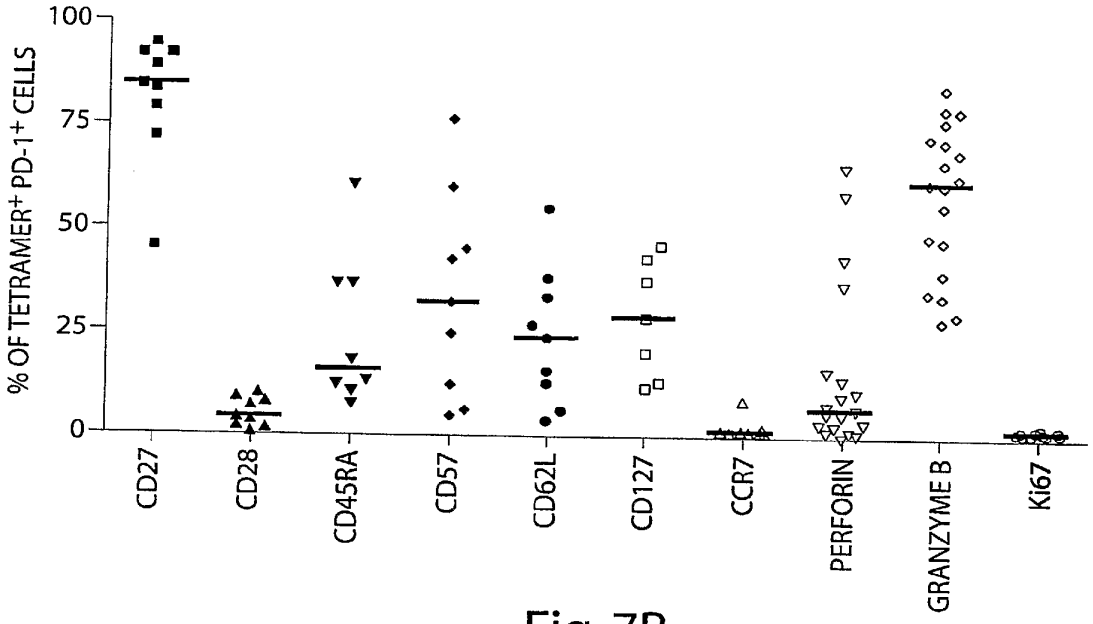

% OF TETRAMER+ PD-1+ CELLS
100
75
50
25
0
CD27
CD28
CD45RA
CD57
CD62L
CD127
CCR7
PERFORIN
GRANZYME B
Ki67

Fig. 7B

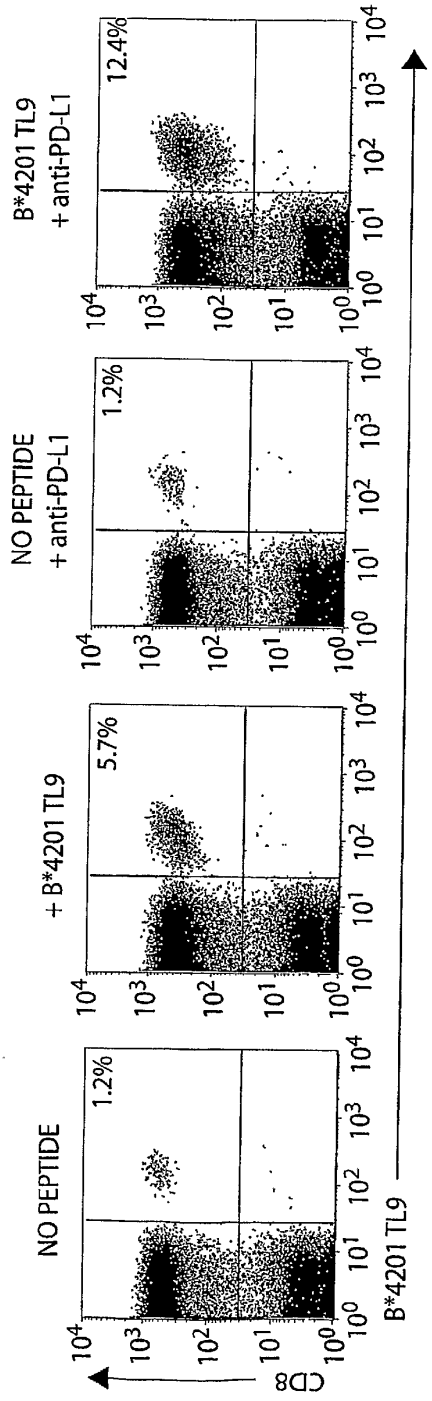


Fig. 8A

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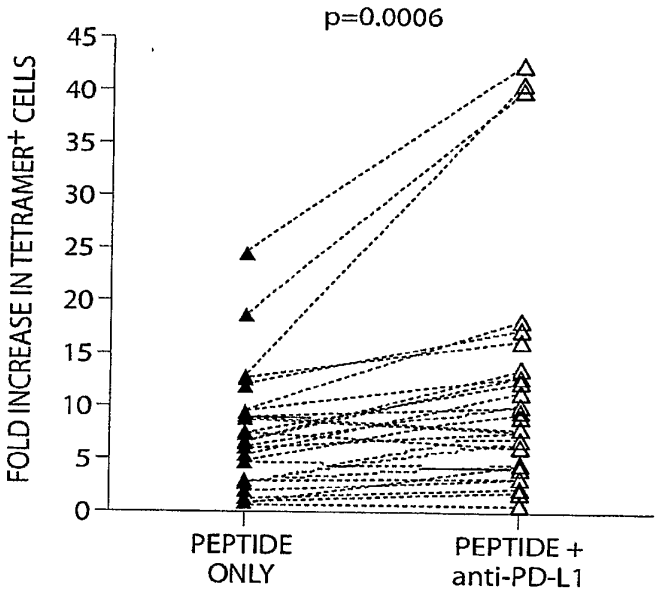

p=0.0006
FOLD INCREASE IN TETRAMER+ CELLS
45
40
35
30
25
20
15
10
5
0
PEPTIDE ONLY
PEPTIDE + anti-PD-L1

Fig. 8B

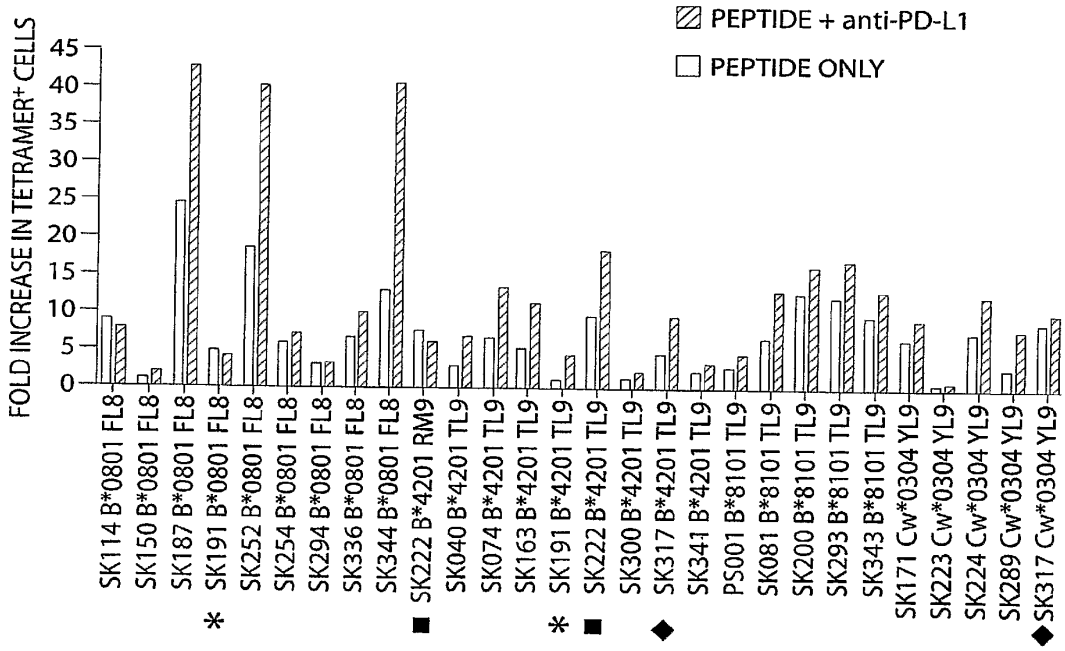


Fig. 8C

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Soluble human program death protein-1-IgV and preparation method thereof

Page bookmark	CN101215329 (A) - Soluble human program death protein-1-IgV and preparation method thereof
Inventor(s):	YINGQI ZHANG [CN]; SHOUZHEN WU [CN]; WEIHUA WANG [CN]; WEI HAN [CN]; CHUNJIE BAO [CN]; XIAOCHANG XUE [CN]; MENG LI [CN]; XIN QIN [CN] ±
Applicant(s):	FOURTH MILITARY MEDICAL UNIV [CN] ±
Classification:	- international: ***C07K14/705; C12N15/12; C12N15/70***
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Application number:	CN2008117236 20080104
Priority number(s):	CN2008117236 20080104
Also published as:	CN101215329 (B)

Abstract of CN101215329 (A)

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The invention discloses soluble programmed dead protein-1-IgV and a preparation process. The protein sequence of is showed as followed: PPTFFPAL LVVTEGDNAT FTCSFSNTSE SFVLNWYRMS PSNQTDKLAA FPEDRSQPGQ DSRFRVTQLP NGRDFHMSVV RARRNDSGTY LCGAISLAPK AQIKESLRAE LRVTERRAEV PTAHPSP. The invention obtains gene of Hpd-1-IgV through applying artificial synthesis according to the structure of human PD-1, target gene is cloned into pQE-30 pronucleus expression carrier to transform colibacillus and is expressed in colibacillus with high efficiency, bacterioclasis shows that target protein is existed in inclusion body form, and purified soluble programmed dead protein-1-IgV is finally obtained through inclusion body scouring, affinity chromatography, gel column chromatography and dialysis renaturation.; The soluble programmed dead protein-1-IgV can respectively combine with mPD-L1/Fc and hPD-L1/Fc in extra-corporal shPD-1IgV and on cell level and molecular level, and has certain tumor killing effect in internal shPD-1IgV. Therefore, shPD-1IgV has excellent internal and external biological activity, provides experiment basis for curing tumor, establishes experiment foundation, and has potential application prospect.

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权利要求书 2 页 说明书 18 页 附图 10 页

[54] 发明名称

可溶性人程序性死亡蛋白 - 1 - lgV 及其制备方法

[57] 摘要

本发明公开了可溶性人程序性死亡蛋白 - 1 - IgV 及其制备方法，可溶性人程序性死亡蛋白 - 1 - IgV 的蛋白序列如下：PPTFFPAL LVVTEGDNAT FTCSFSNTSE SFVLNWYRMS PSNQTDKLAA FPEDR-SQPGQ DSRFRVTQLPNGRDFHMSVV RARRNDSGTY LCGAISLAPK AQIKESLRAE LRVTERRAEV PTAHP-SP。本发明根据人 PD - 1 的结构，应用人工合成的方法获得了 hPD - 1 - IgV 的基因，目的基因克隆入 pQE - 30 原核表达载体，转化大肠杆菌，在大肠杆菌中高效表达，裂菌显示目的蛋白以包涵体形式存在，经包涵体洗涤、亲和层析、凝胶柱层析和透析复性，即可获得纯化的可溶性人程序性死亡蛋白 - 1 - IgV。该可溶性人程序性死亡蛋白 - 1 - IgV 在体外shPD - 1IgV在细胞水平和分子水平上可分别与 mPD - L1/Fc 和 hPD - L1/Fc 结合。在体内 shPD - 1IgV 具有一定的抑瘤效果。因此，shPD - 1IgV 具有良好的体内外生物学活性，为治疗肿瘤提供了实验依据，奠定了实验基础，具有潜在的应用前景。

1．一种可溶性人程序性死亡蛋白-1-IgV，其特征在于，其蛋白序列如下：

PPTFFPAL LVVTEGDNAT FTCSFSNTSE SFVLNWYRMS PSNQTDKLAA FPEDRSQPGQ DSRFRVTQLP NGRDFHMSVV RARRNDSGTY LCGAISLAPK AQIKESLRAE LRVTERRAEV PTAHPSP。

2．权利要求 1 所述的可溶性人程序性死亡蛋白-1-IgV 的制备方法，其特征在于，根据人 PD-1 的结构，应用人工合成的方法获得了 hPD-1-IgV 的基因，目的基因克隆入 pQE-30 原核表达载体，转化大肠杆菌，在大肠杆菌中高效表达，裂菌显示目的蛋白以包涵体形式存在，经包涵体洗涤、亲和层析、凝胶柱层析和透析复性，即可获得纯化的可溶性人程序性死亡蛋白-1-IgV。

3．如权利要求 2 所述的方法，其特征在于，所述的表达及纯化的步骤如下：

1）pQE-30/ hPD-1-IgV工程菌的诱导表达

将含有pQE-30-hPD-1IgV重组质粒的菌株，接种于10ml含Amp的LB培养液中，于37℃培养过夜，次日按1%的比例转接于10 ml含Amp的LB培养液中，于37℃振荡培养至对数生长期A_{600nm}=0.4-0.6时，加入IPTG至终浓度为1 mmol/L诱导表达，于37℃振荡培养3-5h，离心收集菌体，SDS-PAGE电泳进行鉴定；

2）目的蛋白包涵体的分离和纯化

取10g诱导的菌体，按1g湿重对7ml的比例，用裂解缓冲液STE重悬，裂解缓冲液为：50mmol/L Tris.Cl，50mmol/L NaCl，1mmol/L EDTA，裂解缓冲液STE重悬后于-20℃冻过夜，次日于室温水浴中融化，以溶菌酶法裂菌，接着再采用300W超声破菌，其中超声5s，间隔10s，共进行100次；超声完成后，12000rpm/min、4℃离心20 min，弃除上清，沉淀用包涵体洗涤液A重悬，包涵体洗涤液A为：1%TritonX-100，1mmol/L EDTA，1%DOC，50mmol/LTris.Cl pH8.5，100mmol/L NaCl，12000r/min、4℃离心20min，弃上清；用Ni-NTA柱亲和层析纯化所溶解的沉淀，按1g菌体加10ml裂菌缓冲液的比例将菌体重悬，冰浴条件下进行超声裂菌，12000rpm，离心15min，弃上清，1g沉淀加10ml6mol/L尿素，0.1mol/LNaH_2PO_4，0.01mol/LTris，4℃搅拌过夜，12000rpm，离心15min，共离心2次，收集上清待用；经洗涤的包涵体以1g湿重对10ml的比例，溶于6mol/L尿素，0.1mol/LNaH_2PO_4，0.01mol/L Tris的缓冲液中，4℃搅拌过夜，12000r/min、离心30min，收集上清，即为目的蛋白粗提液；以SephacrylS-300凝胶层析，以工作液充分平衡后，所述的工作液为：6mol/L尿素，0.1mol/LNaH_2PO_4，0.01mol/LTris；目的蛋白粗提液2ml上

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柱，以工作液洗脱，流速1ml/min，收集含有目的蛋白的峰，然后进行亲和层析纯化；

用6mol/L尿素，0.1mol/LNaH_2PO_4，0.01mol/LTris充分平衡Ni柱，先用含10mmol咪唑的6 M尿素，0.1mol/LNaH_2PO_4，0.01mol/LTris洗脱杂蛋白，再用含250mmol咪唑的6mol/L尿素，0.1mol/LNaH_2PO_4，0.01mol/LTris洗脱目的蛋白，收集洗脱峰，纯化产物透析至2M尿素，以0.1mol/LNaH_2PO_4， 0.01mol/LTris复性，终浓度1mg/ml，最后透析入0.02mol/LPBS中，用Lowery法测定蛋白浓度；

3）目的蛋白的复性

应用包涵体溶解液稀释亲和层析后的样品，使蛋白浓度约为0.1mg/ml，梯度透析，透析外液加入$CuSO_4$和β－巯基乙醇作为氧化还原系统，4℃透析，每6小时换液一次，梯度为，4M尿素，2M尿素，1M尿素，最后透析到双蒸水，离心留上清，冻干，HPLC检测蛋白纯度。

可溶性人程序性死亡蛋白-1-IgV 及其制备方法

技术领域

本发明属于医药生物技术领域，涉及基因合成、蛋白表达纯化、体外活性检测、动物体内抑瘤检测等技术，特别涉及一种可溶性人程序性死亡蛋白-1-IgV（hPD-1-IgV）及其制备方法。

背景技术

机体免疫系统可以识别“自我/非我”，对“非我”进行免疫监视、识别和应答，以至将其消除。然而，这种机体强大的免疫保护系统，在肿瘤面前却显得束手无策。肿瘤在人体强大的免疫功能作用下仍能发展、转移，表明肿瘤具有自己的保护机制。肿瘤细胞可以通过对自身表面抗原的修饰及改变肿瘤组织周围的微环境来逃避机体的免疫识别与攻击，这就是肿瘤的免疫逃逸。肿瘤存在着许多逃逸免疫系统识别和攻击的机制，包括：HLA-I类抗原和免疫共刺激分子的表达下调或丢失；肿瘤抗原的表达下调、丢失或突变；肿瘤细胞分泌免疫抑制性可溶性因子；肿瘤细胞膜上表达免疫抑制性分子；诱导具有免疫抑制功能的调节性T淋巴细胞等。其中，肿瘤来源的可溶性免疫抑制因子，以影响树突状细胞（DC）以及淋巴T细胞的功能为主。这些因子致使髓样细胞的不正常分化，导致全身性成熟DC数量下降、不成熟DC（ iDC）数量增加及不成熟髓样细胞（iMCs）的增加。主要有：白细胞介素-10（IL-10），转化生长因子－β（TGF-β），前列腺素E2PGE2）和趋化因子（如CCL2/MCP-1）等。在抗肿瘤免疫效应中，$CD8^+$T细胞起主要作用，通过对肿瘤抗原的识别，可以直接杀伤肿瘤细胞，但肿瘤局部的微环境包含大量的细胞因子，这些细胞因子可单独地或协同地影响CTL活化或肿瘤细胞对CTL杀伤的敏感性，共刺激免疫抑制性分子在这个过程中发挥重要的作用，它们要么使CD8 + T细胞不能被活化，要么使活化的$CD8^+$T细胞功能丧失或则使$CD8^+$T细胞凋亡，这些共刺激免疫抑制性分子主要有：CTLA4/B7、PD-1/PD-L1、ICOS/B7-H2、BTLA/B7-H4、TIM3等。

PD-1分子2004年12月在第八届国际人类白细胞分化抗原会议上被命名为CD279。最早是通过削减杂交技术从小鼠处于凋亡状态的杂交瘤及造血祖细胞系克隆，被认为与细胞凋亡相关而命名为程序性死亡-1（programmed death-1）。它是免疫球蛋白超家族的激活诱导的抑制性受体，通过其胞浆区的两个酪氨酸残基与下游的信号分子作用而发挥对免疫应答的负性调控功能。PD-1分子可在淋巴样组织和实质器官组织部位双重抑制淋巴

细胞的活化， 在维持机体的外周耐受方面发挥重要的作用。研究表明，PD-1与其配体PD-L1（B7-H1）相互作用，组成PD-1/ PD-L1通路，并且，PD-1/ PD-L1通路可能参与了肿瘤免疫逃避、炎症、自身免疫性疾病、移植耐受和病毒感染等的发生过程。

申请人在分析 CCR5 结构的基础上以 PD-1 IgV 为研究目标，利用基因工程手段和蛋白质纯化技术，获得纯化的重组蛋白样品。外检测该融合蛋白体内能够与 PD-L1 结合，以融合蛋白对荷瘤小鼠进行抑瘤实验的观察。我们的发明进行了 PD-1 IgV 融合蛋白的实验基础研究，为进一步的肿瘤治疗提供了实验依据和奠定了基础。

1 Programmed death-1（PD-1）分子研究进展

1.1 PD-1 的发现

PD-1分子2004年12月在第八届国际人类白细胞分化抗原会议上被命名为CD279。最早是通过削减杂交技术从小鼠处于凋亡状态的杂交瘤及造血祖细胞系克隆，被认为与细胞凋亡相关而命名为程序性死亡-1（programmed death-1）。它是免疫球蛋白超家族的激活诱导的抑制性受体，通过其胞浆区的两个酪氨酸残基与下游的信号分子作用而发挥对免疫应答的负性调控功能。PD-1分子可在淋巴样组织和实质器官组织部位双重抑制淋巴细胞的活化， 在维持机体的外周耐受方面发挥重要的作用。研究表明，PD-1与其配体PD-L1（B7-H1）相互作用，组成PD-1/ PD-L1通路，并且，PD-1/ PD-L1通路可能参与了肿瘤免疫逃避、炎症、自身免疫性疾病、移植耐受和病毒感染等的发生过程。

1.2 PD-1的结构

PD-1分子相对分子质量是55，000免疫球蛋白超家族I型跨膜糖蛋白，共有288个氨基酸组成（氨基酸组成如下所示）。

001 MQIPQAPWPV VWAVLQLGWR PGWFLDSPDR PWNPPTFFPA LLVVTEGDNA TFTCSFSNTS

061 ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT

121 YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV VGVVGGLLGS

181 LVLLVWVLAV ICSRAARGTI GARRTGQPLK EDPSAVPVFS VDYGELDFQW REKTPEPPVP

241 CVPEQTEYAT IVFPSGMGTS SPARRGSADG PRSAQPLRPE DGHCSWPL

PD-1分子胞外区包含一个IgV样结构域，有4个重要的N 连接糖基化位点，并被重度糖基化。PD-1分子最显著的特征是胞浆区的尾部含有两个酪氨酸残基，其中N末端的酪

氨酸残基与其他的氨基酸残基共同组成了一个ITIM（免疫受体酪氨酸抑制基序）。ITIM存在于许多抑制性受体中，其共同特征是含有一个6个氨基酸残基（Ile/Val/Leu/Ser）-X-Tyr-X-X-（Leu/Val）的共同序列，在免疫反应的负调控方面发挥重要作用。最近的实验证实，其C末端的酪氨酸残基也可与下游的一系列信号转导分子作用而抑制淋巴细胞的活化。

1.3 PD-1的分布

PD-1分子在T、B细胞抗原受体激发后诱导性表达，未成熟的T、B细胞在胸腺和骨髓内发育的特定阶段也有表达。一些肿瘤细胞系已被证实有PD-1蛋白的表达，如Jurkat淋巴瘤，应用佛波酯可明显诱导其上调表达。部分肿瘤细胞系有PD-1mRNA的表达，如Daudi（Burkitt 淋巴瘤），HL260（急性前髓系白血病）等。

1.4 PD-1的配体

PD-L1（B7-H1）和PD-L2（B7-DC）是PD-1的两个配体，其全长的cDNA是通过同源性筛选从胎盘的cDNA文库中获得。这两个分子均定位于人染色体9p24.2，起始于同一方向，间隔42kb。它们同属B7家族，因而同此家族的其他成员一样，成熟的PD-L分子包含有IgV样区、IgC样区、跨膜区及一个短的胞浆区尾部。在胞外区有4个保守的半胱氨酸残基，参与Ig样结构中二硫键的形成。与PD-L2相比，PD-L1的胞浆区在人鼠之间更为保守。PD-L1和PD-L2mRNA不仅可以表达在实质器官组织部位，如心脏、肺和胎盘等，而且在抗原递呈细胞上也有表达，如激活的B细胞、单核细胞和树突状细胞。最近的研究还发现，在胸腺上皮细胞有PD-L1mRNA的表达。PD-L1在蛋白水平上的表达已在激活的T、B及单核细胞上得到证实。

1.5 PD-1/PD-L通路的作用

1.5.1 PD-1与PD-L的作用对体外T细胞功能的影响

PD-1与PD-L1相互作用可抑制T细胞的增殖，同时抑制其对细胞因子IL-2、IFN-γ和IL-10的产生。研究发现PD-L1与PD-1对T细胞功能的影响与TCR与CD28信号的强度有关。在亚适量TCR信号强度下，PD-L1对T细胞的增殖抑制作用明显；而在最适量的TCR信号时，仅在没有CD28共刺激的情况下，才对T细胞增殖发挥抑制作用。PD-L2与PD-1相互作用也可抑制TCR所介导的T细胞增殖和产生IL-4、IL-10和IFN-γ。在较低抗原浓度下，PD-L2与PD-1作用可抑制TCR及B7-2介导的预激活的$CD4^+$T细胞的增殖，并减少Th1和Th2型细胞因子的产生。但随着抗原浓度的升高，其对细胞增殖的抑制下降，但仍能抑制细胞因子的产生。进一步的研究证明，低浓度抗原可刺激PD-1高水平表达，随着抗原浓度的升高，其表达下降。因此，PD-L/PD-1的相互作用对弱的抗原刺激反应更敏感，

这类抗原主要产生于自身免疫性疾病和肿瘤的发生过程中。但另外的研究认为，在亚适量的TCR信号下，PD-L2（B72DC）可有力地刺激体外T细胞的增殖及细胞因子（主要是IFN-γ）的产生。有人发现，在体外应用抗CTLA-4抗体Fab片段阻断其效应时，可对同一抗原特异性的不同的T细胞亚群产生刺激或抑制效应，这种不同的效应取决于T细胞的激活状态和TCR的信号强度。

1.5.2 PD-1与PD-L之间的负调控作用

PD-1的胞内区含有酪氨酸抑制性基序，通过募集含有酪氨酸磷酸酶活性及SH2结构域的蛋白因子传递负调节信号。2000年，Freeman等报道，B7-H1与PD-1结合可减少T细胞增殖；2002年，Iwai等研究发现B7-H1与PD-1结合可以促进肿瘤细胞的免疫逃逸。虽然，通过接种或过继转移的方法均可以增加循环血液中肿瘤抗原特异性$CD8^+$T细胞的数量，但随着肿瘤的不断进展，肿瘤组织常常会出现对$CD8^+$T细胞效应功能的破坏或损伤。PD-1与B7-H1相互作用后，可能通过抑制细胞周期的进展来介导T细胞活化反应的抑制效应。2004年，Shengdian Wang等人通过突变实验发现，B7-H1通过其IgV样区与PD-1的IgV样区相互结合而发挥作用（图2，3）。同时，Christian Blank等发现干预B7-H1与PD-1间的相互作用，可以增强肿瘤抗原特异性$CD8^+$T细胞在肿瘤微环境中的功能。同时，有很多文献资料表明，阻断B7-H1，可以诱导肿瘤组织的退行性发展，但并不仅仅依赖PD-1途径。

1.5.3 PD-1与PD-L参与T、B细胞负调控作用机制

对体外转染PD-1或FcγRII-PD-1融合蛋白基因的B淋巴瘤细胞系的研究表明，PD-1可抑制B细胞受体的刺激性信号。BCR与PD-1的交联可抑制Ca^{2+}内流以及下游信号激活分子如Syk、磷脂酰肌醇-3激酶、磷脂酶-3和Vav的酪氨酸残基的磷酸化。但是，这种抑制效应不需要PD-1胞浆区ITIM基序内的酪氨酸残基参与，而是通过C端的酪氨酸残基与SHP-2酪氨酸磷酸酶结合实现的。此外，PD-1也可通过抑制丝裂原激活的蛋白激酶（MAPK）的酪氨酸残基磷酸化而抑制B淋巴瘤细胞系的增殖。另外，对Jurkat细胞系的研究也表明TCR与PD-1的交联可引起SHP-2的磷酸化并向PD-1的胞浆区募集。体外应用预激活的T细胞表明PD-L/PD-1途径并不引起T细胞的凋亡，而仅使细胞周期停滞于G0/G1期。以改良的酵母双杂交技术发现，PD-1的胞浆区的ITIM酪氨酸残基磷酸化后可与酪氨酸磷酸酶SHP-1的羧基端的SH2结构域结合。因此，PD-1胞浆区两个酪氨酸残基磷酸化后可分别与SHP-1和SHP-2结合发挥负性免疫调控功能。

2 PD-1与疾病

2.1 PD-1与肿瘤

用可溶性PD-1阻断B7-H1可改善小鼠体内抗肝癌细胞（H22肿瘤细胞）免疫反应。体外实验证实，用可溶性PD-1阻断B7-H1可改善部分淋巴细胞的早期活化。PD-L1在多种组织的肿瘤细胞上高表达，它与PD-1的相互作用显示了肿瘤免疫逃避的潜在的机理。B7-H1与PD-1之间的作用，抑制了激活的T细胞的作用，可能是通过抑制细胞周期实现的，肿瘤相关的B7-H1提高了抗原特异性T细胞的凋亡，致使肿瘤逃避免疫监视。体内实验表明，在鼠P815肿瘤上表达的B7-H1促进了激活的肿瘤反应T细胞的凋亡，并促使体内与免疫生成B7-H1（+）肿瘤的生长。PD-1与PD-L1相互作用降低了TCR介导的淋巴细胞的增殖和细胞因子的产生。PD-1表达在多种人与鼠的肿瘤细胞上，并且被IFN-γ刺激上调。研究发现，以可溶性PD-1阻断PD-1/PD-L1通路，增强了肿瘤特异性CTL的杀伤活性。而且，以单克隆抗体阻断PD-1/PD-L1相互作用，逆转了由肿瘤抗原特异性$CD8^+$ T 细胞功能表失引起的肿瘤耐受，加强了抗肿瘤免疫反应。

2.2 PD-1与肿瘤侵润的免疫细胞

Thompson等对RCC（renal cell carcinoma）肿瘤样本以抗PD-1单抗进行免疫组织化学染色，结果发现单核免疫细胞侵润占病人总数的50.9%。PD-1免疫细胞占总病人数的56.6%。而且RCC肿瘤细胞不表达PD-1。PD-1免疫细胞对于B7-H1相关的肿瘤细胞有保护作用。因此，PD-1（+）免疫细胞增强了肿瘤的转移，与肿瘤恶性程度的分级有关。免疫细胞上表达PD-1的水平在高风险RCC肿瘤病人身上是提高的，PD-1/PD-L1促进了RCC肿瘤的发生。B7-H1与B7DC在非小细胞肺癌中表达，并且B7-H1阳性的肿瘤区域内的肿瘤侵润的淋巴细胞（TIL）的数量明显少于B7-H1阴性的肿瘤区，B7H1阳性肿瘤细胞区域表达PD-1的TIL的比例明显低于B7H1阴性肿瘤细胞区域。这或许是由于PD-1/PD-L1通路致使TIL凋亡所致。

2.3 PD-1与自身免疫性疾病

PD-1分子在维持外周耐受发挥了重要的作用， 因而PD-1分子可作为一个免疫反应的负性调控分子参与自身免疫性疾病的发病过程。这一点已在不同遗传背景的PD-1基因敲除小鼠中得到证实。C57BL/6-PD-$1^{-/-}$鼠发生轻微但渐进性的脾肿大，脾脏的B细胞及髓样细胞增多，血清的IgA和IgG2b增加，IgG3增加尤为显著，机体对TI-2抗原的抗IgG3抗体反应显著增强。脾脏的B细胞对抗IgM抗体诱导的增殖反应也增强。C57BL/6-PD-$1^{-/-}$鼠可随年龄的增长而自发地发生狼疮样增殖性肾小球肾炎和关节炎，肾小球有过度的补体C3和IgG3的沉积，踝关节出现颗粒性结节及骨损伤，而同龄同窝对照鼠则表现正常。当同时导入Fas基因无义突变时，小鼠提早发生增殖性狼疮样自身免疫性疾病，疾病的严重程度也大大增加。这一结果表明PD-1和Fas在狼疮样自身免疫性疾病的发生过程中具有

协同作用。自身反应性TCR（2C-TCR）转基因鼠在剔除PD-1基因后可自发发生移植物抗宿主样疾病，部分小鼠10周前死亡。其余则表现为体重减轻，脾肿大，不同程度的皮肤损害：炎症、出血和坏死。病变鼠的心脏、肺及肾脏可发生系统性炎症细胞浸润。2C-TCR+T细胞浸润至表皮的基底层。由于2C-TCR-PD-$1^{-/-}$鼠的T细胞在胸腺的阴性选择过程中未受影响，而外周扩增的2C-TCR^{+}T细胞呈现记忆细胞表型（$CDRBlowCD62LlowCD69_{+}$），因而作者认为PD-1可能参与维持外周耐受。2C-TCR-PD-$1^{+/+}$鼠表现正常，可见PD-1基因缺陷是发生移植物抗宿主样疾病的重要原因。大部分的BALB/c-PD-$1^{-/-}$鼠在10周龄时因患充血性心力衰竭而死亡，但2C-TCR-PD-$1^{+/+}$和BALB/c-PD-$1^{-/-}$-RAG-2鼠则生长良好。胸腔超声检查显示，死亡小鼠的心室腔明显扩张，心室的收缩功能下降。组织学检查显示整个扩张的心室的心肌细胞间遍布IgG及补体C3的沉积，但却没有淋巴细胞的浸润。进一步的研究表明，自身抗体主要是针对一种表达于正常心肌细胞表面的相对分子质量为33000的蛋白，与同遗传背景的PD-$1^{+/+}$对照鼠相比，BALB/c-PD-$1^{-/-}$鼠的血清中也可检测到这种蛋白。这些结果表明，PD-1在防止BALB/c鼠发生自身免疫性扩张性心肌病方面发挥了重要作用。

对不同基因背景的PD-1基因缺陷鼠的研究证实PD-1在体内发挥负性免疫调节作用。C57BLP6-PD-1基因缺陷鼠发生轻度持续性脾肿大，B细胞及髓系细胞增多，血IgG3升高及针对T细胞非依赖性抗原的IgG3反应增强，抗IgM诱导的脾B细胞增生反应增强。C57BLP6-PD-1基因缺陷鼠发生进行性肾小球肾炎及狼疮样关节炎，在肾小球中可检出PAS阳性物质及IgG3沉积。BALBPC-PD-1基因缺陷鼠发生扩张性心肌病，心动超声显示这些鼠心脏明显扩大，收缩功能严重受损，并发现IgG广泛沉积于心肌细胞间，电镜观察发现心肌细胞表面有IgG沉积。此外，血液中出现针对正常心肌表面33 000的蛋白的抗体，提示某些不明原因的人扩张性心肌病可能与自身免疫反应有关。阻断NOD鼠的PD-1或PD-L1会加速处于糖尿病前期的各年龄段雌性鼠糖尿病的发生，在大龄鼠中更为明显。而阻断CTLA-4仅诱使幼龄鼠发病，提示CTLA-4仅在自身免疫反应发生的早期发挥免疫调节作用，而PD-1在自身免疫发生发展的各个阶段均有作用。与对照组相比，阻断PD1/PD-L1后，胰岛炎症评分明显增高，产生IFN-γ的GAD反应性脾细胞增多，但抗胰岛素抗体并不增高。NOD鼠的胰岛细胞上表达PD-L1，不表达PD-L2，提示胰岛细胞自身表达的PD-L1是调节自身反应性T细胞活性的重要因素。在由MOG诱导的EAE（实验性自身免疫性脑脊髓炎）C57BLP6模型研究中，阻断PD-1会使疾病加重，伴随脑组织中炎症细胞浸润增多，同时，针对MOG的自身免疫反应加强，产生IFN-γ的T细胞增多，发型超敏反应增强，血中抗MOG抗体水平升高。与NOD鼠不同的是，阻断PD-L2而非PD-L1

可导致疾病加重。PD-L1在EAE发病早期即开始表达，而PD-L2表达较晚且表达量很少。实验性哮喘模型的研究支持这一结果，在肺组织中PD-L1表达远比PD-L2丰富，而PD-L2阻断可使疾病加重。那么，PD-L1在这些组织中的高表达有何意义仍待进一步研究。Hitachi等在类风湿性关节炎病人的关节积液中发现，$CD4^+$T细胞PD-1表达明显高于$CD8^+$T细胞，$CD4^+PD\text{-}1^+$T 细胞大多数同时表达CTLA-4，而不表达CD26。$CD4^+PD\text{-}1^+$T细胞产生IL-10，与$CD4^+PD\text{-}1^-$T细胞相比，其产生的IL-2明显减少。类风湿性关节炎病人的关节积液含有丰富的$PD\text{-}1^+$T细胞，这一亚群的T细胞是无反应性的。

2.3 PD-1与病毒感染

用微阵分析和流式细胞术，Barber等发现PD-1在LCMV慢性感染鼠的功能耗竭性$CD8^+$T细胞（exhausted CD8 T cell）上高表达，而急性感染的功能记忆性$CD8^+$T细胞上不表达。PD-L1在多种感染的细胞上高表达，在慢性LCMV感染的小鼠上以阻断性单抗阻断PD-1/PD-L1通路，加强了$CD8^+$T细胞功能，激活了CTL活性，导致IFN和TNF的产生。Day等检测了HIV感染的特异性$CD8^+$T细胞的PD-1的表达，他们选择的人群是没有抗HIV治疗的HIV患者。结果发现在HIV特异的CD8 T细胞上PD-1明显高表达，且这种表达与对HIV特异性$CD8^+$T细胞功能破坏程度正相关，并与疾病发生过程相关。阻断PD-1/PD-L1通路增强HIV特异性$CD4^+$、$CD8^+$T细胞功能。

发明内容

本发明的目的在于，构建人程序性死亡蛋白-1-IgV（hPD-1-IgV）融合蛋白，使其在体内外能够与其相应的配体 PD-L1 相互结合，从而阻断体内 PD-1/PD-L1 通路，打破肿瘤免疫逃避，并恢复细胞毒性 T 淋巴细胞的杀伤功能，为研制抗肿瘤药物提供新的思路和途径。

为了实现上述任务，本发明所采用的技术方案是：

一种可溶性人程序性死亡蛋白-1-IgV，其特征在于，其蛋白序列如下：

PPTFFPAL LVVTEGDNAT FTCSFSNTSE SFVLNWYRMS PSNQTDKLAA FPEDRSQPGQ DSRFRVTQLP NGRDFHMSVV RARRNDSGTY LCGAISLAPK AQIKESLRAE LRVTERRAEV PTAHPSP。

上述可溶性人程序性死亡蛋白-1-IgV 的制备方法，其特征在于，根据人 PD-1 的结构，应用人工合成的方法获得了 hPD-1-IgV 的基因，目的基因克隆入 pQE-30 原核表达载体，转化大肠杆菌，在大肠杆菌中高效表达，裂菌显示目的蛋白以包涵体形式存在，经包涵体洗涤、亲和层析、凝胶柱层析和透析复性，即可获得纯化的可溶性人程序性死亡蛋白-1-IgV。

以经纯化后得到的可溶性的目的蛋白 hPD-1-IgV 分别以酶联免疫吸附方法（ELISA）和流式细胞术（FCM）检测 shPD-1IgV 与 PD-L1 的结合，以及检测 shPD-1IgV 与抗 PD-L1 单抗竞争结合 PD-L1。MTT 法检测 CTL 对肿瘤细胞的杀伤。以 shPD-1IgV 治疗荷瘤小鼠，测量肿瘤体积，观察其抑瘤效果。以流式细胞术（FCM）检测荷瘤小鼠各组中外周血中 $CD4^+CD25^+$T（Treg）细胞占总 CD4+T 细胞的比例。结果显示：shPD-1IgV 可分别与商品化的 mPD-L1/Fc 和 hPD-L1/Fc 结合，并且 shPD-1IgV 可与肿瘤细胞表面上表达的 PD-L1 结合，shPD-1IgV 可与抗 PD-L1 单抗竞争结合 mPD-L1/Fc。以 shPD-1IgV 治疗的荷瘤小鼠分离的 CTL 与对照组比较有明显的杀伤肿瘤细胞作用。shPD-1IgV 治疗的荷瘤小鼠与对照组比较表现了明显的抑瘤效果，具有统计学意义，$P<0.05$，而且对照组的荷瘤小鼠外周血中 CD4+CD25+T 细胞占总 CD4+T 细胞的比例明显高于 shPD-1IgV 治疗组，为进一步的肿瘤治疗提供了实验依据和奠定了基础。具体内容是：

1、合成 hPD-1-IgV 基因序列

分析 Swiss-Pro 蛋白质数据库 PD-1 的结构，结合文献，截取 PD-1 胞外断 IgV 结构域氨基酸序列，获得 PD-1 胞外断 IgV 的氨基酸序列（命名为：hPD-1-IgV）：

PPTFFPAL LVVTEGDNAT FTCSFSNTSE SFVLNWYRMS PSNQTDKLAA FPEDRSQPGQ DSRFRVTQLP NGRDFHMSVV RARRNDSGTY LCGAISLAPK AQIKESLRAE LRVTERRAEV PTAHPSP

按照 Gene-bank 公布的人 PD-1-IgV 的基因序列，5 端插入 *Bam*HI 酶切位点，3 端引进中止密码子 TGA 并插入 *Sal*I 酶切位点，依据大肠杆菌偏爱的密码子，合成目的基因。

pQE-30 原核表达载体来自 Jieru Meng，Nan Ma 等（参考文献 Jieru Meng，Nan Ma et al. NGR Enhanced the Anti-Angiogenic Activity of tum-5.[J] J. Biochem. 140, (2006)）。通过 BamHI 和 SalI 双酶切将 hPD-1-IgV 基因克隆入表达载体并克隆入载体 pGEX-4T-1 中。

2．构建重组质粒pQE-30/ hPD-1-IgV

将pGEX-4T-1/ hPD-1-IgV载体转化大肠杆菌DH5α，提取质粒DNA后，将其与质粒pQE-30分别用*Bam*HI和*Sal*I进行双酶切。取上述pGEX-4T-1/ hPD-1-IgV获得的小片段（hPD-1-IgV）与从pQE-30中酶切的大片段相连接。酶切产物用2.0%琼脂糖凝胶电泳分离，分别回收pGEX-4T-1获得的小片段和pQE-30中的大片段。将回收后的小片段和大片段用T4连接酶在16℃连接过夜，转化DH5α感受态细胞，铺板、培养，挑取单克隆，提取质粒，经酶切鉴定和DNA测序，获得含有hPD-1-IgV融合基因原核克隆载体，命名pQE-30/ hPD-1-IgV。

3．重组蛋白的诱导表达、纯化与鉴定

3.1 pQE-30/ hPD-1-IgV工程菌的诱导表达

将含有pQE-30-hPD-1IgV重组质粒的菌株，接种于10ml含Amp的LB培养液中，于37℃培养过夜，次日按1%的比例转接于10 ml含Amp的LB培养液中，于37℃振荡培养至对数生长期（A_{600nm}=0.4-0.6）时，加入IPTG至终浓度为1 mmol/L诱导表达，于37℃振荡培养3-5h。离心收集菌体，SDS-PAGE电泳进行鉴定。

3.2 目的蛋白包涵体的分离和纯化

取10g诱导的菌体，按1g湿重对7ml的比例，用裂解缓冲液STE（50mmol/L Tris.Cl pH 8.0 ，50mmol/L NaCl， 1mmol/L EDTA） 重悬，-20℃冷冻过夜。次日于室温水浴中融化，参照分子克隆第三版溶菌酶法裂菌，接着再采用300W超声破菌，其中超声5s，间隔10s，共进行100次。超声完成后，12000rpm/min，4℃离心20min，弃除上清，沉淀用包涵体洗涤液A（1%TritonX-100，1mmol/L EDTA，1%DOC，50mmol/LTris.Cl pH8.5，100mmol/L NaCl）重悬，12000r/min 4℃离心20min，弃上清。用Ni-NTA柱亲和层析纯化所溶解的沉淀（包涵体）。按1g菌体加10ml裂菌缓冲液的比例将菌体重悬，冰浴条件下进行超声裂菌。12000rpm，离心15min，弃上清，1g沉淀加10ml6mol/L尿素，0.1mol/LNaH_2PO_4，0.01mol/LTris（pH 7.5）。4℃搅拌过夜，12000rpm，离心15min，共离心2次，收集上清待用。经洗涤的包涵体以1g湿重对10ml的比例，溶于6mol/L尿素，0.1mol/LNaH_2PO_4，0.01mol/LTris pH 7.5的缓冲液中，4℃搅拌过夜，12000r/min 离心30min，收集上清，即为目的蛋白粗提液。SephacrylS-300（1.6cm×80cm）凝胶层析，以工作液（6mol/L尿素，0.1mol/LNaH_2PO_4，0.01mol/Ltris pH 7.5）充分平衡后，目的蛋白粗提液2ml上柱，以工作液洗脱，流速1ml/min，收集含有目的蛋白的峰，然后进行亲和层析纯化。用6mol/L尿素，0.1mol/LNaH_2PO_4，0.01mol/LTris（pH 7.5）充分平衡Ni柱，先用含10mmol咪唑的6M尿素，0.1mol/LNaH_2PO_4，0.01mol/LTris（pH 7.5）洗脱杂蛋白，再用含250mmol咪唑的6mol/L尿素，0.1mol/LNaH_2PO_4，0.01mol/LTris（pH 7.5）洗脱目的蛋白。收集洗脱峰，纯化产物透析至2M尿素，0.1mol/LNaH_2PO_4，0.01mol/LTris（pH 7.5）复性，终浓度1mg/ml。最后透析入0.02mol/LPBS（pH 7.2）中，用Lowery法测定蛋白浓度。

3.3 目的蛋白的复性

应用包涵体溶解液稀释亲和层析后的样品，使蛋白浓度约为0.1mg/ml，梯度透析，透析外液加入$CuSO_4$和β－巯基乙醇作为氧化还原系统，4℃透析，每6小时换液一次，梯度为，4M尿素，2M尿素，1M尿素，最后透析到双蒸水，离心留上清，冻干。HPLC检

测蛋白纯度。

附图说明

图 1 是重组表达质粒的构建。其中，图（A）是重组表达质粒 pQE-30-rhPD-1IgV 的酶切鉴定，图中的标记 1：DNA marker（DL2000），2：pQE-30-rhPD-1IgV digested by *Bam*HI 和 *Sal*I；图（B）是 rhPD-1IgV 区的克隆策略；

酶切载体 pGEX-4T-1-rhPD-1IgV 和 pQE-30，经连接获得重组融合表达载体 pQE-30-rhPD-1IgV，以载体 pQE-30-rhPD-1IgV 转化感受态细胞 DH5α，扩增后提取质粒，用 *Bam*HI 和 *Sal*I 进行双酶切鉴定图（A），获得与预期结果一致大小的插入片段。经华诺生物技术服务有限公司测序证实，该插入片段的基因序列图（B）与预期完全一致。

图 2 是蛋白质的表达纯化与鉴定。其中，图（A）中的标记为：1：分子量蛋白质标准，2：pQE-30 空质粒，3：诱导后的 rhPD-1IgV，4：裂菌上清，5：裂菌沉淀。

重组质粒转化大肠杆菌后，经诱导表达，稳定筛选，构建工程菌。经发酵后，收集大量菌体，裂菌显示目的蛋白以包涵体形式存在（图A），经对包涵体洗涤，SephacrylS-300凝胶柱层析（图B）亲和层析（图C），SDS-PAGE显示蛋白单一条带（图D），Western-blot鉴定显示能与抗His单克隆抗体特异性结合（图E）。HPLC分析表明其纯度大于95%。

图3是融合蛋白hPD-1IgV体外分子水平与鼠或人B7-H1/Fc（mB7-H1/Fc或hB7-H1/Fc）结合检测。结合ELISA表明shPD-1IgV能够分别与hB7-H1/Fc和mB7-H1/Fc结合（图A，B）。shPD-1IgV能够与包被每孔100nghB7-H1/Fc和mB7-H1/Fc有效的结合，结合呈现剂量依赖性，表明明shPD-1IgV能够与hB7-H1/Fc和mB7-H1/Fc结合，而且，在1000ngshPD-1IgV的剂量下，shPD-1IgV与hB7-H1/Fc或mB7-H1/Fc结合呈现饱和。竞争ELISA结果显示shPD-1IgV可以阻断hB7-H1/Fc与抗hB7-H1单抗的结合（图C）。100ng抗hB7-H1单抗与100nghB7-H1/Fc共同孵育后，增加shPD-1IgV的剂量可以减少抗hB7-H1单抗与hB7-H1/Fc的结合。其中，shPD-1IgV增加到800ng后，阻断作用趋于平缓。

图4是应用流式细胞术检测融合蛋白hPD-1IgV细胞水平上与B7-H1的结合。其中（A）阴性对照，（B）SP2/0细胞，（C）HT-29细胞；shPD-1IgV能够与细胞表面上的B7-H1结合（图4）。与对照比较，shPD-1IgV能够与SP2/0细胞（小鼠骨髓瘤细胞）表达的B7-H1结合，结合率为12%，而且shPD-1IgV能够与IFN-γ刺激的HT-29细胞表达的B7-H1结合，结合率为15.6%。

图5是MTT法检测融合蛋白hPD-1IgV激起的CTL杀伤活性。与对生理盐水照组比较，治疗组（剂量I组，剂量II组，剂量III组）具有明显的CTL杀伤活性，分别是52.3%，55.4%和56.1%。CTX组的CTL杀伤活性明显高于对照组，但是明显低于治疗组。

表1是各组CTL杀伤活性比较。

表1.各组CTL杀伤活性比较

	12.5：1	25：1	50：1
剂量I组	0.311±0.015	0.461±0.022	1.231±0.134
剂量II组	0.240±0.043	0.447±0.034	1.225±0.162
剂量III组	0.329±0.031	0.488±0.030	1.296±0.104
生理盐水对照组	0.190±0.065	0.247±0.034	0.392±0.043

图6是流式细胞术检测荷瘤小鼠外周血中CD4+CD25+Treg细胞占CD4+T细胞的比例。其中，图（A）是生理盐水对照组，图（B）是剂量I组，图（C）是剂量II组，图（D）是剂量剂量III组，图（E）是对照直方图；与生理盐水对照组比较，治疗组外周血中$CD4^+CD25^+$Treg细胞占$CD4^+$T细胞的比例明显减少（图A，B，C，D，E，），表2 是CD4CD25 Treg百分比。

表 2

CD4CD25 Treg百分比	
剂量I组	24.8±2.876
剂量II组	19.7±2.934
剂量III组	18.2±2.673
对照组	28.3±3.002
CTX组	10.3±3.231

各治疗组中外周血中$CD4^+CD25^+$Treg细胞占$CD4^+$T细胞的比例分别是24.8%、19.7%、18.2%，明显低于对照组的28.3%。统计学分析表明，治疗组各组与对照组比较有明显差异，$P<0.05$。同时，与治疗II组和治疗III组比较，治疗I组有明显差异，$P<0.05$。但是，治疗治疗II组和治疗III组没有统计学差异，$P>0.05$。

图 7 是 hPD-1IgV 融合蛋白对 SP2/0 荷瘤的小鼠的抑瘤作用。图中，$^aP<0.05$，与生理盐水组比较；$^cP<0.05$，与 CTX 组比较；应用 shPD-1IgV 对 BALB/c 荷瘤鼠治疗 10 天，结果显示，与生理盐水组比较，治疗组有显著性差异（$P<0.05$），但是，5mg/Kg 治疗组与10mg/Kg治疗组之间没有统计学意义，但与15mg/Kg治疗组都有统计学意义（$P<0.05$）。同时，治疗组与环磷酰胺（CTX）组比较也有显著性差异（$P<0.05$）。

以下结合附图和实施例对本发明作进一步的详细描述。

具体实施方式

4. 融合蛋白 PD-1-IgV 的构建、表达及纯化

4.1 PD-1-IgV 基因的设计

分析 Swiss-Pro 蛋白质数据库 PD-1 的结构，结合文献，截取 PD-1 胞外断 IgV 结构域氨基酸序列，获得 PD-1 胞外断 IgV 的氨基酸序列（命名为：hPD-1-IgV）。按照 Gene-bank 公布的人 PD-1-IgV 的基因序列，5 端插入 *Bam*HI 酶切位点，3 端引进中止密码子 TGA 并插入 *Sal*I 酶切位点，依据大肠杆菌偏爱的密码子，合成目的基因。合成的基因序列，

GGA TCC CAG TAT ATA AAA GCA AAT TCT AAA TTT ATA GGT ATA ACT GAA GCA GCA GCA GAA TTC GAC CTA TAT GTG GTA GAG TAT GGT AGC AAT ATG ACA ATT GAA TGC AAA TTC CCA GTA GAA AAA CAA TTA GAC CTG GCT GCA CTA ATT GTC TAT TGG GAA ATG GAG GAT AAG AAC ATT ATT CAA TTT GTG CAT GGA GAG GAA GAC CTG AAG GTT CAG CAT AGT AGC TAC AGA CAG CGT GCC CGG CTG TTG AAG GAC CAG CTC TCC CTG GGA AAT GCT GCA CTT CAG ATC ACA GAT GTG AAA TTG CAG GAT GCA GGG GTG TAC CGC TGC ATG ATC AGC TAT GGT GGT GCC GAC TAC AAG CGA ATT ACT GTG AAA GTC AAT TGA GTC GAC

其蛋白序列为：

PPTFFPAL LVVTEGDNAT FTCSFSNTSE SFVLNWYRMS PSNQTDKLAA FPEDRSQPGQ DSRFRVTQLP NGRDFHMSVV RARRNDSGTY LCGAISLAPK AQIKESLRAE LRVTERRAEV PTAHPSP。

目的基因被克隆入 pGEX-4T-1 载体（上海生工生物技术公司合成并提供）。

4.1.1 感受态细胞的制备

取 DH5α 甘油菌种按 1:100 的比例接种入的 LB 培养基中，37℃振荡培养过夜，次日转接一次，继续培养至 OD_{600} 约 0.4 左右。（无菌操作）将菌液冰浴 10 min，离心（3000 rpm×5 min，4℃）后弃去上清，加入 1/2 体积预冷的 100 mmol/L $CaCl_2$，轻轻吹起沉淀，冰浴 40min，离心（3000 rpm×5 min，4℃），弃上清后加入 1/25 体积的含 25%甘油的 100 mmol/L $CaCl_2$，吹起沉淀，分装入 Eppendorf 管中，-70℃保存备用。

4.1.2 感受态细胞的转化、培养

将大肠杆菌感受态细胞从-70℃中取出，冰浴融解5-10分钟，加入含有目的基因的 pUC57，轻微混匀，继续冰浴30分钟，然后铺板，37℃培养过夜。

4.1.3 质粒的提取与鉴定

在质粒转化后37℃过夜培养的培养皿上挑取边缘整齐，生长状态良好的克隆，接种到

10 ml 含Amp的LB培养基中，37℃、200 rpm培养8小时，用质粒提取试剂盒提取质粒，用*Bam*HI和双酶切后，行琼脂糖凝胶电泳观察是否有插入片断以及插入的片断是否与预期的长度一致，将酶切鉴定阳性的克隆送上海博亚公司进行DNA测序。

4.2 重组质粒pQE-30/ hPD-1-IgV的构建、表达、纯化和复性

4.2.1 重组质粒pQE-30/ hPD-1-IgV的构建

将pGEX-4T-1/ hPD-1-IgV载体转化大肠杆菌DH5α，提取质粒DNA后，将其与质粒pQE-30分别用*Bam*HI和*Sal*I进行双酶切。取3.1中所述pGEX-4T-1获得的小片段与从pQE-30中的大片段相连接。酶切产物用2.0%琼脂糖凝胶电泳分离，分别回收pGEX-4T-1获得的小片段和pQE-30中的大片段。将回收后的小片段和大片段用T4连接酶在16℃连接过夜，转化DH5α感受态细胞，铺板、培养，挑取单克隆，提取质粒，经酶切鉴定和DNA测序，获得含有hPD-1-IgV融合基因原核克隆载体，命名pQE-30/ hPD-1-IgV。

4.2.2重组蛋白的诱导表达、纯化与鉴定

4.2.2.1 pQE-30/ hPD-1-IgV工程菌的诱导表达

将含有pQE-30-hPD-1IgV重组质粒的菌株，接种于10ml含Amp的LB培养液中，于37℃培养过夜，次日按1%的比例转接于10 ml含Amp的LB培养液中，于37℃振荡培养至对数生长期（A_{600nm}=0.4～0.6）时，加入IPTG至终浓度为1 mmol/L诱导表达，于37℃振荡培养3-5h。离心收集菌体，SDS-PAGE电泳进行鉴定。

4.2.2.2 目的蛋白包涵体的分离和纯化

取10g诱导的菌体，按1g湿重对7ml的比例，用裂解缓冲液STE（50mmol/L Tris.Cl pH 8.0，50mmol/L NaCl，1mmol/L EDTA）重悬，-20℃冷冻过夜。次日于室温水浴中融化，参照分子克隆第三版溶菌酶法裂菌，接着再采用300W超声破菌，其中超声5s，间隔10s，共进行100次。超声完成后，12000rpm/min，4℃，离心20min，弃除上清，沉淀用包涵体洗涤液A（1%TritonX-100，1mmol/L EDTA，1%DOC，50mmol/LTris.Cl pH8.5，100mmol/L NaCl） 重悬，12000r/min 4℃离心20min，弃上清。用Ni-NTA柱亲和层析纯化所溶解的沉淀（包涵体）。按1g菌体加10ml裂菌缓冲液的比例将菌体重悬，冰浴条件下进行超声裂菌。12000rpm，离心15min，弃上清，1g沉淀加10ml6mol/L尿素，0.1mol/LNaH_2PO_4，0.01mol/LTris（pH 7.5）。4℃搅拌过夜，12000rpm，离心15min，共离心2次，收集上清待用。经洗涤的包涵体以1g湿重对10ml的比例，溶于6mol/L尿素，0.1mol/LNaH_2PO_4，0.01mol/LTris pH 7.5的缓冲液中，4℃搅拌过夜，12000r/min 离心30min，收集上清，即为目的蛋白粗提液。SephacrylS-300（1.6cm×80cm）凝胶层析，以工作液（6mol/L尿素，0.1mol/LNaH_2PO_4，0.01mol/LTris pH 7.5）充分平衡后，目的蛋白粗提液2ml上柱，以工

作液洗脱，流速1ml/min，收集含有目的蛋白的峰，然后进行亲和层析纯化。用6mol/L尿素，0.1mol/LNaH_2PO_4，0.01mol/LTris（pH 7.5）充分平衡Ni柱，先用含10mmol咪唑的6M尿素，0.1mol/LNaH_2PO_4，0.01mol/LTris（pH 7.5）洗脱杂蛋白，再用含250mmol咪唑的6mol/L尿素，0.1mol/LNaH_2PO_4，0.01mol/LTris（pH 7.5）洗脱目的蛋白。收集洗脱峰，纯化产物透析至2M尿素，0.1mol/LNaH_2PO_4，0.01mol/LTris（pH 7.5）复性，终浓度1mg/ml。最后透析入0.02mol/LPBS（pH 7.2）中，用Lowery法测定蛋白浓度。

4.2.3 目的蛋白的复性

应用包涵体溶解液稀释亲和层析后的样品，使蛋白浓度约为0.1mg/ml，梯度透析，透析外液加入$CuSO_4$和β－巯基乙醇作为氧化还原系统，4℃透析，每6小时换液一次，梯度为，4M尿素，2M尿素，1M尿素，最后透析到双蒸水，离心留上清，冻干。HPLC检测蛋白纯度。

5.融合蛋白 hPD-1-IgV 体外活性检测

5.1 融合蛋白 hPD-1-IgV 体外分子水平上的检测

5.1.1 ELISA 检测 shPD-1IgV 与 hB7H1/Fc 结合活性

取复性后的 shPD-1IgV 倍比稀释后包被 96 孔板，每个浓度设 3 个复孔，4℃包被过夜，封闭 2h，洗涤 6 次*2 分钟/次，每孔加入 100ng hB7-H1/Fc，室温 1h，洗涤 6 次*2 分钟/次，加入抗 hB7-H1 单抗，室温 1h，洗涤 6 次，每次 2 分钟，加抗鼠二抗，室温 30min，洗涤 6 次，每次 2 分钟/次，OPD 显色后检测 A_{490nm} 值，实验中以无关 his 融合蛋白为对照。方法步骤如下：

包被：取 96 孔 ELISA 板，将倍比稀释后的 shPD-1IgV 融合蛋白溶解于包被缓冲液中，4℃包被过夜；

倒掉包被液，加入封闭液，室温封闭 2 h；

弃去封闭液，用洗涤液洗 6 次，每次 2 min；

分别小鼠抗人 B7-H1 单抗（500μg /mL）100μL/孔，室温孵育 1h；

弃去单抗，用洗涤液洗 6 次，每次 3 min；

加入 HRP 标记的抗小鼠二抗，100μL/孔，室温孵育 1h；

弃去二抗，用洗涤液洗 6 次，每次 3 min；

加入底物显色液，100μL/孔，室温，显色 10-20 min；

加入终止液，50μL/孔，终止反应；

酶标仪读数，测定每孔在 490nm 的吸光值。

5.1.2 ELISA 检测 shPD-1IgV 与 mB7H1/Fc 结合活性

96 孔板包被不同浓度的 mB7-H1/Fc，每个浓度设 3 个复孔，4℃包被过夜，封闭 2h，洗涤 6 次*2 分钟/次，每孔加入 500ugshPD-1IgV，室温 1h，洗涤 6 次*2 分钟/次，加入抗 his 单抗，室温 1h，洗涤 6 次*2 分钟/次，加抗鼠二抗，室温 30min，洗涤 6 次*2 分钟/次，OPD 显色后检测 A_{490nm} 值，实验中以无关 his 融合蛋白为对照。

5.1.3 ELISA检测shPD-1IgV对hB7-H1/Fc与抗hB7H1单抗的竞争活性

96孔板包被100ng hB7-H1/Fc，设3个复孔，4℃包被过夜，封闭2h，洗涤6次*2分钟/次，每孔加入倍比稀释的shPD-1IgV和100ng抗hB7-H1单抗，室温1h，洗涤6次*2分钟/次，加抗鼠二抗，室温30min，洗涤6次*2分钟/次，OPD显色后检测A_{490nm}值，实验中以无关his融合蛋白为对照。

5.2 流式细胞术检测细胞水平上检测融合蛋白hPD-1IgV与B7H1结合活性

人HT-29细胞贴壁后，加入终浓度为20ng/mL的IFN-γ，刺激2天后HT-29细胞高表达B7-H1分子；小鼠SP2/0细胞（小鼠骨髓瘤细胞）低表达B7-H1蛋白。以shPD-1IgV融合蛋白流式细胞术检测与HT-29细胞和SP2/0细胞表达的B7-H1结合。方法步骤如下：

分别制备 HT-29 细胞和 SP2/0 细胞悬液，用 10%FCS RPMI1640 调整细胞浓度为 5×10^6-1×10^7/ml。取 40μl 细胞悬液加入预先有特异性抗 His mAb（5-50μl）的小玻璃管或塑料离心管，再加 50μl 1：20（用 DPBS 稀释）灭活正常兔血清，4℃ 30min。用洗涤液洗涤 2 次，每次加洗涤液 2ml 左右（1000rpm×5min）。弃上清，加入 50μl 工作浓度的羊抗鼠荧光标记物，充分振摇，4℃30min。用洗涤液洗涤 2 次，每次加液 2ml 左右（1000rpm*5min）。加 500μl 固定液。

试剂配制如下：

DPBS（10×，贮存液）：

NaCl：80g，KCl：2g，Na_2HPO4：11.5g，KH_2PO4：2g，蒸馏水加至 1000mL，临用前稀释。

洗涤液：DPBS×1：900 mL，FCS：50 mL，4% NaN_3：50 mL。

固定液：DPBS×1：1000 mL，Glucose：20g，Formaldehyde：10 mL，NaN_3：0.2g。

6. 融合蛋白 hPD-1-IgV 体内活性观察

6.1 接种肿瘤细胞

BALB/c小鼠，共40只，体重：18-22g，雄性。取对数生长期状态的SP2/0肿瘤细胞，按照1×10^7个细胞/只接种至小鼠背部皮下。每天观察小鼠体内肿瘤体积变化，待观察到形成肿瘤后进行分组。

6.2 荷瘤小鼠分组

选取肿瘤体积大小均一的荷瘤小鼠，剔除肿瘤体积太大或太小的荷瘤小鼠。将其随机分5组，每组6只。生理盐水组对照组；剂量I组：每kg鼠重5mg shPD-1IgV融合蛋白；剂量II组：每kg鼠重10mg shPD-1IgV融合蛋白；剂量III组：每kg鼠重15mgshPD-1IgV融合蛋白；设环磷酰胺阳性对照组。

6.3 给药方式和剂量

腹腔注射，生理盐水组每12小时给生理盐水一次，治疗组（剂量I组，剂量II组，剂量III组）按照剂量每12小时给药一次，环磷酰胺（CTX）组每24小时给药一次。连续10天，每天测量肿瘤的长径和短径，按下列公式计算肿瘤体积TV （mm^3） = $a\times b^2\times\pi/6$，其中，a为肿瘤长径，b为与a垂直的短径。

6.4 流式细胞术检测外周血中$CD4^+CD25^+$Treg细胞占$CD4^+$T细胞的比例

各组荷瘤小鼠，眼球取血，每毫升血中加1.5-2.4mgEDTA防止凝血。取100μl抗凝血，分别加抗CD4和CD25的直标抗体，室温避光20-30min。加2ml红细胞裂解液（82.9g/LNH_4Cl，10g/L$KHCO_3$，370mg/LEDTA），轻轻混匀，室温放置10min。4℃，1000r/min离心5min，弃上清。加2ml洗涤液（8g/LNaCl，0.2g/LKCl，1.15g/LNa_2HPO_4，0.2g/LKH_2PO_4，5%FCS，4%NaN_3），轻轻混匀，4℃，1000r/min 离心5min，弃上清。重复一次。加500μl固定液（8g/LNaCl，0.2g/LKCl，1.15g/LNa_2HPO_4，0.2g/LKH_2PO_4，2%葡萄糖，1%甲醛，0.02% NaN_3）。FCM检测外周血中$CD4^+CD25^+$Treg细胞占$CD4^+$T细胞的比例。

6.5 MTT法检测CTL杀伤活性

a. 脾细胞的分离：最后一次测量肿瘤体积后，断颈法处死小鼠，无菌条件下打开腹腔取脾。将脾浸入生理盐水中， 在80目钢网上轻轻研磨， 收集脾细胞。1500r/min离心15min，弃上清。加入红细胞裂解液（Tris-NH_4Cl，0.16mol/L的NH_4Cl和0.17mol/L的Tris按9：1的比例混合，调pH=7.2），作用1min，迅速加入生理盐水，洗2遍。用含100U/ml青霉素，100μg/ml链霉素，10%FBS，50U/mlIL-2的RPMI1640重悬，台盼蓝计数后待用。

b. CTL的活化：肿瘤细胞经50μg/ml丝裂霉素C预处理，与脾细胞共培养，10%FCS，RPMI培养基，100U/ml青霉素，100μg/ml链霉素。在第0，2，4天加入IL-2（2units/ml）。5天后，收集非贴壁细胞，用来测定杀伤活性。

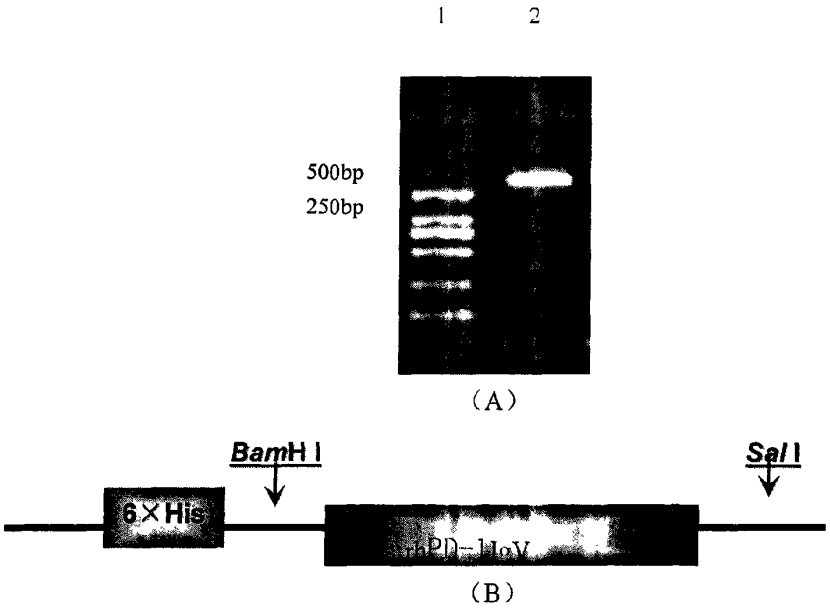
c. MTT检测杀伤活性：将肿瘤细胞以每孔5000个细胞的浓度加入到96孔板中。待细胞贴壁后，将制备的脾细胞按12.5：1和25：1以及50：1的比例加入到上述96孔板中。每个浓度设3个复孔。37℃，5%CO_2孵箱培养72h。弃去培养基，每孔加入50μlMTT反应液

（0.5mg/ml无血清1640培基）。37℃，5%CO_2孵箱培养4h。弃去MTT反应液，每孔加入100μlDMSO，室温振荡10min。酶标仪测490nm处的吸光值（A值）。计算杀伤率。

6.6 SPSS统计分析对统计数据进行t检验分析，方差分析。

可溶性人程序性死亡蛋白-1-IgV 的蛋白序列

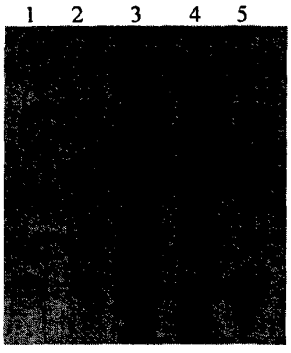
PPTFFPAL LVVTEGDNAT FTCSFSNTSE SFVLNWYRMS PSNQTDKLAA FPEDRSQPGQ DSRFRVTQLP NGRDFHMSVV RARRNDSGTY LCGAISLAPK AQIKESLRAE LRVTERRAEV PTAHPSP。


1
2
500bp
250bp
BamH I
Sal I
6×His
PD-HoV

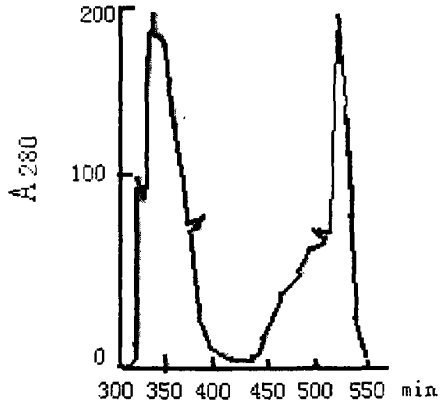
（A）

（B）

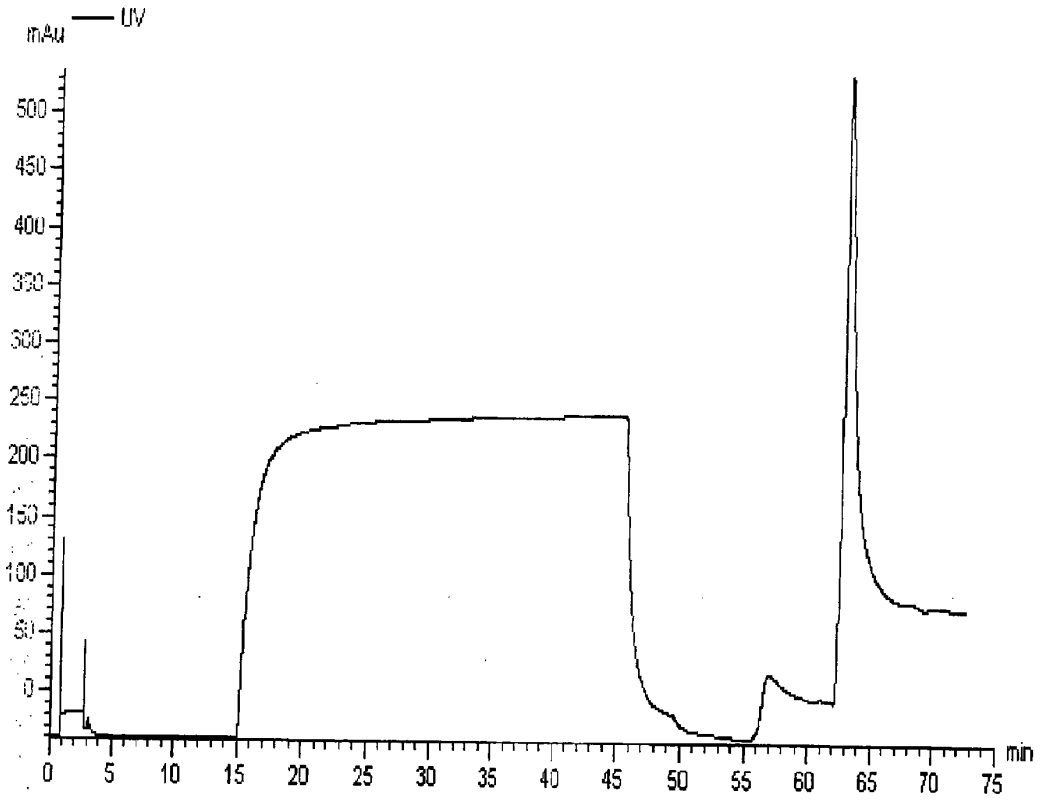
图 1


1 2 3 4 5

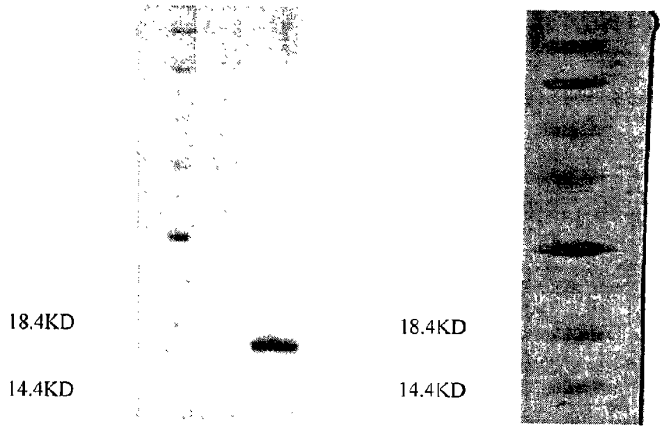
（A）


200
100
0
A 280
300 350 400 450 500 550 min

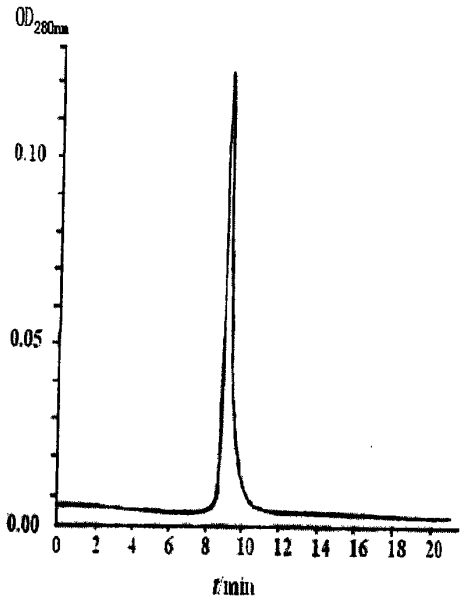
（B）


mAu
UV
500
450
400
350
300
250
200
150
100
50
0
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
min

(C)

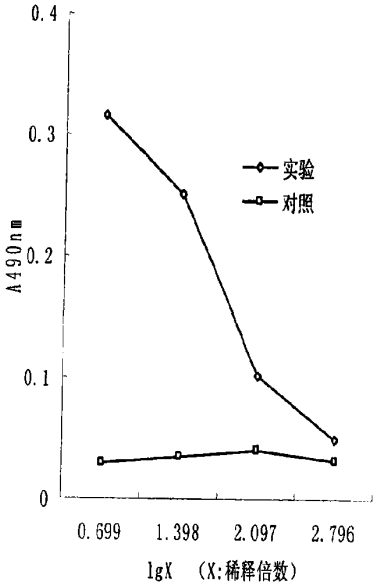

18.4KD
14.4KD
18.4KD
14.4KD

(D) (E)

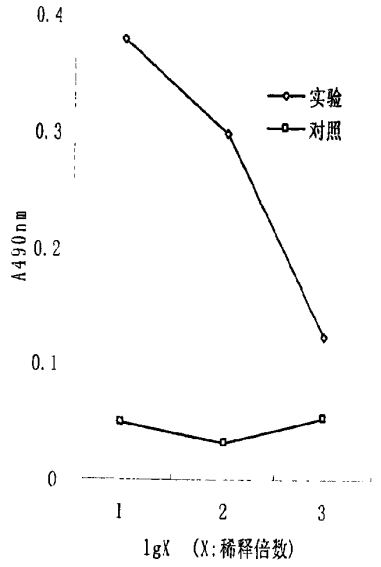


(F)

图 2

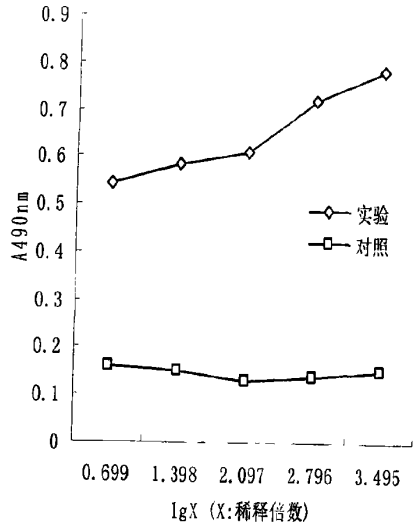


(A)



(B)

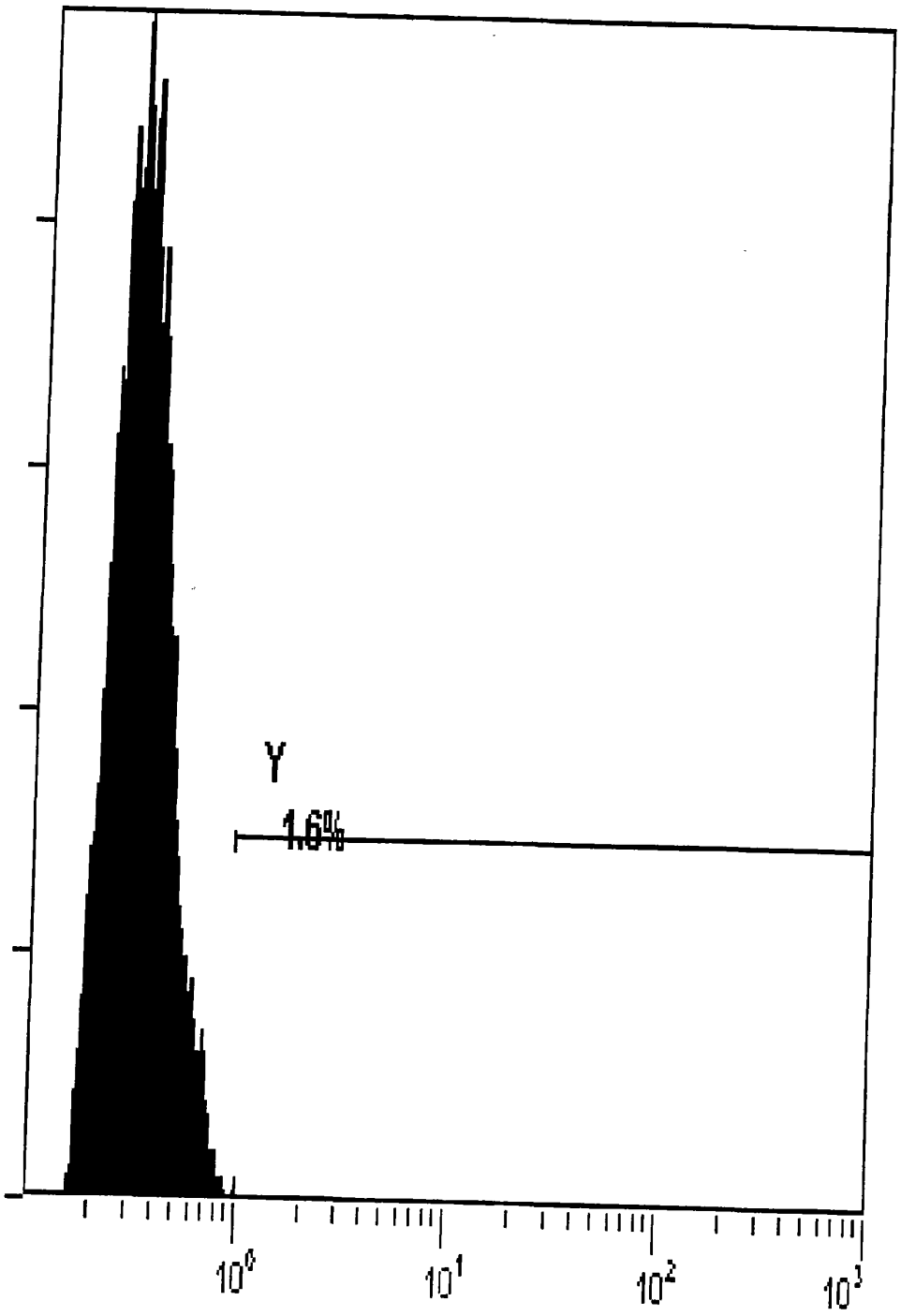
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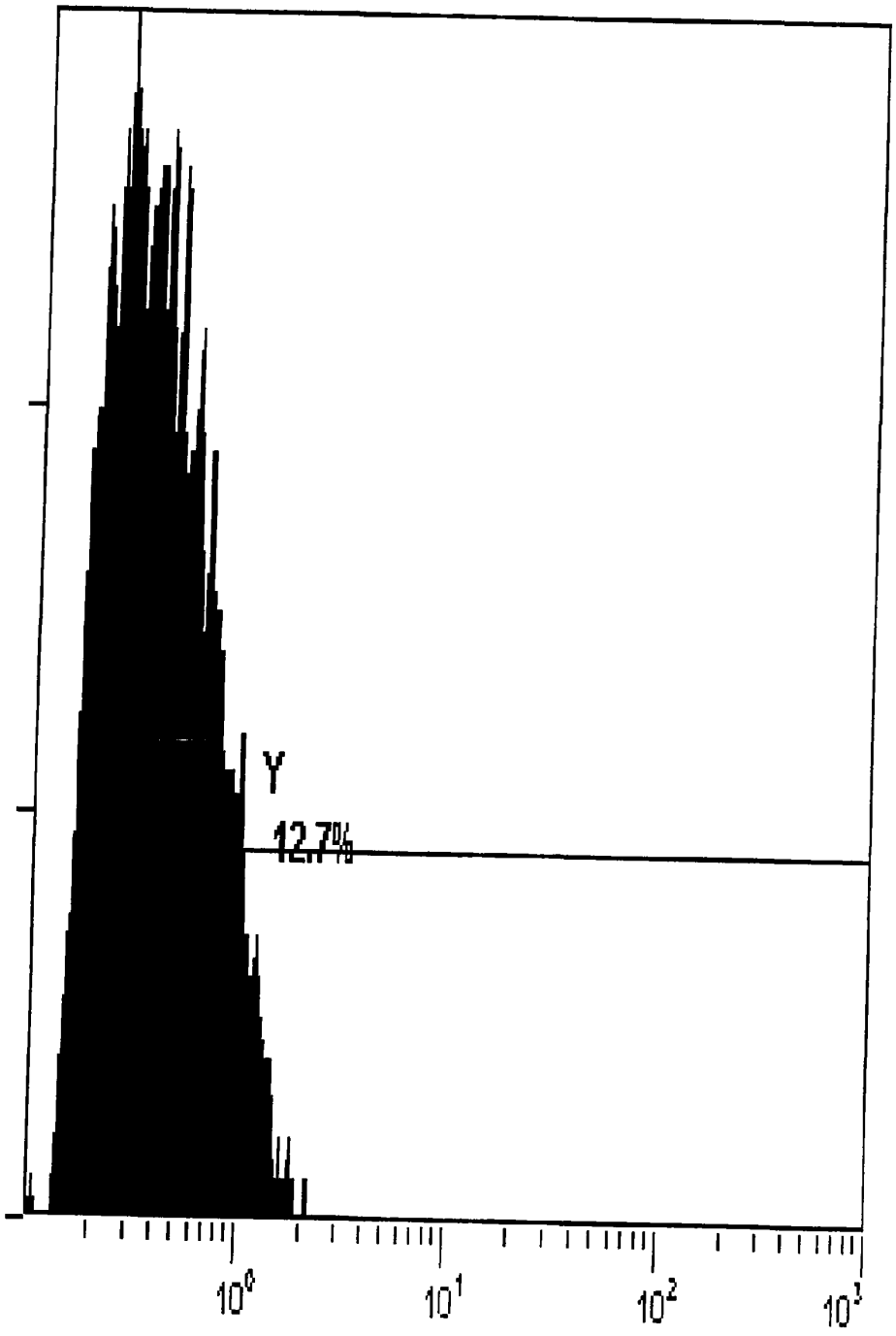
(C)

图 3

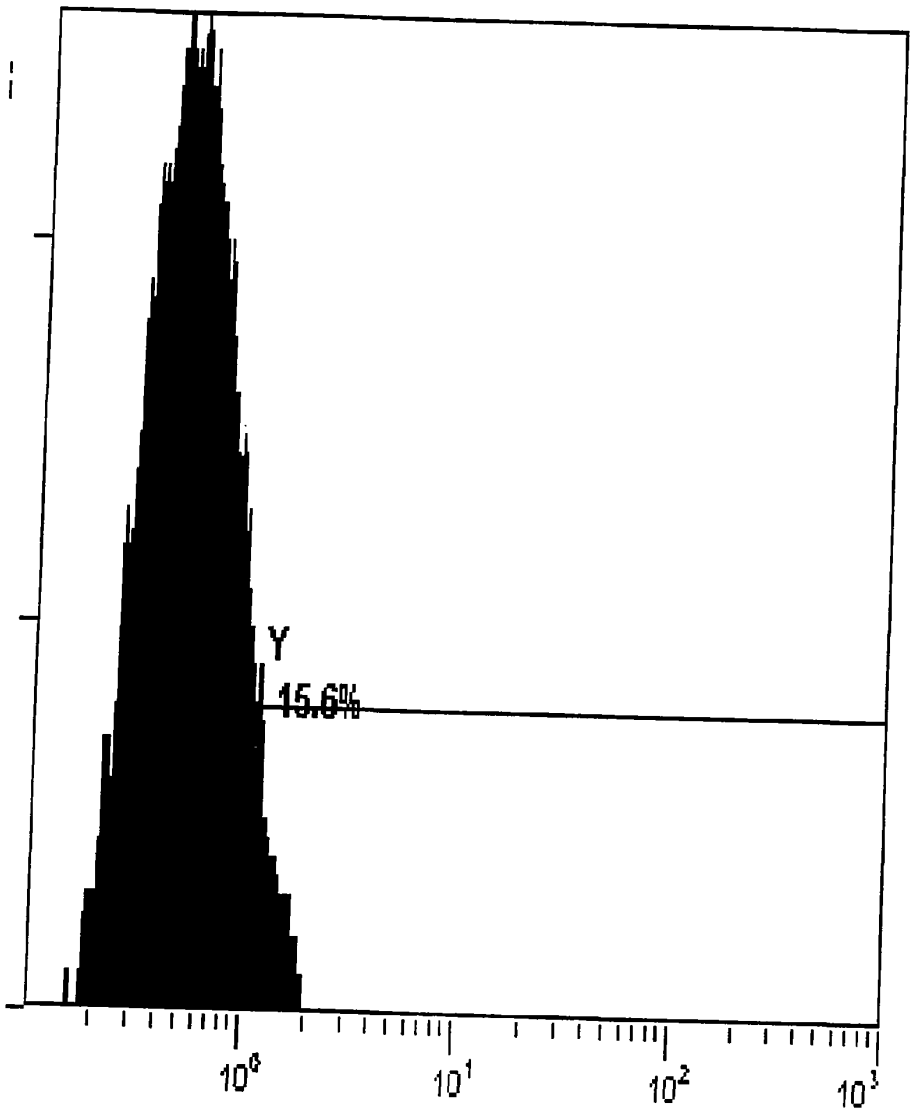
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（A）



（B）



（C）

图 4

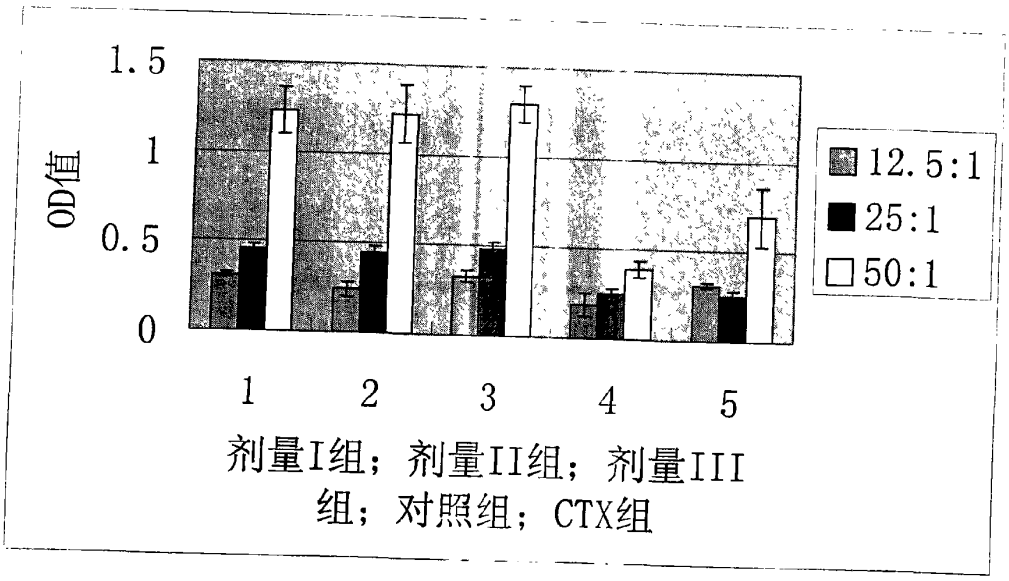
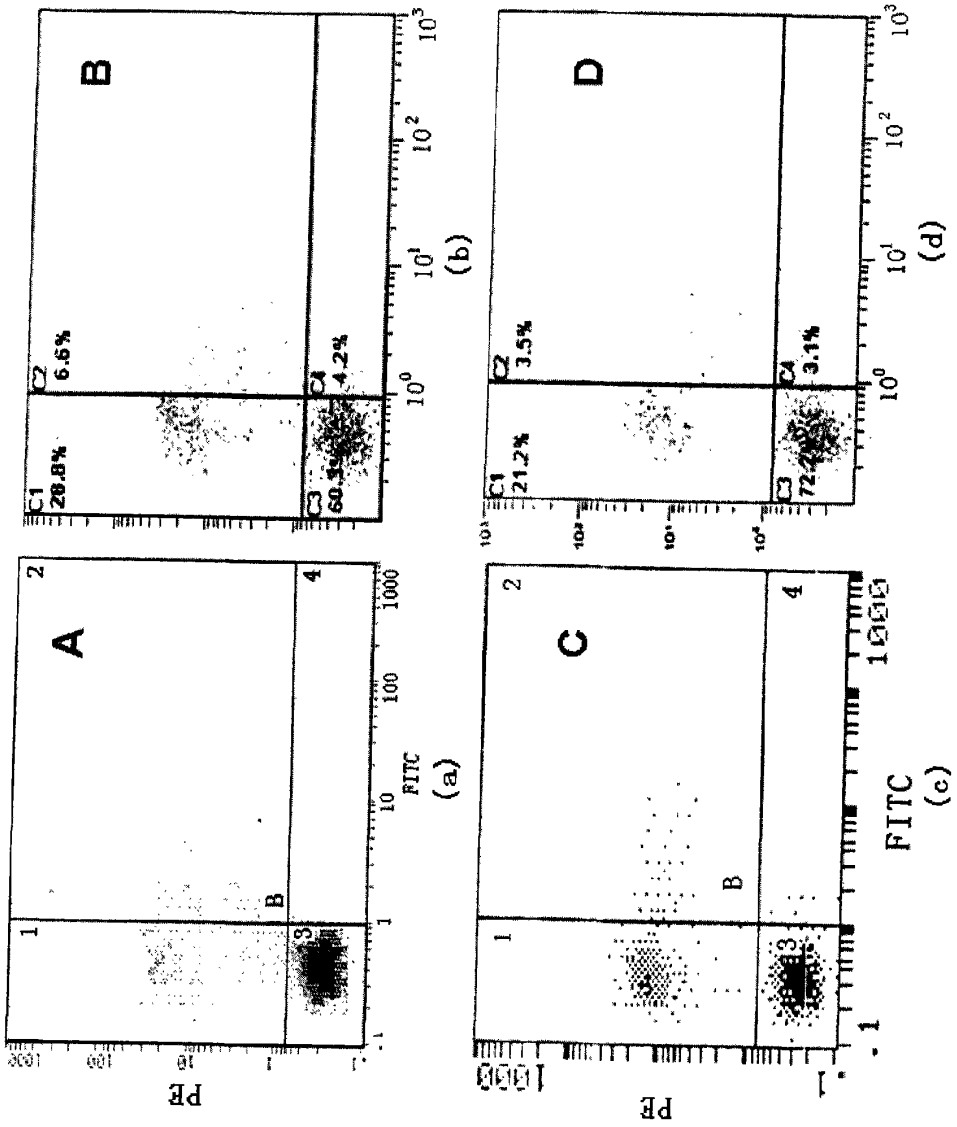
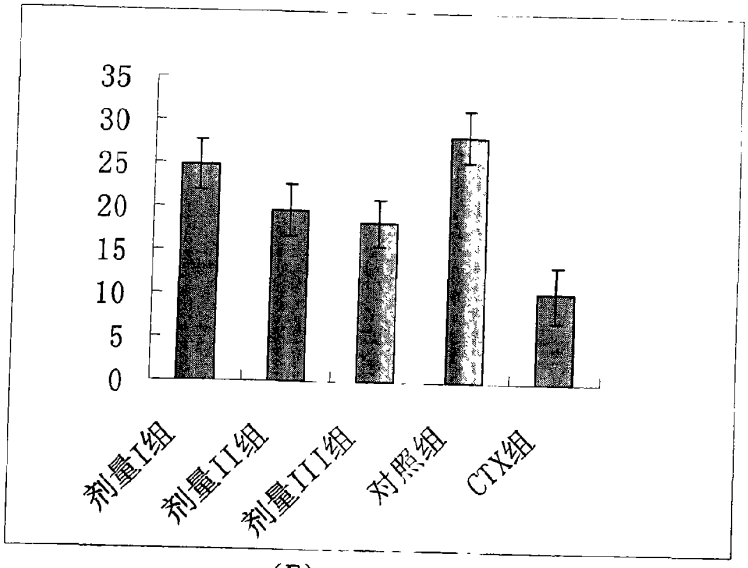


图 5



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35
30
25
20
15
10
5
0
剂量I组
剂量II组
剂量III组
对照组
CTX组

(E)

图 6

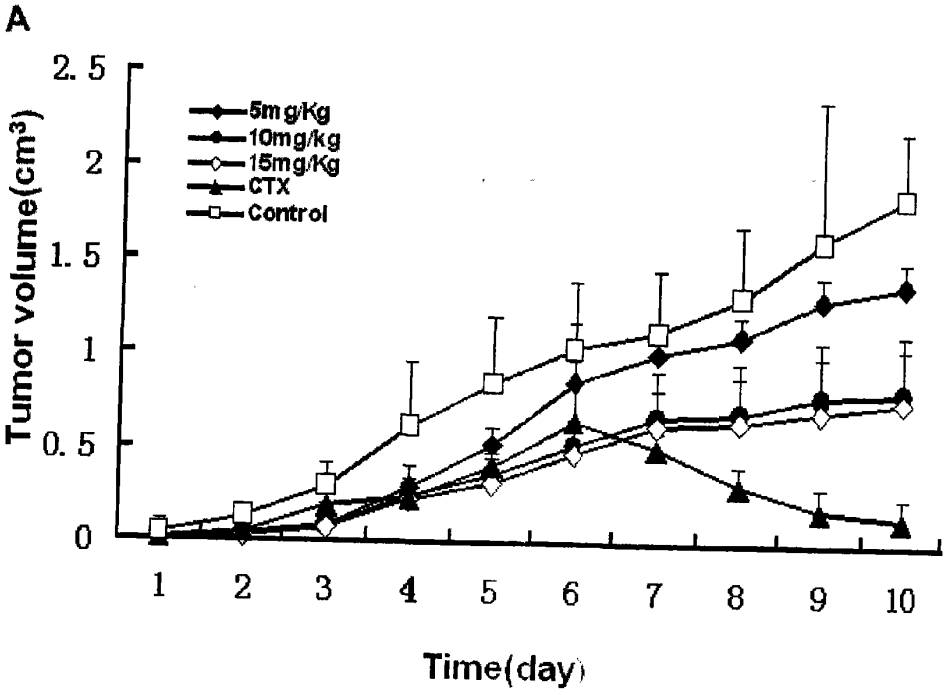

A
2.5
2
1.5
1
0.5
0
Tumor volume(cm³)
5mg/Kg
10mg/kg
15mg/Kg
CTX
Control
1
2
3
4
5
6
7
8
9
10
Time(day)

图 7

Electronic Acknowledgement Receipt	
EFS ID:	29797591
Application Number:	14400667
International Application Number:	
Confirmation Number:	8185
Title of Invention:	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
First Named Inventor/Applicant Name:	John P. Cogswell
Customer Number:	115018
Filer:	Ji Eun Kim/Dana Bennett Jackson
Filer Authorized By:	Ji Eun Kim
Attorney Docket Number:	3338.0630002/ELE/C-K/AHB
Receipt Date:	17-JUL-2017
Filing Date:	12-NOV-2014
Time Stamp:	14:00:26
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	33380630002ids.pdf	116320 441bad2a6e7b03af6b640cafdbefaba73db39661	no	3
Warnings:					

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	33380630002sbform.pdf	99714 113f1eeaa88fcdcb77d01b6b8bc1bae4f1325e71	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Foreign Reference	FP2_WO2006133396A2.pdf	4156563 b586831c1924e87b660bb2b91eed0638fde2144c	no	86
Warnings:					
Information:					
4	Foreign Reference	FP1_CN101215329A.pdf	1663678 d82b86bf6941aed9f775e65c2915425d66441070	no	32
Warnings:					
Information:					
5	Non Patent Literature	NPL3_Wang_2011_.pdf	1543201 e56663507c2ed99be2d0031177b8326310a124ac	no	4
Warnings:					
Information:					
			Total Files Size (in bytes):	7579476	

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

Inventors: COGSWELL *et al.*	Confirmation No.: 8185
Applicant: Bristol-Myers Squibb Company	Art Unit: 1644
Application No.: 14/400,667	Examiner: OUSPENSKI, Ilia I.
§ 371(c) Date: November 12, 2014	Atty. Docket: 3338.0630002/ELE/C-K/BGC

Title: **CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING**

Second Supplemental Information Disclosure Statement

Mail Stop Amendment

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

Commissioner:

Listed on the accompanying IDS Form PTO/SB/08a and 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 February 6, 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 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(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.

Under Statement 37 C.F.R. § 1.97(e)(1), I hereby state that each item of information contained in this Information Disclosure Statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this Information Disclosure Statement. 37 C.F.R. § 1.97(e)(1).

Document **US17**, 2017/0028040 A1, is an English language equivalent of CN-102836441-A, cited on the Office Action issued May 12, 2017 of corresponding Chinese Patent Application No. 201380037764.1.

Document **FP2**, WO-2006133396-A2, is an English language equivalent of CN-101355965-A, cited on the Office Action issued May 12, 2017 of corresponding Chinese Patent Application No. 201380037764.1.

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

Document **FP1**, CN-101215329-A, is in a foreign language. An unverified English language abstract is appended to the first page of document **FP1**.

Document **NPL3**, Wang (2011), is in a foreign language. An English language abstract is provided on the first page of document **NPL3**.

Copies of documents **FP1-FP2** and **NPL3** are submitted. However, in accordance with 37 C.F.R. § 1.98(a)(2)(ii), no copy of U.S. patent application publication cited as document **US17** on the attached IDS Form is submitted.

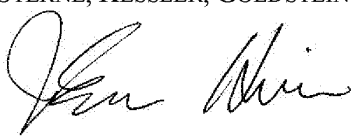
It is respectfully requested that the Examiner initial and return a copy of the enclosed IDS Form, and indicate in the official file wrapper of this patent application that the documents have been considered.

No fee is submitted under 37 C.F.R. § 1.97(c)(1).

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: **July 17, 2017**

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5483585_1

Atty. Dkt. No. 3338.0630002/ELE/C-K/BGC

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/400,667	11/12/2014	John P. Cogswell	3338.0630002/ELE/C-K/AHB	8185

115018 7590 05/02/2017
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	

MAIL DATE	DELIVERY MODE
05/02/2017	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. 14/400,667	Applicant(s) COGSWELL ET AL.	
	Examiner ILIA OUSPENSKI	Art Unit 1644	AIA (First Inventor to File) Status No

-- 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 *02/06/2017*.

☐ 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) *1,4,10,14-16 and 20-35* is/are pending in the application.

5a) Of the above claim(s) *1,4,10,16,20 and 21* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *14,15 and 22-35* 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 *11/12/2014* 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 is being examined under the pre-AIA first to invent provisions.

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

2. Applicant's amendment and remarks filed on 02/06/2017 are acknowledged. Claims 1, 4, 10, 14-16 and 20-35 are pending.

3. Applicant's election without traverse of the invention of Group IV (claims 14, 15, 22 and 23, and new claims 24-35) in the reply filed on 02/06/2017 is acknowledged.

Claims 1, 4, 10, 16, 20 and 21 are withdrawn from further consideration by the Examiner under 37 C.F.R. § 1.142(b) as being drawn to nonelected inventions.

Claims 14, 15, and 22-35 are presently under consideration.

4. The following is a quotation of the appropriate paragraphs of pre-AIA **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 --

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

5. Claims 14, 15, and 22-35 are rejected under pre-AIA **35 U.S.C. 102(b)** as being anticipated by the data on clinical trial NCT01024231 available at clinicaltrials.gov, as evidenced by Ascierto et al. (Seminars in Oncology, October 2010, 37(5): 508-516).

According to ClinicalTrials.gov, accessed online on 04/27/2017, a Phase I study NCT01024231, "Dose-escalation Study of Combination BMS-936558 (MDX-1106) and Ipilimumab in Subjects with Unresectable Stage III or Stage IV Malignant Melanoma," was initiated in 2009. Ascierto et al. cite in Table 2 (page 514) a Phase I study NCT01024231, "Dose-escalation study of combination BMS-936558 (MDX-1106) and ipilimumab in subjects with unresectable stage III or stage IV malignant melanoma," thus providing evidence that the description of this trial was publicly available at least as early as October 2010.

The instant claims are directed to a method of treating cancer comprising administering to the subject an anti-PD-1 antibody and an anti-CTLA4 antibody according to the following regimen:

(i) induction phase, comprising

(a) at least 2 combined doses of both antibodies, followed by

(b) at least 2 doses of anti-PD-1 antibody alone; and

(ii) maintenance phase, comprising

at least 4 combined doses of both antibodies.

The data on clinical trial NCT01024231 at clinicaltrials.gov contains the following description of the administration regimen in Cohorts 1-5:

anti-CTLA4 antibody ipilimumab every 3 weeks for 9 weeks in induction and every 12 weeks for 84 weeks in maintenance, and

anti-PD-1 antibody nivolumab every 3 weeks for 21 weeks in induction and every 12 weeks for 84 weeks in maintenance.

In other words, the administration regimen is as follows:

(i) induction phase, comprising

(a) 3 combined doses of both antibodies, followed by

(b) 4 doses of anti-PD-1 antibody alone; and

(ii) maintenance phase, comprising

6 combined doses of both antibodies.

In the instant claims, intervals between the doses are 2, 3 or 4 weeks in the induction phase and 8, 12 or 16 weeks in the maintenance phase; in the NCT01024231 trial, the intervals are 3 weeks in the induction phase and 12 weeks in the maintenance phase.

In the instant claims, the antibody doses are 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 5 mg/kg or 10 mg/kg; in the NCT01024231 trial, the doses are 0.3 mg/kg, 1 mg/kg, 3 mg/kg and 10 mg/kg.

Accordingly, the administration regimen of the NCT01024231 trial is entirely within the scope of the instant claims, and thus the description of the NCT01024231 trial anticipates the claimed invention.

6. 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.

7. Claims 14, 15, and 22-35 are provisionally rejected on the ground of nonstatutory **double patenting** as being unpatentable over claims 22-33 of copending Application No. 15/122827, published as US 2017/0088626.

Although the claims at issue are not identical, they are not patentably distinct from each other because claims 22-33 of the '827 application are directed to a method of treating cancer comprising administering anti-PD-1 and anti-CTLA4 antibodies according to the same regimen as recited in the instant claims.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

8. Conclusion: no claim is allowed.

9. The following references are cited or record as they are deemed to be pertinent to the present invention:

Curran et al. (Proc. Natl. Acad. Sci. USA (2010) 107: 4275-4280) teach the advantages of combining anti-PD-1 and anti-CTLA4 antibodies for cancer treatment based on pre-clinical data.

Honjo et al. (US Patent No. 8,728,474) and Korman et al. (US Patent No. 9,358,289) claim methods of treating cancer comprising administering a combination of anti-CTLA4 and anti-PD-1 antibodies.

The following prior art references cite or mention clinical trial NCT01024231:
Sharma et al. (Nature Reviews: Cancer (2011) 11: 805-812),

Natarajan et al. (Drugs (2011) 71: 1233-1250),
May et al. (Clin. Cancer Res. (2011) 17: 5233–5238),
Wolchok J.D. (Pigment Cell Res. (2011) v. 24, p. 1005)
Postow et al. (Cancer J. (Mar-Apr 2012) 18: 153–159) and
Sznol M. (Semin. Oncol. (April 2012) 39:192-203).

10. Any inquiry concerning this communication or earlier communications from the examiner should be directed to ILIA OUSPENSKI whose telephone number is (571)272-2920. The examiner can normally be reached on Monday-Friday 9 - 5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel E. Kolker can be reached on 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 OUSPENSKI/
ILIA OUSPENSKI, Ph.D.
Primary Examiner
Art Unit 1644

Notice of References Cited	Application/Control No. 14/400,667	Applicant(s)/Patent Under Reexamination COGSWELL ET AL.	
	Examiner ILIA OUSPENSKI	Art Unit 1644	Page 1 of 3

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-8,728,474 B2	05-2014	Honjo; Tasuku	A61K31/7088	424/141.1
*	B	US-9,358,289 B2	06-2016	Korman; Alan J.	C07K16/18	1/1
*	C	US-2017/0088626 A1	03-2017	JURE-KUNKEL; Maria	C07K16/2896	1/1
	D	US-				
	E	US-				
	F	US-				
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	J	US-				
	K	US-				
	L	US-				
	M	US-				

FOREIGN PATENT DOCUMENTS

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	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	NCT01024231, "Dose-escalation Study of Combination BMS-936558 (MDX-1106) and Ipilimumab in Subjects with Unresectable Stage III or Stage IV Malignant Melanoma." Accessed at ClinicalTrials.gov on 04/27/2017; 4 pages.
	V	Ascierto et al. (Seminars in Oncology, October 2010, 37(5): 508-516).
	W	Curran et al. (Proc. Natl. Acad. Sci. USA (2010) 107: 4275-4280).
	X	Sharma et al. (Nature Reviews: Cancer (2011) 11: 805-812).

*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.

Notice of References Cited	Application/Control No. 14/400,667	Applicant(s)/Patent Under Reexamination COGSWELL ET AL.	
	Examiner ILIA OUSPENSKI	Art Unit 1644	Page 2 of 3

U.S. PATENT DOCUMENTS

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	C	US-				
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FOREIGN PATENT DOCUMENTS

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NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Natarajan et al. (Drugs (2011) 71: 1233-1250).
	V	May et al. (Clin. Cancer Res. (2011) 17: 5233–5238).
	W	Wolchok J.D. (Pigment Cell Res. (2011) v. 24, p. 1005).
	X	Postow et al. (Cancer J. (Mar-Apr 2012) 18: 153–159).

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Notice of References Cited	Application/Control No. 14/400,667	Applicant(s)/Patent Under Reexamination COGSWELL ET AL.	
	Examiner ILIA OUSPENSKI	Art Unit 1644	Page 3 of 3

U.S. PATENT DOCUMENTS

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FOREIGN PATENT DOCUMENTS

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NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Sznol M. (Semin. Oncol. (April 2012) 39:192-203).
	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.

Substitute for form 1449/PTO

FIRST SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	14/400,667
§ 371(c) Date	November 12, 2014
First Named Inventor	COGSWELL, John P.
Art Unit	1644
Examiner Name	OUSPENSKI, ILIA I
Attorney Docket Number	3338.0630002/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-7595048-B2	09-29-2009	HONJO, Tasuku *et al.*	
	US2	US-8383796-B2	02-26-2013	KORMAN, Alan J. *et al.*	
	US3	US-8779105-B2	07-15-2014	KORMAN, Alan J. *et al.*	
	US4	US-9084776-B2	07-21-2015	KORMAN, Alan J. *et al.*	
	US5	US-9102725-B2	08-11-2015	KORMAN, Alan J. *et al.*	
	US6	US-9212224-B2	12-15-2015	COGSWELL, John P. *et al.*	
	US7	US-9273135-B2	03-01-2016	KORMAN, Alan J. *et al.*	
	US8	US-9358289-B2	06-07-2016	KORMAN, Alan J. *et al.*	
	US9	US-9387247-B2	07-12-2016	KORMAN, Alan J. *et al.*	
	US10	US-9492539-B2	11-15-2016	KORMAN, Alan J. *et al.*	
	US11	US-9492540-B2	11-15-2016	KORMAN, Alan J. *et al.*	
	US12	US-20060110383-A1	05-25-2006	HONJO, Tasuku *et al.*	
	US13	US-20100015642-A1	01-21-2010	KWON, Eugene D. *et al.*	
	US14	US-20160075782-A1	03-17-2016	KORMAN, Alan J. *et al.*	
	US15	US-20160090417-A1	03-31-2016	COGSWELL, John P. *et al.*	
	US16	US-20160362495-A1	12-15-2016	KORMAN, Alan 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]
	NPL1	International Preliminary Report on Patentability and Written Opinion for International Application No. PCT/US2013/040764, The International Bureau of WIPO, Geneva, Switzerland, issued on November 18, 2014, 10 pages.	☐
	NPL2	International Search Report for International Application No. PCT/US2013/040764, European Patent Office, Netherlands, mailed on October 31, 2013, 8 pages.	☐

5007466_1

Examiner Signature	/ILIA I OUSPENSKI/	Date Considered	04/24/2017

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BIB DATA SHEET

CONFIRMATION NO. 8185

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
14/400,667	11/12/2014 RULE	424	1644	3338.0630002/ELE/C-K/AHB

APPLICANTS

Bristol-Myers Squibb Company, Princeton, NJ;

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Xi-Tao Wang, Wellesley, MA;
Jon M. Wigginton, Rockville, MD;

**** CONTINUING DATA ***************************

This application is a 371 of PCT/US2013/040764 05/13/2013
which claims benefit of 61/647,442 05/15/2012
and claims benefit of 61/790,747 03/15/2013

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

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****

01/22/2015

Foreign Priority claimed	35 USC 119(a-d) conditions met	Verified and Acknowledged	Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
☐ Yes ☑ No	☐ Yes ☑ No	/ILIA I OUSPENSKI/ Examiner's Signature	☐ Initials	PA	34	22	5

ADDRESS

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

TITLE

CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING

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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14400667	COGSWELL ET AL.
	Examiner	Art Unit
	ILIA OUSPENSKI	1644

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
Priority applications	4/30/2017	io
WEST: attached	4/30/2017	io
ProQuest: same as WEST	4/30/2017	io
Inventor search	4/30/2017	io

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

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Sheet 1 of 6

COMPLETE IF KNOWN	
Application Number	14/400,667
Filing Date	11/12/2014
First Named Inventor	JOHN P. COGSWELL
Art Unit	1644
Examiner Name	
Attorney Docket Number	11981-US-PCT

U.S. PATENT DOCUMENTS

Examiner Initials	Cite No.	Document Number	Publication date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1A	2009/0317368	12-24-2009	Chen	
	1B	6,803,192	10-12-2004	Chen	
	1C	6,808,710	10-26-2004	Wood et al.	
	1D	6,984,720	01-10-2006	Korman et al.	
	1E	7,635,757	12-22-2009	Freeman et al.	
	1F	7,943,743	05-17-2011	Korman et al.	
	1G	8,008,449	08-30-2011	Korman et al.	
	1H	8,168,757	05-01-2012	Finnefrock et al.	
	1I	8,217,149	07-10-2012	Irving et al.	

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	2A	WO 00/37504	06-29-2000	Pfizer, Inc. et al.		☐
	2B	WO 01/14424	03-01-2001	Medarex, Inc.		☐
	2C	WO 01/14556	03-01-2001	Dana-Farber Cancer Institute, Inc.		☐
	2D	WO 01/14557	03-01-2001	Dana-Farber Cancer Institute, Inc. et al.		☐
	2E	WO 01/21631	03-29-2001	Millennium Pharmaceuticals, Inc.		☐
	2F	WO 01/39722	06-07-2001	Mayo Foundation for Medical Education and Research		☐
	2G	WO 02/078731	10-10-2002	Wyeth		☐
	2H	WO 02/079499	10-10-2002	Wyeth et al.		☐
	2I	WO 02/086083	10-31-2002	Mayo Foundation for Medical Education and Research		☐
	2J	WO 03/042402	05-22-2003	Dana-Farber Cancer Institute, Inc. et al.		☐
	2K	WO 2004/004771	01-15-2004	Ono Pharmaceutical Co., Ltd.		☒ (English Abstract only)
	2L	WO 2004/056875	07-08-2004	Wyeth et al.		☐
	2M	WO 2004/072286	08-26-2004	Ono Pharmaceutical Co., Ltd.		☒ (English Abstract only)
	2N	WO 2006/042237	04-20-2006	Mayo Foundation for Medical Education and Research		☐
	2O	WO 2006/121168	11-16-2006	Ono Pharmaceutical Co., Ltd. et al.		☐
	2P	WO 2007/005874	01-11-2007	Medarex, Inc.		☐
	2Q	WO 2011/066389	06-03-2011	MedImmune, Limited et al.		☐
	2R	WO 2012/145493	10-26-2012	Amplimmune, Inc.		☐
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Sheet	3	of	6

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	3A	Androsky, D.J. et al., "Programmed Death Ligand 1 is Expressed by Non-Hodgkin Lymphomas and Inhibits the Activity of Tumor-Associated T Cells", Clinical Cancer Research, Vol. 17, No. 13, pp. 4232-4244 (2011)	
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	3H	Dasanu, C.A. et al., "Immune alterations and emerging immunotherapeutic approaches in lung cancer", Expert Opin. Biol. Ther., Vol. 12, No. 7, pp. 923-937 (2012)	
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	3K	Dong, H. et al., "Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion", Nature Medicine, Vol. 8, No. 8, pp. 793-800 (2002)	
	3L	Flies, D.B. et al., "Blockade of the B7-H1/PD-1 Pathway for Cancer Immunotherapy", Yale Journal of Biology and Medicine, Vol. 84, pp. 409-421 (2011)	
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Application Number	**14/400,667**
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First Named Inventor	**JOHN P. COGSWELL**
Art Unit	**1644**
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Attorney Docket Number	**11981-US-PCT**

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	4A	Gadiot, J. et al., "Overall Survival and PD-L1 Expression in Metastasized Malignant Melanoma", Cancer, Vol. 117, pp. 2192-2201 (2011)	
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	4M	Kim, P.S. et al., "Features of responding T cells in cancer and chronic infection", Current Opinion in Immunology, Vol. 22, pp. 223-230 (2010)	

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Application Number	14/400,667
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First Named Inventor	JOHN P. COGSWELL
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Attorney Docket Number	11981-US-PCT

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	5A	Konishi, J. et al., "B7-H1 Expression on Non-Small Cell Lung Cancer Cells and Its Relationship with Tumor-Infiltrating Lymphocytes and Their PD-1 Expression", Clinical Cancer Research, Vol. 10, pp. 5094-5100 (2004)	
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	5D	Pardoll, D.M., "The blockade of immune checkpoints in cancer immunotherapy", Nature Reviews: Cancer, Vol. 12, pp. 252-264 (2012)	
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	6A	Wolchok, J.D. et al., "Ipilimumab monotherapy in patients with pretreated advanced melanoma: a randomised, double-blind, multicentre, phase 2, dose-ranging study", Lancet Oncol., Vol. 11, pp. 155-164 (2010)	
	6B	Wong, R.M. et al., "Programmed death-1 blockade enhances expansion and functional capacity of human melanoma antigen-specific CTLs", International Immunology, Vol. 19, No. 10, pp. 1223-1234 (2007)	
	6C	Zou, W. et al., "Inhibitory B7-family molecules in the tumour microenvironment", Nature Reviews: Immunology, Vol. 8, pp. 467-477 (2008)	

Examiner Signature	/ILIA I OUSPENSKI/	Date Considered	04/24/2017

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☐	L10	L8 and (cogswell-j$ or goldberg-s$ or gupta-a$ or jure-kunkel-m$ or wang-x$ or wigginton-j$).in.	6
☐	L8	(L7 same (dosage$ or dosing$ or dose$ or regimen$ or schedul$ or once$ or twice$ or day$ or daily$ or week$ or month$ or frequen$ or "mg/kg"))	138
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☐	L5	((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 nivolumab or pembrolizumab) or ((pd-1 or pd1 or pdhd1 or cd279) near3 antibod$))	2819

END OF SEARCH HISTORY

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 8185
COGSWELL *et al.*	Art Unit: 1644
Appl. No.: 14/400,667	Examiner: OUSPENSKI, Ilia I.
Filed: November 12, 2014	Atty. Docket: 3338.0630002/ELE/C-K/AHB
For: CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING	

Reply to Restriction Requirement and Preliminary Amendment Under 37 C.F.R. § 1.115

Mail Stop Amendment

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

Sir:

In reply to the Restriction Requirement dated December 5, 2016, Applicants submit the following Amendments and Remarks.

Amendments to the Claims begin on page 2 of this paper.

Remarks begin on page 9 of this paper.

It is not believed that extensions of time or fees for net addition of claims 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 fees required therefor (including fees for net addition of claims) 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. (Withdrawn) A method of treating a subject afflicted with a cancer comprising administering to the subject a therapeutically effective amount of an antibody or an antigen-binding portion thereof that disrupts the interaction between Programmed Death-1 (PD-1) and Programmed Death Ligand-1 (PD-L1), wherein the antibody or antigen-binding portion thereof binds specifically to PD-1 or to PD-L1.

2-3. (Canceled)

4. (Withdrawn) A method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is a suitable candidate for immunotherapy, the selecting comprising: (i) providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; and (iii) selecting the subject as a suitable candidate based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and (b) administering a composition comprising a therapeutically effective amount of an anti-PD-1 antibody to the selected subject.

5-9. (Canceled)

10. (Withdrawn) A method for treatment of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is not suitable for anti-PD-1 antibody immunotherapy, the selecting comprising: (i) providing a test tissue sample obtained from a

patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; and (iii) selecting the subject as not suitable for anti-PD-1 antibody immunotherapy based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is less than a predetermined threshold level; and (b) administering a standard-of-care therapeutic other than an anti-PD-1 antibody to the selected subject.

11-13. (Canceled)

14. (Currently Amended) A method of treating a subject afflicted with a cancer comprising administering to the subject:

(a) an antibody or an antigen-binding portion thereof that specifically binds to and inhibits Programmed Death-1 (PD-1) ("anti-PD-1 antibody"); and

(b) an antibody or an antigen-binding portion thereof that specifically binds to and inhibits Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) ("anti-CTLA-4 antibody");

each antibody being administered at a dosage ranging from 0.1 mg/kg to 20.0 mg/kg body weight in a concurrent regimen comprising:

(i) an induction dosing schedule comprising combined administration of the anti-PD-1 antibody and the anti-CTLA-4 ~~antibodies~~ antibody 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~~ 10, or 12 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, 10, or 12 doses; followed by

(ii) ~~(ii)~~ a maintenance dosing schedule comprising combined administration of the anti-PD-1 antibody and the anti-CTLA-4 antibody ~~antibodies~~ at a dosing frequency of at least once every 8, 12 or 16 weeks, or at least once a quarter, for at least 4, 6, 8, 10, 12 or 16 doses.

15. (Currently Amended) The method of claim 14, wherein the anti-PD-1 antibody and the anti-CTLA-4 antibody ~~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.

16. (Withdrawn) A method of treating a subject afflicted with a cancer, the subject having previously been treated with an anti-CTLA-4 antibody, which method comprises administering to the subject in a sequenced regimen an antibody or an antigen-binding portion thereof that specifically binds to and inhibits PD-1 at a dosage ranging from 0.1 to 20.0 mg/kg

body weight and at a dosing frequency of at least once every week, at least once every 2, 3 or 4 weeks, or at least once a month, for up to 6 to up to 72 doses.

17-19. (Canceled)

20. (Withdrawn) A kit for treating a subject afflicted with a cancer, the kit comprising: (a) a dosage ranging from 0.1 to 20.0 mg/kg body weight of an antibody or an antigen-binding portion thereof that specifically binds to and inhibits PD-1; (b) a dosage ranging from 0.1 to 20.0 mg/kg body weight of an antibody or an antigen-binding portion thereof that specifically binds to and inhibits CTLA-4; and (c) instructions for using the anti-PD-1 and anti-CTLA-4 antibodies the concurrent regimen method of claim 14.

21. (Withdrawn) A kit for treating a subject afflicted with a cancer, the kit comprising: (a) a dosage ranging from 0.1 to 20.0 mg/kg body weight of an antibody or an antigen-binding portion thereof that specifically binds to and inhibits PD-1; and (b) instructions for using the anti-PD-1 antibody in the sequenced regimen method of claim 16.

22. (Previously presented) The method of claim 15, wherein the anti-PD-1 antibody is nivolumab.

23. (Previously presented) The method of claim 15, wherein the anti-CTLA-4 antibody is ipilimumab.

24. (New) The method of claim 14, wherein each of the anti-PD-1 antibody and the anti-CTLA-4 antibody is individually administered at a dosage of 0.1, 0.3, 0.5, 1, 3, 5, 10 or 20 mg/kg.

25. (New) The method of claim 14, wherein the dosage of each of the anti-PD-1 antibody and anti-CTLA-4 antibody is kept constant during the induction dosing schedule or the maintenance dosing schedule.

26. (New) The method of claim 14, wherein the anti-CTLA-4 antibody is administered during the induction dosing schedule once every 3 weeks for a total of 4 doses.

27. (New) The method of claim 14, wherein the anti-PD-1 antibody is administered during the maintenance dosing schedule at 3 mg/kg every 2 to 12 weeks.

28. (New) A method of treating a subject afflicted with a cancer comprising administering to the subject 3 mg/kg of an anti-PD-1 antibody and 1 mg/kg of an anti-CTLA-4 antibody.

29. (New) The method of claim 28, further comprising subsequently administering 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, 10, or 12 doses.

30. (New) The method of claim 14, wherein the concurrent regimen comprises:

(i) an induction dosing schedule comprising combined administration of the anti-PD-1 antibody and the anti-CTLA-4 antibody at a dosing frequency of once every 3 weeks for 4 doses, followed by administration of the anti-PD-1 antibody alone at a dosing frequency of once every 3 weeks for 4 doses; followed by

(ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 antibody and the anti-CTLA-4 antibody at a dosing frequency of once every 2 to 12 weeks for up to 8 doses.

31. (New) The method of claim 14, wherein the treatment provides a reduction in tumor size, reduction in tumor growth, elimination of the tumor, reduction in number of metastatic lesions over time, complete response, partial response, or stable disease.

32. (New) The method of claim 14, wherein the cancer is selected from liver cancer, hepatocellular carcinoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, breast cancer, lung cancer, squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, cutaneous or intraocular malignant melanoma, renal cancer, renal cell carcinoma, uterine cancer, ovarian cancer, colorectal cancer, colon cancer, colorectal cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, castration-resistant prostate 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, gastric cancer, 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 childhood, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the CNS, primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, asbestos-induced cancers, and a hematologic malignancy.

33. (New) The method of claim 35, wherein the hematologic malignancy is selected from 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, lymphocytic lymphoma, 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.

34. (New) The method of claim 14, wherein the cancer is the cancer is an advanced, recurring, metastatic, and/or refractory cancer.

35. (New) The method of claim 14, wherein the treatment is further combined with one of more of chemotherapy, radiation, surgery, hormone deprivation, angiogenesis inhibitors, and an anti-PD-L1 antibody or antigen-binding portion thereof.

Remarks

Status of the Claims

Upon entry of the foregoing amendments, claims 1, 4, 10, 14-16, and 20-35 are pending in the application, with claims 1, 4, 10, 14, 16, and 28 being the independent claims. Claims 1, 4, 10, 16, 20, and 21 are sought to be withdrawn. Claims 2-3, 5-9, 11-13, and 17-18 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein. Claim 19 was previously canceled. New claims 24 to 35 are sought to be added. Support for the newly submitted claims can be found throughout the specification as originally filed. See U.S. 20150125463, which publication corresponds to the subject application, at paragraphs [0154], [0162]-[0164], [0166], [0168], [0169], [0172], [0173], [0177], and [0191] and Example 15. These changes to the claims are believed to introduce no new matter, and their entry is respectfully requested.

Restriction Requirement

In reply to the Restriction Requirement dated December 5, 2016, requesting an election of a single invention to prosecute in the above-referenced patent application, Applicants hereby provisionally elect to prosecute the subject matter of **Group IV,** represented by **claims 14, 15, 22 and 23 (and new claims 24-35)**. Applicants reserve the right to pursue the non-elected subject matter in one or more divisional applications. This election is made without prejudice to or disclaimer of the other claims or subject matter disclosed.

This election is made **without traverse**.

Conclusion

Prompt and favorable consideration of this Preliminary Amendment is respectfully requested. Applicants believe 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.

Respectfully submitted,

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

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

Date: February 6, 2017

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: COGSWELL *et al.*	Confirmation No.: 8185
Applicant: Bristol-Myers Squibb Company	Art Unit: 1644
Application No.: 14/400,667	Examiner: OUSPENSKI, Ilia I.
§ 371(c) Date: November 12, 2014	Atty. Docket: 3338.0630002/ELE/C-K/AHB

Title: **CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING**

First Supplemental Information Disclosure Statement

Mail Stop Amendment

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

Commissioner:

Listed on the accompanying IDS Forms PTO/SB/08a and 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 First Supplemental Information Disclosure Statement is a continuation of the numbering in Applicant's Information Disclosure Statement filed on January 21, 2015 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(b). This Information Disclosure Statement is being filed within three months of the date of filing of a national application other than a continued prosecution application (CPA), OR within three months of the date of entry of the national stage as set forth in 37 C.F.R. § 1.491 in an international application, OR before the mailing date of a first Office Action on the merits OR 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 **NPL1-NPL2** 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-US16** on the attached IDS Forms 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.

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
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Date: Feb. 6, 2017

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Substitute for form 1449/PTO

FIRST SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	14/400,667
§ 371(c) Date	November 12, 2014
First Named Inventor	COGSWELL, John P.
Art Unit	1644
Examiner Name	OUSPENSKI, ILIA I
Attorney Docket Number	3338.0630002/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-7595048-B2	09-29-2009	HONJO, Tasuku *et al.*	
	US2	US-8383796-B2	02-26-2013	KORMAN, Alan J. *et al.*	
	US3	US-8779105-B2	07-15-2014	KORMAN, Alan J. *et al.*	
	US4	US-9084776-B2	07-21-2015	KORMAN, Alan J. *et al.*	
	US5	US-9102725-B2	08-11-2015	KORMAN, Alan J. *et al.*	
	US6	US-9212224-B2	12-15-2015	COGSWELL, John P. *et al.*	
	US7	US-9273135-B2	03-01-2016	KORMAN, Alan J. *et al.*	
	US8	US-9358289-B2	06-07-2016	KORMAN, Alan J. *et al.*	
	US9	US-9387247-B2	07-12-2016	KORMAN, Alan J. *et al.*	
	US10	US-9492539-B2	11-15-2016	KORMAN, Alan J. *et al.*	
	US11	US-9492540-B2	11-15-2016	KORMAN, Alan J. *et al.*	
	US12	US-20060110383-A1	05-25-2006	HONJO, Tasuku *et al.*	
	US13	US-20100015642-A1	01-21-2010	KWON, Eugene D. *et al.*	
	US14	US-20160075782-A1	03-17-2016	KORMAN, Alan J. *et al.*	
	US15	US-20160090417-A1	03-31-2016	COGSWELL, John P. *et al.*	
	US16	US-20160362495-A1	12-15-2016	KORMAN, Alan 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]
	NPL1	International Preliminary Report on Patentability and Written Opinion for International Application No. PCT/US2013/040764, The International Bureau of WIPO, Geneva, Switzerland, issued on November 18, 2014, 10 pages.	☐
	NPL2	International Search Report for International Application No. PCT/US2013/040764, European Patent Office, Netherlands, mailed on October 31, 2013, 8 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.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 11981-WO-PCT	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2013/040764	International filing date *(day/month/year)* 13 May 2013 (13.05.2013)	Priority date *(day/month/year)* 15 May 2012 (15.05.2012)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant BRISTOL-MYERS SQUIBB COMPANY		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	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 Article 35(2) with regard to novelty, inventive step or 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

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 18 November 2014 (18.11.2014)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Simin Baharlou e-mail: pt09.pct@wipo.int

Form PCT/IB/373 (January 2004)

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/US2013/040764	13.05.2013	15.05.2012

International Patent Classification (IPC) or both national classification and IPC
INV. C07K16/28

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 D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Fellows, Edward Telephone No. +49 89 2399-7460

Form PCT/ISA/237 (Cover Sheet) (July 2009)

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 43bis.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 filed or furnished:

   a. (means)

      ☐ on paper

      ☒ in electronic form

   b. (time)

      ☒ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

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 in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of

☐ the entire international application

☒ claims Nos. 4-21

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international search *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for the whole application or for said claims Nos. 4-21

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has, within the applicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-3

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	1-3
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-3
Industrial applicability (IA)	Yes:	Claims	1-3
	No:	Claims	

2. Citations and explanations

see separate sheet

1 **Concerning section IV**

Unity

1.1 This authority considers that there are 5 inventions covered by the claims indicated as follows:

Invention 1: claims 1-3 (completely)

A method of treating a subject with cancer comprising administering to the subject a therapeutically effective amount of an antibody, or an antigen-binding portion thereof, that disrupts the interaction between PD-1 and PD-L1 wherein the antibody or antigen-binding portion thereof binds specifically to PD-1 or PD-L1 and related subject-matter.

Invention 2: claims 4-9 and 22 (completely)

A method for immunotherapy of a subject with cancer comprising the testing of a tissue sample, assessing the portion of cells in the sample that express PD-L1 and administering a composition comprising a therapeutically effective amount of an anti-PD-1 antibody to a selected subject and related subject-matter.

Invention 3: claim 10 (completely)

A method for immunotherapy of a subject with cancer comprising selecting a subject who is not suitable of anti-PD-1 antibody immunotherapy and administering to the subject a standard-of-care therapeutic other than an anti-PD-1 antibody.

Invention 4: claims 11-13 (completely)

A monoclonal antibody, or an antigen-binding portion thereof, that binds specifically to a cell surface-expressed PD-L1 in a sample and related subject-matter.

Invention 5: claims 14-20 (completely)

A method of treating a subject with cancer comprising administering to the subject an antibody, or an antigen-binding portion thereof, that specifically binds and inhibits PD-1 and an antibody, or an antigen-binding portion thereof, that specifically binds and inhibits CTLA-4, each antibody be administered at a specific dosage range in a concurrent regimen and related subject-matter.

1.2 They are not so linked as to form a single general inventive concept (Rule13.1 PCT) for the following reasons:

1.3 The present application relates to methods of treating subjects with cancer through the detection of suitable patients for immunotherapy comprising the use of antibodies that disrupt the interaction of PD-1/PD-1L.

1.4 The technical relationship and thus common general concept within the meaning of Rule 13.2 PCT linking inventions 1-5 can be seen as the provision of methods of treating subjects with cancer through the detection of suitable patients for immunotherapy comprising the use of antibodies that disrupt the interaction of PD-1/PD-1L.

1.5 However, documents **D1** and **D2** disclose methods for detecting the presence of human PD- L1 in a sample, or measuring the amount of human PD-L1, 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 (cf. D1 page 79 last paragraph and D2 page 58 last paragraph). Both documents also disclose methods of inhibiting growth of tumor cells in a subject, **D1** disclosing administering to the subject a therapeutically effective amount of an anti-PD-L1 antibody, or antigen-binding portion thereof that disrupts the interaction with PD-1 (cf. D1 page 80 lines 15-21) and **D2** disclosing administering to the subject a therapeutically effective amount of an anti-PD-1 antibody, or antigen-binding portion thereof that disrupts interaction with PD-L1 (cf. D2 page 59 third paragraph) since it is well known that 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 (cf. D1 and D2, taken separately, examples). **D1** further discloses the *in vivo* efficacy of combination therapy of anti-CTLA-4 and anti-PD- L1 antibodies on tumor establishment and growth (cf. D1 examples) resulting in tumor free mice. **D2** further discloses tumor immunity in mice following single antibody therapy (anti-PD-1) or combination antibody therapy (anti-CTLA-4 and anti-PD-1; cf. D2 examples).

1.6 Therefore, since the aforementioned common general concept linking inventions 1-5 lacks novelty with regards to documents **D1** and **D2** taken separately it cannot be viewed as a single general inventive concept within the meaning of Rule 13.1 PCT.

1.7 Such knowledge destroys the linking units and since no other technical feature can be distinguished which in the light of the prior art (including common general knowledge in the field) could be regarded as special common

technical feature that defines a contribution over the prior art, the ISA is of the opinion that there is no single inventive concept (Rule 13.1 PCT) underlying the different inventions in the sense of Rule 13.2 PCT.

1.8 Consequently, there is lack of unity and the different inventions not belonging to a common inventive concept are formulated as different inventions pursuant to Article 17(3)(e) PCT.

1.9 Since the Applicant did not pay additional search fees for the other inventions, an opinion will only be formulated for the subject-matter of the invention searched, i.e. invention 1 as specified above.

2 **Concerning section V**

Reference is made to the following documents:

D1 WO 2007/005874 A2 (MEDAREX INC [US]; KORMAN ALAN J [US]; SELBY MARK J [US]; WANG CHANGYU) 11 January 2007 (2007-01-11)

D2 WO 2006/121168 A1 (ONO PHARMACEUTICAL CO [JP]; MEDAREX INC [US]; KORMAN ALAN J; SRINIVASA) 16 November 2006 (2006-11-16)

D3 WO 2004/056875 A1 (WYETH CORP [US]; CAMBRIDGE ANTIBODY TECH [GB]; COLLINS MARY [US]; WOOD) 8 July 2004 (2004-07-08)

D4 HIRANO FUMIYA ET AL: "Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 65, no. 3, 1 February 2005 (2005-02-01), pages 1089-1096, XP002419626, ISSN: 0008-5472

D5 DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; April 2011 (2011-04), DODSON LINDZY F ET AL: "Potential targets for pancreatic cancer immunotherapeutics", Database accession no. PREV201100567322 ; &

IMMUNOTHERAPY,
vol. 3, no. 4, April 2011 (2011-04), pages 517-537,
ISSN: 1750-743X, DOI: 10.2217/IMT.11.10

D6 DATABASE BIOSIS [Online]
BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; July 2011 (2011-07),
ANDORSKY DAVID J ET AL: "Programmed Death Ligand 1 Is Expressed by Non-Hodgkin Lymphomas and Inhibits the Activity of Tumor-Associated T Cells",
Database accession no. PREV201100493026 ; & CLINICAL CANCER RESEARCH,
vol. 17, no. 13, July 2011 (2011-07), pages 4232-4244,
ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-10-2660

Novelty and inventive step (Articles 33(1), 33(2) and 33(3) PCT).

Novelty

2.1 Documents **D1**-**D3** disclose methods for detecting the presence of human PD-1 in a sample, or measuring the amount of human PD-L1, 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 (cf. D1 page 79 last paragraph; D2 page 58 last paragraph and D3 page 4 [013]). The documents also disclose methods of inhibiting growth of tumor cells in a subject, **D1** disclosing administering to the subject of a therapeutically effective amount of an anti-PD-L1 antibody, or antigen-binding portion thereof that disrupts the interaction with PD-1 (cf. D1 page 80 lines 15-21) and **D2** and **D3** disclosing administering to the subject a therapeutically effective amount of an anti-PD-1 antibody, or antigen-binding portion thereof that disrupts interaction with PD-L1 (cf. D2 page 59 third paragraph and D3 page 25 [084]) since it is well known that 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 (cf. D1 and D2, taken separately, examples). **D1** further discloses the *in vivo* efficacy of combination therapy of anti-CTLA-4 and anti-PD-L1 antibodies on tumor establishment and growth (cf. D1 examples) resulting in tumor free mice. **D2** further discloses tumor immunity in mice following single antibody therapy (anti-PD-1) or combination antibody therapy (anti-CTLA-4 and anti-

PD-1; cf. D2 examples). All three of the aforementioned documents disclose the administration of such antibodies or combinations of such antibodies wherein 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 (cf. D1 page 75 before last paragraph, D2 page 55 second paragraph and D3 page 42 [0130]).

2.2 Document **D1**-**D3**, taken separately, anticipate the subject-matter of claims 1-3 which therefore do not meet the requirements of Articles 33(2) PCT.

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.
11981-WO-PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US2013/040764	13 May 2013 (13-05-2013)	15 May 2012 (15-05-2012)
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 7 sheets.

☐ 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:
      ☒ 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. ☒ 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,**
   ☒ 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,**
   ☒ 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. 7
      ☒ 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. ☐ none of the figures is to be published with the abstract

Form PCT/ISA/210 (first sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/040764

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 and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   ☐ on paper

X ☐ in electronic form

   b. (time)

X ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purpose of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in 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/US2013/040764

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

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:

see additional sheet

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 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.:

1-3

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.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/040764

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28
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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/005874 A2 (MEDAREX INC [US]; KORMAN ALAN J [US]; SELBY MARK J [US]; WANG CHANGYU) 11 January 2007 (2007-01-11) pages 79,80,75; example all	1-3
X	WO 2006/121168 A1 (ONO PHARMACEUTICAL CO [JP]; MEDAREX INC [US]; KORMAN ALAN J; SRINIVASA) 16 November 2006 (2006-11-16) pages 58,59,55; example all	1-3
X	WO 2004/056875 A1 (WYETH CORP [US]; CAMBRIDGE ANTIBODY TECH [GB]; COLLINS MARY [US]; WOOD) 8 July 2004 (2004-07-08) pages 4,25,42; example all	1-3

-/--

[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
18 July 2013	31/10/2013

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	Fellows, Edward

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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.
A	HIRANO FUMIYA ET AL: "Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 65, no. 3, 1 February 2005 (2005-02-01), pages 1089-1096, XP002419626, ISSN: 0008-5472 the whole document ----- DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; April 2011 (2011-04), DODSON LINDZY F ET AL: "Potential targets for pancreatic cancer immunotherapeutics", XP002703660, Database accession no. PREV201100567322 the whole document & IMMUNOTHERAPY, vol. 3, no. 4, April 2011 (2011-04), pages 517-537, ISSN: 1750-743X, DOI: 10.2217/IMT.11.10 ----- DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; July 2011 (2011-07), ANDORSKY DAVID J ET AL: "Programmed Death Ligand 1 Is Expressed by Non-Hodgkin Lymphomas and Inhibits the Activity of Tumor-Associated T Cells", XP002703661, Database accession no. PREV201100493026 the whole document & CLINICAL CANCER RESEARCH, vol. 17, no. 13, July 2011 (2011-07), pages 4232-4244, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-10-2660 -----	1-3 1-3 1-3

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2013/040764

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007005874 A2	11-01-2007	AU 2006265108 A1	11-01-2007
		BR PI0613361 A2	04-01-2011
		CA 2612241 A1	11-01-2007
		CN 101248089 A	20-08-2008
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		CA 2607147 A1	16-11-2006
		CN 103059138 A	24-04-2013
		EP 1896582 A1	12-03-2008
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		IL 169152 A	30-11-2010
		JP 4511943 B2	28-07-2010
		JP 2006521783 A	28-09-2006
		JP 2010189395 A	02-09-2010

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2013/040764

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
		MX PA05006828 A	08-09-2005
		US 2004213795 A1	28-10-2004
		US 2006210567 A1	21-09-2006
		US 2008311117 A1	18-12-2008
		US 2010028330 A1	04-02-2010
		WO 2004056875 A1	08-07-2004

Form PCT/ISA/210 (patent family annex) (April 2005)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-3

   A method of treating a subject with cancer comprising administering to the subject a therapeutically effective amount of an antibody, or an antigen-binding portion thereof, that disrupts the interaction between PD-1 and PD-L1 wherein the antibody or antigen-binding portion thereof binds specifically to PD-1 or PD-L1 and related subject-matter.

   ---

2. claims: 4-9, 21

   A method for immunotherapy of a subject with cancer comprising the testing of a tissue sample, assessing the portion of cells in the sample that express PD-L1 and administering a composition comprising a therapeutically effective amount of an anti-PD-1 antibody to a selected subject and related subject-matter.

   ---

3. claim: 10

   A method for immunotherapy of a subject with cancer comprising selecting a subject who is not suitable of anti-PD-1 antibody immunotherapy and administering to the subject a standard-of-care therapeutic other than an anti-PD-1 antibody.

   ---

4. claims: 11-13

   A monoclonal antibody, or an antigen-binding portion thereof, that binds specifically to a cell surface-expressed PD-L1 in a sample and related subject-matter.

   ---

5. claims: 14-20

   A method of treating a subject with cancer comprising administering to the subject an antibody, or an antigen-binding portion thereof, that specifically binds and inhibits PD-1 and an antibody, or an antigen-binding portion thereof, that specifically binds and inhibits CTLA-4, each antibody be administered at a specific dosage range in a concurrent regimen and related subject-matter.

Electronic Patent Application Fee Transmittal

Application Number:	14400667
Filing Date:	12-Nov-2014
Title of Invention:	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
First Named Inventor/Applicant Name:	John P. Cogswell
Filer:	Ji Eun Kim/Patrick Duffy
Attorney Docket Number:	3338.0630002/ELE/C-K/AHB

Filed as Large Entity

Filing Fees for U.S. National Stage under 35 USC 371

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
INDEPENDENT CLAIMS IN EXCESS OF 3	1614	1	420	420
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**420**

Electronic Acknowledgement Receipt	
EFS ID:	28278233
Application Number:	14400667
International Application Number:	
Confirmation Number:	8185
Title of Invention:	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
First Named Inventor/Applicant Name:	John P. Cogswell
Customer Number:	115018
Filer:	Ji Eun Kim/Patrick Duffy
Filer Authorized By:	Ji Eun Kim
Attorney Docket Number:	3338.0630002/ELE/C-K/AHB
Receipt Date:	06-FEB-2017
Filing Date:	12-NOV-2014
Time Stamp:	18:04:46
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$420
RAM confirmation Number	020717INTEFSW18055700
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	Miscellaneous Incoming Letter	33380630002_cl.pdf	168629 d91add9f320598e812f170c4302a6ad4d8c2d5e8	no	2
Warnings:					
Information:					
2		33380630002_filing.pdf	449400 47b3a707d5eecd262c184a8303cacba4fe956a79	yes	14

Multipart Description/PDF files in .zip description

Document Description	Start	End
Preliminary Amendment	1	1
Claims	2	8
Applicant Arguments/Remarks Made in an Amendment	9	10
Transmittal Letter	11	13
Information Disclosure Statement (IDS) Form (SB08)	14	14

Warnings:					
Information:					
3	Non Patent Literature	NPL1_IPRP_PCTUS2013040764.pdf	383836 47034692c16a1a7d01e06f36ca3f586fa36f1531	no	10
Warnings:					
Information:					
4	Non Patent Literature	NPL2_ISR_PCTUS2013040764.pdf	257660 0534f35b72c6eddc4cce3405a2d9e138ede13f96	no	8
Warnings:					
Information:					

5	Fee Worksheet (SB06)	fee-info.pdf	30866 fc6d5ef9d3c57fcbfa5af717a1e8ff539b526587	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	1290391	

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.

JI-EUN KIM
DIRECTOR
(202) 772-8619
CKIM@SKGF.COM

Sterne Kessler Goldstein Fox
ATTORNEYS AT LAW

February 6, 2017

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

Confirmation No. 8185
Art Unit 1644
Attn: Mail Stop Amendment

Re: U.S. Utility Patent Application
Appl. No. 14/400,667; 371(c) Date: November 12, 2014
For: **CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING**
Inventors: COGSWELL *et al.*
Our Ref: 3338.0630002/ELE/C-K/AHB

Commissioner:

Transmitted herewith for appropriate action are the following documents:

1. Online Credit Card Payment Authorization in the amount of $420.00 to cover the excess claim fee;

2. Reply to Restriction Requirement and Preliminary Amendment;

3. First Supplemental Information Disclosure Statement;

4. Form PTO/SB/08a and Form PTO/SB/08b equivalent (1 sheet) listing 18 documents (US1-US16 and NPL1-NPL2);

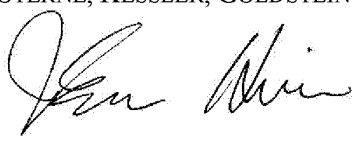
5. Copies of cited documents (NPL1-NPL2).

In the event that extensions of time are necessary to prevent abandonment of this patent application, then such extensions of time are hereby petitioned.

Fee payment is provided through online credit card payment. 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

C-K/AHB/kc
Enclosures

5043380_1

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/400,667	Filing Date 11/12/2014	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)		
FOR	NUMBER FILED	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 $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ 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	02/06/2017	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 21	Minus	** 22	= 0	X $80 =	0
	Independent (37 CFR 1.16(h))	* 5	Minus	***5	= 0	X $420 =	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 $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ 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.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** 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.

SLIE
TAMIE JARRETT

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.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: COGSWELL *et al.*	Confirmation No.: 8185
Applicant: Bristol-Myers Squibb Company	Art Unit: 1644
Application No.: 14/400,667	Examiner: Ouspenski, Ilia I.
371(c) Date: November 12, 2014	Atty. Docket: 3338.0630002/ELE/C-K/A-B

Title: **CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING**

Authorization to Treat a Reply as Incorporating an Extension of Time Under 37 C.F.R. § 1.136(a)(3)

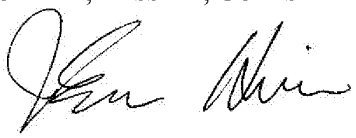
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Commissioner:

The U.S. Patent and Trademark Office is hereby authorized to treat any concurrent or future reply that requires a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. The U.S. Patent and Trademark Office is hereby authorized to charge all required extension of time fees to our Deposit Account No. 19-0036, if such fees are not otherwise provided for in such reply.

Respectfully submitted,

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



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

Date: January 3, 2017

1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600
4945770_1.docx

Electronic Acknowledgement Receipt

EFS ID:	27954478
Application Number:	14400667
International Application Number:	
Confirmation Number:	8185
Title of Invention:	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
First Named Inventor/Applicant Name:	John P. Cogswell
Customer Number:	115018
Filer:	Ji Eun Kim/Patrick Duffy
Filer Authorized By:	Ji Eun Kim
Attorney Docket Number:	3338.0630002/ELE/C-K/BMD
Receipt Date:	03-JAN-2017
Filing Date:	12-NOV-2014
Time Stamp:	15:31:26
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Authorization for Extension of Time all replies	33380630002_authorization.pdf	98110 cc3a9dece3033c77efef0a9e04fce7f2405ebd23	no	1

Warnings:

Information:	
Total Files Size (in bytes):	98110

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.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/400,667	11/12/2014	John P. Cogswell	3338.0630002/ELE/C-K/BMD	8185

115018 7590 12/05/2016
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	

MAIL DATE	DELIVERY MODE
12/05/2016	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. 14/400,667	Applicant(s) COGSWELL ET AL.	
	Examiner ILIA OUSPENSKI	Art Unit 1644	AIA (First Inventor to File) Status No

-- 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 2 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 *11/12/2014*.

☐ 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) *1-18 and 20-23* 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) _____ is/are rejected.

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

9)☒ Claim(s) *1-18 and 20-23* 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 is being examined under the pre-AIA first to invent provisions.

2. Applicant's preliminary amendment filed on 11/12/2014 is acknowledged. Claims 1-18 and 20-23 are pending.

Restriction Requirement

2. Restriction is required under 35 U.S.C. 121 and 372.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

I. Claims 1-3, drawn to a method of treating a subject afflicted with cancer comprising administering to the subject an antibody that disrupts the interaction between PD-1 and PD-L1, wherein the antibody binds specifically to PD-1 or to PD-L1.

II. Claims 4-9, drawn to a method for immunotherapy of a subject afflicted with cancer, comprising (a) selecting a subject that is a suitable candidate for immunotherapy based on an assessment that the proportion of cells in a test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and (b) administering a composition comprising an anti-PD-1 antibody to the selected subject.

III. Claims 10-13, drawn to a method for treatment of a subject afflicted with cancer comprising (a) selecting the subject as not suitable for anti-PD-1 antibody immunotherapy based on an assessment that the proportion of cells in a test tissue sample that express PD-L1 on the cell surface is less than a predetermined threshold level; and (b) administering a standard-of-care therapeutic other than an anti-PD-1

antibody to the selected subject.

IV. Claims 14, 15, 22 and 23, drawn to a method of treating a subject afflicted with a cancer comprising administering to the subject (a) an antibody that specifically binds to and inhibits PD-1; and (b) an antibody that specifically binds to and inhibits CTLA-4.

V. Claims 16-18, drawn to a method of treating a subject afflicted with cancer, the subject having previously been treated with an anti-CTLA-4 antibody, comprising administering to the subject an antibody that specifically binds to and inhibits PD-1.

VI. Claim 20, drawn to a kit comprising an antibody that specifically binds to and inhibits PD- 1 and an antibody that specifically binds to and inhibits CTLA-4.

VII. Claim 21, drawn to a kit comprising an antibody that specifically binds to and inhibits PD- 1.

In accordance with 37 CFR 1.499, Applicant is required, in response to this action, to elect a single invention to which the claims must be restricted. Applicant is advised that the response to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed (37 CFR 1.143).

3. The inventions listed as Groups I-VII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

(a). The inventions of Groups I-II and IV-VII are deemed to have no special technical feature that defines the contribution over the prior art of Honjo et al. (US 7,595,048).

The technical feature linking Groups I-II and IV-VII is a method of treating cancer comprising administering an anti-PD-1 antibody.

Honjo teaches a method for treatment of cancer, wherein an anti-PD-1 antibody is administered to a subject with cancer (e.g. claim 1), thereby anticipating the technical feature linking Groups I-II and IV-VII.

Therefore, the teachings of prior art anticipate the technical feature linking Groups I-II and IV-VII, and as such, the claimed inventions do not contribute a special technical feature when viewed over the prior art. Thus they do not have a single general inventive concept and so lack unity of invention.

(b). The inventions of Groups II and III are deemed to have no special technical feature that defines the contribution over the prior art of Honjo et al. (US 7595048).

The technical feature linking Groups II and III is a method of selecting a subject for cancer immunotherapy based on an assessment of the proportion of cells in a test tissue sample that express PD-L1 on the cell surface.

Honjo teaches a method for treatment of cancer, wherein an anti-PD-1 antibody is administered to a subject with cancer in which PD-L1 or PD-L2 is over-expressed (e.g. claim 1), which inherently comprises a step of selecting a subject for immunotherapy based on an assessment of expression of PD-L1 and PD-L1 in a test tissue sample, thereby anticipating the technical feature linking Groups II and III.

Therefore, the teachings of prior art anticipate the technical feature linking Groups II and III, and as such, the claimed inventions do not contribute a special technical feature when viewed over the prior art. Thus they do not have a single general inventive concept and so lack unity of invention.

4. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 C.F.R. § 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filed petition under 37 C.F.R. § 1.48(b) and by the fee required under 37 C.F.R. § 1.17(h).

5. Any inquiry concerning this communication or earlier communications from the examiner should be directed to ILIA OUSPENSKI whose telephone number is (571)272-2920. The examiner can normally be reached on Monday-Friday 9 - 5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel E. Kolker can be reached on 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 OUSPENSKI/
ILIA OUSPENSKI, Ph.D.
Primary Examiner
Art Unit 1644

Notice of References Cited	Application/Control No. 14/400,667	Applicant(s)/Patent Under Reexamination COGSWELL ET AL.	
	Examiner ILIA 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-7,595,048 B2	09-2009	Honjo; Tasuku	A61K31/7088	424/142.1
	B	US-				
	C	US-				
	D	US-				
	E	US-				
	F	US-				
	G	US-				
	H	US-				
	I	US-				
	J	US-				
	K	US-				
	L	US-				
	M	US-				

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.

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/400,667	11/12/2014	John P. Cogswell	3338.0630002/ELE/C-K/BMD

CONFIRMATION NO. 8185

115018
Sterne, Kessler, Goldstein & Fox P.L.L.C.
1100 New York Avenue NW
Washington, DC 20005

POA ACCEPTANCE LETTER

OC000000086428343

Date Mailed: 10/12/2016

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 10/07/2016.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/khoang/

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Alexandria, Virginia 22313-1450
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/400,667	11/12/2014	John P. Cogswell	11981-US-PCT

CONFIRMATION NO. 8185

POWER OF ATTORNEY NOTICE

23914
HENRY HADAD
BRISTOL-MYERS SQUIBB COMPANY
PATENT DEPARTMENT
P O BOX 4000
PRINCETON, NJ 08543-4000



Date Mailed: 10/12/2016

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 10/07/2016.

- The Power of Attorney to you in this application has been revoked by the applicant. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/khoang/

Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82A (07-13)
Approved for use through 11/30/2014. OMB 0651-0051
U.S. Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE
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TRANSMITTAL FOR POWER OF ATTORNEY TO ONE OR MORE REGISTERED PRACTITIONERS

NOTE: This form is to be submitted with the Power of Attorney by Applicant form (PTO/AIA/82B) to identify the application to which the Power of Attorney is directed, in accordance with 37 CFR 1.5, unless the application number and filing date are identified in the Power of Attorney by Applicant form. If neither form PTO/AIA/82A nor form PTO/AIA82B identifies the application to which the Power of Attorney is directed, the Power of Attorney will not be recognized in the application.

Application Number	14/400,667
Filing Date	May 13, 2013 (Int'l Filing Date)
First Named Inventor	John P. COGSWELL
Title	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
Art Unit	1644
Examiner Name	OUSPENSKI, ILIA I.
Attorney Docket Number	3338.0630002/ELE/C-K/BMD

SIGNATURE of Applicant or Patent Practitioner

Signature	/Ji-Eun Kim/	Date (Optional)	Oct. 7, 2016
Name	Ji-Eun Kim	Registration Number	68,504
Title (if Applicant is a juristic entity)	Attorney for Applicant		
Applicant Name (if Applicant is a juristic entity)	Bristol-Myers Squibb Company		

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. If more than one applicant, use multiple forms.

[X] *Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. 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 3 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.

Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82B (07-13)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date

(Note: The boxes above may be left blank if information is provided on form PTO/AIA/82A.)

[X] I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above: **115018**

OR

[ ] I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

Please recognize or change the correspondence address for the application identified in the attached transmittal letter or the boxes above to:

[X] The address associated with the above-mentioned Customer Number

OR

[ ] The address associated with Customer Number: **115018**

OR

Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

BRISTOL-MYERS SQUIBB COMPANY

[ ] Inventor or Joint Inventor (title not required below)

[ ] Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

[X] Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

[ ] Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature		Date (Optional)	July 31, 2015
Name	Paul D. Golian		
Title	Vice President & Assistant General Counsel, Bristol-Myers Squibb Company		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

[X] Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. 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 3 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.

Electronic Acknowledgement Receipt

EFS ID:	27154951
Application Number:	14400667
International Application Number:	
Confirmation Number:	8185
Title of Invention:	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
First Named Inventor/Applicant Name:	John P. Cogswell
Customer Number:	23914
Filer:	Ji Eun Kim/Jeanette Yando
Filer Authorized By:	Ji Eun Kim
Attorney Docket Number:	11981-US-PCT
Receipt Date:	07-OCT-2016
Filing Date:	12-NOV-2014
Time Stamp:	16:05:43
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	33380630002-CLUSPTO.pdf	184309 f99ef35e3bf14abbaf03773bddb6401fe91284b5	no	1

Warnings:

Information:					
2	Power of Attorney	33380630002-POAFINAL.pdf	436118 948f865f0156c2f62fe223f986e1443e05f68bd3	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	620427	

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.

JI-EUN KIM
DIRECTOR
(202) 772-8619
CKIM@SKGF.COM

Sterne Kessler Goldstein Fox
ATTORNEYS AT LAW

October 7, 2016

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

Confirmation No. 8185
Art Unit 1644

Re: U.S. Utility Patent Application
Appl. No. 14/400,667; Int'l Filing Date: May 13, 2013
For: **CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING**
Inventors: COGSWELL *et al.*
Our Ref: 3338.0630002/ELE/C-K/BMD

Commissioner:

Transmitted herewith for appropriate action is a document entitled Power of Attorney by Applicant (PTO/AIA/82B) and the Transmittal for Power of Attorney form (PTO/AIA/82A) which is submitted electronically.

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 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) 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

C-K/BMD/jly
Enclosure(s)

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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

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/400,667	11/12/2014	John P. Cogswell	11981-US-PCT

CONFIRMATION NO. 8185

23914
HENRY HADAD
BRISTOL-MYERS SQUIBB COMPANY
PATENT DEPARTMENT
P O BOX 4000
PRINCETON, NJ 08543-4000

PUBLICATION NOTICE



Title:CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING

Publication No.US-2015-0125463-A1
Publication Date:05/07/2015

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Office of Public Records. The Office of Public Records can be reached by telephone at (703) 308-9726 or (800) 972-6382, by facsimile at (703) 305-8759, by mail addressed to the United States Patent and Trademark Office, Office of Public Records, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently http://pair.uspto.gov/. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

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

U.S. APPLICATION NUMBER NO.	FIRST NAMED INVENTOR	ATTY. DOCKET NO.
14/400,667	John P. Cogswell	11981-US-PCT

23914
HENRY HADAD
BRISTOL-MYERS SQUIBB COMPANY
PATENT DEPARTMENT
P O BOX 4000
PRINCETON, NJ 08543-4000

INTERNATIONAL APPLICATION NO.	
PCT/US2013/040764	
I.A. FILING DATE	PRIORITY DATE
05/13/2013	05/15/2012

CONFIRMATION NO. 8185
371 ACCEPTANCE LETTER

OC000000072975918

Date Mailed: 01/26/2015

NOTICE OF ACCEPTANCE OF APPLICATION UNDER 35 U.S.C 371 AND 37 CFR 1.495

The applicant is hereby advised that the United States Patent and Trademark Office, in its capacity as a Designated / Elected Office (37 CFR 1.495), has ACCEPTED the above identified international application for national patentability examination in the United States Patent and Trademark Office.

The United States Application Number assigned to the application is shown above. A Filing Receipt will be issued for the present application in due course. **THE DATE APPEARING ON THE FILING RECEIPT AS THE "FILING DATE or 371(c) DATE" IS THE DATE ON WHICH THE LAST OF THE 35 U.S.C. 371 (c)(1) and (c)(2) REQUIREMENTS HAS BEEN RECEIVED IN THE OFFICE. THIS DATE IS SHOWN BELOW.** The filing date of the above identified application is the international filing date of the international application (Article 11(3) and 35 U.S.C. 363)

11/12/2014
DATE OF RECEIPT OF 35 U.S.C.
371(c)(1) and (c)(2) REQUIREMENTS

The following items have been received:

- Copy of the International Application filed on 11/12/2014
- Copy of the International Search Report filed on 11/12/2014
- Preliminary Amendments filed on 11/12/2014
- Information Disclosure Statements filed on 11/12/2014
- Sequence Listing txt file (EFS or physical medium) filed on 11/12/2014
- Inventor's Oath or Declaration filed on 11/12/2014
- Sequence Listing (PDF or paper) filed on 11/12/2014
- U.S. Basic National Fees filed on 11/12/2014
- Priority Documents filed on 11/12/2014
- Power of Attorney filed on 11/12/2014
- Specification filed on 11/12/2014
- Claims filed on 11/12/2014
- Abstracts filed on 11/12/2014
- Drawings filed on 11/12/2014
- Authorization to Permit Access filed on 11/12/2014
- Application Data Sheet (37 CFR 1.76) filed on 11/12/2014

FORM PCT/DO/EO/903 (371 Acceptance Notice)

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

LAMONT M HUNTER

Telephone: (571) 272-0308

FORM PCT/DO/EO/903 (371 Acceptance Notice)

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 NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
14/400,667	11/12/2014		3280	11981-US-PCT	22	5

CONFIRMATION NO. 8185

23914
HENRY HADAD
BRISTOL-MYERS SQUIBB COMPANY
PATENT DEPARTMENT
P O BOX 4000
PRINCETON, NJ 08543-4000

FILING RECEIPT

OC000000072975917

Date Mailed: 01/26/2015

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

John P. Cogswell, Yardley, PA;
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Ashok Kumar Gupta, Clarksburg, MD;
Maria Jure-Kunkel, Plainsboro, NJ;
Xi-Tao Wang, Wellesley, MA;
Jon M. Wigginton, Rockville, MD;

Applicant(s)

Bristol-Myers Squibb Company, Princeton, NJ

Assignment For Published Patent Application

Bristol- Myers Squibb Company, Princeton, NJ

Power of Attorney: The patent practitioners associated with Customer Number 23914

Domestic Priority data as claimed by applicant

This application is a 371 of PCT/US2013/040764 05/13/2013
which claims benefit of 61/647,442 05/15/2012
and claims benefit of 61/790,747 03/15/2013

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access - A proper **Authorization to Permit Access to Application by Participating Offices** (PTO/SB/39 or its equivalent) has been received by the USPTO.

If Required, Foreign Filing License Granted: 01/22/2015

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/400,667**

Projected Publication Date: 05/07/2015

Non-Publication Request: No

Early Publication Request: No
Title

CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING

Preliminary Class

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

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 U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 14/400,667

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	280
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	480
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	720
TOTAL CLAIMS (37 CFR 1.16(i))	22 minus 20 =	* 2			OR	x 80 =	160
INDEPENDENT CLAIMS (37 CFR 1.16(h))	5 minus 3 =	* 2				x 420 =	840
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).						800
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	3280

APPLICATION AS AMENDED - PART II

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

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

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** 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 found in the appropriate box in column 1.

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

Substitute for Form PTO-1360
(For use with Form PTO/SB/06)

Application Number	Filing Date
14400667	

Applicant(s) John Cogswell

* May be used for additional claims or amendments

CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT	
	Indep	Depend	Indep	Depend	Indep	Depend
1	1		1			
2		1		1		
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Total Indep	7		5		0	
Total Depend	15	↵	17	↵	0	↵
Total Claims	22		22		0	

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	Indep	Depend	Indep	Depend	Indep	Depend
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PTO/SB/08A (8/03)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT(S)

(use as many sheets as necessary)

Sheet	1	of	6

COMPLETE IF KNOWN	
Application Number	**14/400,667**
Filing Date	**11/12/2014**
First Named Inventor	**JOHN P. COGSWELL**
Art Unit	**1644**
Examiner Name	
Attorney Docket Number	**11981-US-PCT**

U.S. PATENT DOCUMENTS

Examiner Initials	Cite No.	Document Number	Publication date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1A	2009/0317368	12-24-2009	Chen	
	1B	6,803,192	10-12-2004	Chen	
	1C	6,808,710	10-26-2004	Wood et al.	
	1D	6,984,720	01-10-2006	Korman et al.	
	1E	7,635,757	12-22-2009	Freeman et al.	
	1F	7,943,743	05-17-2011	Korman et al.	
	1G	8,008,449	08-30-2011	Korman et al.	
	1H	8,168,757	05-01-2012	Finnefrock et al.	
	1I	8,217,149	07-10-2012	Irving et al.	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609: Draw a line through citation if not in conformance and not considered. Include a copy of this form with the next communication to applicant

PTO/SB/08A (8/03)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT(S)

(use as many sheets as necessary)

Sheet	2	of	6

COMPLETE IF KNOWN	
Application Number	14/400,667
Filing Date	11/12/2014
First Named Inventor	JOHN P. COGSWELL
Art Unit	1644
Examiner Name	
Attorney Docket Number	11981-US-PCT

FOREIGN PATENT DOCUMENTS

Examiner Initials	Cite No.	Foreign Patent Document	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Check box if English language translation is attached
	2A	WO 00/37504	06-29-2000	Pfizer, Inc. et al.		☐
	2B	WO 01/14424	03-01-2001	Medarex, Inc.		☐
	2C	WO 01/14556	03-01-2001	Dana-Farber Cancer Institute, Inc.		☐
	2D	WO 01/14557	03-01-2001	Dana-Farber Cancer Institute, Inc. et al.		☐
	2E	WO 01/21631	03-29-2001	Millennium Pharmaceuticals, Inc.		☐
	2F	WO 01/39722	06-07-2001	Mayo Foundation for Medical Education and Research		☐
	2G	WO 02/078731	10-10-2002	Wyeth		☐
	2H	WO 02/079499	10-10-2002	Wyeth et al.		☐
	2I	WO 02/086083	10-31-2002	Mayo Foundation for Medical Education and Research		☐
	2J	WO 03/042402	05-22-2003	Dana-Farber Cancer Institute, Inc. et al.		☐
	2K	WO 2004/004771	01-15-2004	Ono Pharmaceutical Co., Ltd.		☒ (English Abstract only)
	2L	WO 2004/056875	07-08-2004	Wyeth et al.		☐
	2M	WO 2004/072286	08-26-2004	Ono Pharmaceutical Co., Ltd.		☒ (English Abstract only)
	2N	WO 2006/042237	04-20-2006	Mayo Foundation for Medical Education and Research		☐
	2O	WO 2006/121168	11-16-2006	Ono Pharmaceutical Co., Ltd. et al.		☐
	2P	WO 2007/005874	01-11-2007	Medarex, Inc.		☐
	2Q	WO 2011/066389	06-03-2011	MedImmune, Limited et al.		☐
	2R	WO 2012/145493	10-26-2012	Amplimmune, Inc.		☐
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*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609: Draw a line through citation if not in conformance and not considered. Include a copy of this form with the next communication to applicant

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT(S)

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Sheet	3	of	6

COMPLETE IF KNOWN	
Application Number	14/400,667
Filing Date	11/12/2014
First Named Inventor	JOHN P. COGSWELL
Art Unit	1644
Examiner Name	
Attorney Docket Number	11981-US-PCT

NON PATENT LITERATURE DOCUMENTS

Examiner Initials	Cite No.	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	Check box if English language Translation is attached
	3A	Androsky, D.J. et al., "Programmed Death Ligand 1 is Expressed by Non-Hodgkin Lymphomas and Inhibits the Activity of Tumor-Associated T Cells", Clinical Cancer Research, Vol. 17, No. 13, pp. 4232-4244 (2011)	
	3B	Blank, C. et al., "Interaction of PD-L1 on tumor cells with PD-1 on tumor-specific T cells as a mechanism of immune evasion: implications for tumor immunotherapy", Cancer Immunol. Immunother., Vol. 54, pp. 307-314 (2005)	
	3C	Blank, C. et al., "PD-L1/B7H-1 Inhibits the Effector Phase of Tumor Rejection by T Cell Receptor (TCR) Transgenic $CD8^+$ T Cells", Cancer Research, Vol. 64, pp. 1140-1145 (2004)	
	3D	Brahmer, J.R. et al., "Phase I Study of Single-Agent Anti-Programmed Death-1 (MDX-1106) in Refractory Solid Tumors: Safety, Clinical Activity, Pharmacodynamics, and Immunologic Correlates", Journal of Clinical Oncology, Vol. 28, No. 19, pp. 3167-3175 (2010)	
	3E	Brahmer, J.R. et al., "Safety and Activity of Anti-PD-L1 Antibody in Patients with Advanced Cancer", The New England Journal of Medicine, Vol. 366, No. 26, pp. 2455-2465 (2012)	
	3F	Brown, J.A. et al., "Blockade of Programmed Death-1 Ligands on Dendritic Cells Enhances T Cell Activation and Cytokine Production", The Journal of Immunology, Vol. 170, pp. 1257-1266 (2003)	
	3G	Carter, L.L. et al., "PD-1:PD-L inhibitory pathway affects both $CD4^+$ and $CD8^+$ T cells and is overcome by IL-2", Eur. J. Immunol., Vol. 32, pp. 634-643 (2002)	
	3H	Dasanu, C.A. et al., "Immune alterations and emerging immunotherapeutic approaches in lung cancer", Expert Opin. Biol. Ther., Vol. 12, No. 7, pp. 923-937 (2012)	
	3I	Dodson, L.F. et al., "Potential targets for pancreatic cancer immunotherapeutics", Immunotherapy, Vol. 3, No. 4, pp. 517-537 (2011)	
	3J	Dong, H. et al., "B7-H1 pathway and its role in the evasion of tumor immunity", J. Mol. Med., Vol. 81, pp. 281-287 (2003)	
	3K	Dong, H. et al., "Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion", Nature Medicine, Vol. 8, No. 8, pp. 793-800 (2002)	
	3L	Flies, D.B. et al., "Blockade of the B7-H1/PD-1 Pathway for Cancer Immunotherapy", Yale Journal of Biology and Medicine, Vol. 84, pp. 409-421 (2011)	
	3M	Freeman, G.J. et al., "Engagement of the PD-1 Immunoinhibitory Receptor by a Novel B7 Family Member Leads to Negative Regulation of Lymphocyte Activation", J. Exp. Med., Vol. 192, No. 7, pp. 1027-1034 (2000)	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT(S)

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Sheet 4 of 6

COMPLETE IF KNOWN	
Application Number	**14/400,667**
Filing Date	**11/12/2014**
First Named Inventor	**JOHN P. COGSWELL**
Art Unit	**1644**
Examiner Name	
Attorney Docket Number	**11981-US-PCT**

NON PATENT LITERATURE DOCUMENTS

Examiner Initials	Cite No.	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	Check box if English language Translation is attached
	4A	Gadiot, J. et al., "Overall Survival and PD-L1 Expression in Metastasized Malignant Melanoma", Cancer, Vol. 117, pp. 2192-2201 (2011)	
	4B	Gajewski, T.F. et al., "Gene Signature in Melanoma Associated with Clinical Activity: A Potential Clue to Unlock Cancer Immunotherapy", The Cancer Journal, Vol. 16, No. 4, pp. 399-403 (2010)	
	4C	Garbe, C. et al., "Diagnosis and treatment of melanoma. European consensus-based interdisciplinary guideline — Update 2012", European Journal of Cancer, Vol. 48, pp. 2375-2390 (2012)	
	4D	Hamanishi, J. et al., "Programmed cell death 1 ligand 1 and tumor-infiltrating $CD8^+$ T lymphocytes are prognostic factors of human ovarian cancer", Proc. Natl. Acad. Sci., Vol. 104, No. 9, pp. 3360-3365 (2007)	
	4E	Hamid, O. et al., "Anti-programmed death-1 and anti-programmed death-ligand 1 antibodies in cancer therapy", Expert Opin. Biol. Ther., Vol. 13, No. 6, pp. 847-861 (2013)	
	4F	He, Y.-F. et al., "Blocking Programmed Death-1 Ligand-PD-1 Interactions by Local Gene Therapy Results in Enhancement of Antitumor Effect of Secondary Lymphoid Tissue Chemokine", The Journal of Immunology, Vol. 173, pp. 4919-4928 (2004)	
	4G	Hino, R. et al., "Tumor Cell Expression of Programmed Cell Death-1 Ligand 1 is a Prognostic Factor for Malignant Melanoma", Cancer, Vol. 116, pp. 1757-1766 (2010)	
	4H	Hirano, F. et al., "Blockade of B7-H1 and PD-1 by Monoclonal Antibodies Potentiates Cancer Therapeutic Immunity", Cancer Res., Vol. 65, No. 3, pp. 1089-1096 (2005)	
	4I	Holt, G.E. et al., "Immune Modulation as a Therapeutic Strategy for Non-Small-Cell Lung Cancer", Clinical Lung Cancer, Vol. 9, Suppl. 1, pp. S13-S19 (2008)	
	4J	Holt, G.E. et al., "Immunotherapy as a strategy for the treatment of non-small-cell lung cancer", Therapy, Vol. 8, No. 1, pp. 43-54 (2011)	
	4K	Iwai, Y. et al., "Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade", Proc. Natl. Acad. Sci., Vol. 99, No. 19, pp. 12293-12297 (2002)	
	4L	Iwai, Y. et al., "PD-1 blockade inhibits hematogenous spread of poorly immunogenic tumor cells by enhanced recruitment of effector T cells", International Immunology, Vol. 17, No. 2, pp. 133-144 (2004)	
	4M	Kim, P.S. et al., "Features of responding T cells in cancer and chronic infection", Current Opinion in Immunology, Vol. 22, pp. 223-230 (2010)	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT(S)

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Sheet 5 of 6

COMPLETE IF KNOWN	
Application Number	14/400,667
Filing Date	11/12/2014
First Named Inventor	JOHN P. COGSWELL
Art Unit	1644
Examiner Name	
Attorney Docket Number	11981-US-PCT

NON PATENT LITERATURE DOCUMENTS

Examiner Initials	Cite No.	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	Check box if English language Translation is attached
	5A	Konishi, J. et al., "B7-H1 Expression on Non-Small Cell Lung Cancer Cells and Its Relationship with Tumor-Infiltrating Lymphocytes and Their PD-1 Expression", Clinical Cancer Research, Vol. 10, pp. 5094-5100 (2004)	
	5B	Lipson, E.J. et al., "Durable Cancer Regression Off-Treatment and Effective Reinduction Therapy with an Anti-PD-1 Antibody", Clinical Cancer Research, Vol. 19, No. 2, pp. 462-468 (2012)	
	5C	Mellman, I. et al., "Cancer immunotherapy comes of age", Nature, Vol. 480, pp. 480-489 (2011)	
	5D	Pardoll, D.M., "The blockade of immune checkpoints in cancer immunotherapy", Nature Reviews: Cancer, Vol. 12, pp. 252-264 (2012)	
	5E	Parry, R.V. et al., "CTLA-4 and PD-1 Receptors Inhibit T-Cell Activation by Distinct Mechanisms", Molecular and Cellular Biology, Vol. 25, No. 21, pp. 9543-9553 (2005)	
	5F	Schreiber, R.D. et al., "Cancer Immunoediting: Integrating Immunity's Roles in Cancer Suppression and Promotion", Science, Vol. 331, pp. 1565-1570 (2011)	
	5G	Sharma, P. et al., "Novel cancer immunotherapy agents with survival benefit: recent successes and next steps", Nature Reviews: Cancer, Vol. 11, pp. 805-812 (2011)	
	5H	Shepherd, F.A. et al., "Immunotherapy for Non-small Cell Lung Cancer: Novel Approaches to Improve Patient Outcome", Journal of Thoracic Oncology, Vol. 6, No. 10, pp. 1763-1773 (2011)	
	5I	Sompuram, S.R. et al., "Antibodies Immunoreactive with Formalin-Fixed Tissue Antigens Recognize Linear Protein Epitopes", Am. J. Clin. Pathol., Vol. 125, pp. 82-90 (2006)	
	5J	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, Vol. 4, No. 127, 127ra37 (2012), and Supplementary Materials, http://stm.sciencemag.org/content/suppl/2012/03/26/4.127.127ra37.DC1.html	
	5K	Topalian, S.L. et al., "Cancer Immunotherapy Comes of Age", Journal of Clinical Oncology, Vol. 29, No. 36, pp. 4828-4836 (2011)	
	5L	Topalian, S.L. et al., "Safety, Activity, and Immune Correlates of Anti-PD-1 Antibody in Cancer", The New England Journal of Medicine, Vol. 366, No. 26, pp. 2443-2454 (2012)	
	5M	Topalian, S.L. et al. "Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity", Current Opinion in Immunology, Vol. 24, pp. 207-212 (2012)	

Examiner Signature		Date Considered	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT(S)

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Sheet	6	of	6

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Application Number	**14/400,667**
Filing Date	**11/12/2014**
First Named Inventor	**JOHN P. COGSWELL**
Art Unit	**1644**
Examiner Name	
Attorney Docket Number	**11981-US-PCT**

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	6A	Wolchok, J.D. et al., "Ipilimumab monotherapy in patients with pretreated advanced melanoma: a randomised, double-blind, multicentre, phase 2, dose-ranging study", Lancet Oncol., Vol. 11, pp. 155-164 (2010)	
	6B	Wong, R.M. et al., "Programmed death-1 blockade enhances expansion and functional capacity of human melanoma antigen-specific CTLs", International Immunology, Vol. 19, No. 10, pp. 1223-1234 (2007)	
	6C	Zou, W. et al., "Inhibitory B7-family molecules in the tumour microenvironment", Nature Reviews: Immunology, Vol. 8, pp. 467-477 (2008)	

Examiner Signature		Date Considered	

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Electronic Acknowledgement Receipt

EFS ID:	21260541
Application Number:	14400667
International Application Number:	
Confirmation Number:	8185
Title of Invention:	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
First Named Inventor/Applicant Name:	John P. Cogswell
Customer Number:	23914
Filer:	Melissa Miller/Lisa Swidra
Filer Authorized By:	Melissa Miller
Attorney Docket Number:	11981-US-PCT
Receipt Date:	21-JAN-2015
Filing Date:	
Time Stamp:	08:39:49
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
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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.

11981-US-PCT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF **JOHN COGSWELL, ET AL**
FOR: **CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING**
APPLICATION NO: **14/400667** APPLICATION DATE: **November 12, 2014**
ART UNIT: EXAMINER:
CONFIRMATION NO: 8185 USPTO CUSTOMER NO: **23914**

TRANSMITTED VIA EFS-WEB
Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Sir:

In accordance with 37 C.F.R. §1.56, Applicant directs the Examiner's attention to the references cited on the attached forms PTO/SB/08A and PTO/SB/08B.

The Examiner is requested to consider the foregoing references in relation to this application and to indicate that each reference was considered by returning an initialed copy of the PTO/SB/08A and PTO/SB/08B forms.

The listed references can be found in related patent application no. 13/892,671, filed May 13, 2013, and copies are available therein. However, Applicants are willing to send copies of any or all of these references at the Examiner's request.

Applicant believes this Information Disclosure Statement is being filed such that no fees are required 37 C.F.R. § 1.97(b) (3). If this belief is erroneous, please charge any requisite fees to Deposit Account No. 19-3880 in the name of Bristol-Myers Squibb Company

Respectfully submitted,

Bristol-Myers Squibb Company
Patent Department
P.O. Box 4000
Princeton, NJ 08543-4000

MELISSA MILLER
Attorney for Applicant
Reg. No. 52,988
Phone: 609-252-4323
Date:

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            20                  25                  30
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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
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Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105
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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
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Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
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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
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Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
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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

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Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
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Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
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            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
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Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
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            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
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Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
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            20                  25                  30

Gly Phe Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Trp Ile Thr Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Leu
    50                  55                  60

Gln Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr Ser Thr Val Tyr
65                  70                  75                  80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
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Ser Val Lys Val Ser Cys Lys Thr Ser Gly Asp Thr Phe Ser Thr Tyr
            20                  25                  30


Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45


Gly Gly Ile Ile Pro Ile Phe Gly Lys Ala His Tyr Ala Gln Lys Phe
    50                  55                  60


Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
                85                  90                  95


Ala Arg Lys Phe His Phe Val Ser Gly Ser Pro Phe Gly Met Asp Val
            100                 105                 110


Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
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Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15
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Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30

Asp Val His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Met
        35                  40                  45

Gly Trp Leu His Ala Asp Thr Gly Ile Thr Lys Phe Ser Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Arg Asp Thr Ser Ala Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Arg Ile Gln Leu Trp Phe Asp Tyr Trp Gly Gln Gly Thr
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Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82A (07-13)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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TRANSMITTAL FOR POWER OF ATTORNEY TO ONE OR MORE REGISTERED PRACTITIONERS

NOTE: This form is to be submitted with the Power of Attorney by Applicant form (PTO/AIA/82B) to identify the application to which the Power of Attorney is directed, in accordance with 37 CFR 1.5, unless the application number and filing date are identified in the Power of Attorney by Applicant form. If neither form PTO/AIA/82A nor form PTO/AIA82B identifies the application to which the Power of Attorney is directed, the Power of Attorney will not be recognized in the application.

Application Number	14/400,667
Filing Date	November 12, 2014
First Named Inventor	JOHN P. COGSWELL
Title	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
Art Unit	NOT YET ASSIGNED
Examiner Name	NOT YET ASSIGNED
Attorney Docket Number	11981-US-PCT

SIGNATURE of Applicant or Patent Practitioner

Signature	Melissa Miller	Date (Optional)	11/3/14
Name	MELISSA MILLER	Registration Number	52,988
Title (if Applicant is a juristic entity)	SENIOR CORPORATE COUNSEL-PATENTS		
Applicant Name (if Applicant is a juristic entity)	BRISTOL-MYERS SQUIBB COMPANY		

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. If more than one applicant, use multiple forms.

☐ *Total of _____ forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. 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 3 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.

Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82B (07-13)
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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 displays a valid OMB control number

POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date

(Note: The boxes above may be left blank if information is provided on form PTO/AIA/82A.)

☑ I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above: 23914

OR

☐ I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

Please recognize or change the correspondence address for the application identified in the attached transmittal letter or the boxes above to:

☑ The address associated with the above-mentioned Customer Number

OR

☐ The address associated with Customer Number:

OR

Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

Bristol-Myers Squibb Company

☐ Inventor or Joint Inventor (title not required below)

☐ Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

☑ Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

☐ Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature		Date (Optional)	November 1, 2013
Name	Henry Hadad		
Title	Senior Vice President & Deputy General Counsel, IP, Bristol-Myers Squibb Company		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

Total of forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. 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 3 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.

Electronic Acknowledgement Receipt

EFS ID:	20690706
Application Number:	14400667
International Application Number:	
Confirmation Number:	8185
Title of Invention:	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
First Named Inventor/Applicant Name:	John P. Cogswell
Customer Number:	23914
Filer:	Melissa Miller/Nell Bartholomew
Filer Authorized By:	Melissa Miller
Attorney Docket Number:	11981 USPCT
Receipt Date:	13-NOV-2014
Filing Date:	
Time Stamp:	16:05:32
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Power of Attorney	20141113_POA_11981USPCT.pdf	2037380 114bee386e813a7a1776cea864611745e2ec367d	no	2

Warnings:	
Information:	

Total Files Size (in bytes):	2037380

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.

PTO-1390 (06-13)
Approved for use through 6/30/2013. OMB 0651-0021
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A SUBMISSION UNDER 35 U.S.C. 371	Attorney Docket No. 11981-US-PCT
	U.S. Application No. (if known, see 37 CFR 1.5) UNKNOWN

International Application No.	International Filing Date	Priority Date Claimed
PCT/US2013/040764	May 13, 2013	May 15, 2012

Title of Invention
CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING

First Named Inventor
COGSWELL JOHN

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). NOTE: The express request under 35 U.S.C. 371(f) will not be effective unless the requirements under 35 U.S.C. 371(c)(1), (2), and (4) for payment of the basic national fee, copy of the International Application and English translation thereof (if required), and the oath or declaration of the inventor(s) have been received.
2. ☐ A copy of the International Application (35 U.S.C. 371(c)(2)) is attached hereto (not required if the International Application was previously communicated by the International Bureau or was filed in the United States Receiving Office (RO/US)).
3. An English language translation of the International Application (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
4. An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4))
   a. ☐ is attached.
   b. ☑ was previously filed in the international phase under PCT Rule 4.17(iv).

Items 5 to 8 below concern amendments made in the international phase.

PCT Article 19 and 34 amendments

5. ☐ Amendments to the claims under PCT Article 19 are attached (not required if communicated by the International Bureau) (35 U.S.C. 371(c)(3)).
6. ☐ English translation of the PCT Article 19 amendment is attached (35 U.S.C. 371(c)(3)).
7. ☐ English translation of annexes (Article 19 and/or 34 amendments only) of the International Preliminary Examination Report is attached (35 U.S.C. 371(c)(5)).

Cancellation of amendments made in the international phase

8a. ☐ Do not enter the amendment made in the international phase under PCT Article 19.

8b. ☐ Do not enter the amendment made in the international phase under PCT Article 34.

NOTE: A proper amendment made in English under Article 19 or 34 will be entered in the U.S. national phase application absent a clear instruction from applicant not to enter the amendment(s).

The following items 9 to 17 concern a document(s) or information included.

9. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
10. ☑ A preliminary amendment.
11. ☑ An Application Data Sheet under 37 CFR 1.76.
12. ☐ A substitute specification. NOTE: A substitute specification cannot include claims. See 37 CFR 1.125(b).
13. ☐ A power of attorney and/or change of address letter.
14. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13*ter*.3 and 37 CFR 1.821-1.825.
15. ☐ Assignment papers *(cover sheet and document(s))*. Name of Assignee: _____
16. ☐ 37 CFR 3.73(c) Statement *(when there is an Assignee)*.

This collection of information is required by 37 CFR 1.414 and 1.491-1.492. 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 15 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: Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

PTO-1390 (06-13)
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U.S. APPLN. No. (if known – see 37 CFR 1.5)	INTERNATIONAL APPLICATION No.	ATTORNEY DOCKET No.
UNKNOWN	PCT/US2013/040764	11981-US-PCT

17. [✓] Other items or information:

Declaration for the purposes of US submission.

The following fees have been submitted.	CALCULATIONS	PTO USE ONLY
18. [✓] Basic national fee (37 CFR 1.492(a)) $280	$ 280	
19. [✓] Examination fee (37 CFR 1.492(c)) If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4) ..... $0 All other situations $720	$ 720	
20. [✓] Search fee (37 CFR 1.492(b)) If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4) ..... $0 Search fee (37 CFR 1.445(a)(2)) has been paid on the international application to the USPTO as an International Searching Authority ..... $120 International Search Report prepared by an ISA other than the US and provided to the Office or previously communicated to the US by the IB ..... $480 All other situations $600	$ 480	
TOTAL OF 18, 19, and 20 =	$ 1480	
[ ] Additional fee for specification and drawings filed in paper over 100 sheets (excluding sequence listing in compliance with 37 CFR 1.821(c) or (e) in an electronic medium or computer program listing in an electronic medium) (37 CFR 1.492(j)). Fee for each additional 50 sheets of paper or fraction thereof $400	$ 800	

Total Sheets	Extra Sheets	Number of each addition 50 or fraction thereof (round up to a whole number)	RATE
178 - 100 =	/ 50 =	2	x $400

Surcharge of $140.00 for furnishing any of the search fee, examination fee, or the oath or declaration after the date of commencement of the national stage (37 CFR 1.492(h)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	22 - 20 =	2	x $80	$ 160	
Independent claims	5 - 3 =	2	x $420	$ 840	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $780	$	
Processing fee of $140.00 for furnishing the English translation later than 30 months from the earliest claimed priority date (37 CFR 1.492(i)). +				$	
TOTAL OF ABOVE CALCULATIONS =				$ 1800	
[ ] **Applicant asserts small entity status.** See 37 CFR 1.27. Fees above are reduced by ½.					
[ ] **Applicant certifies micro entity status.** See 37 CFR 1.29. Fees above are reduced by ¾. Applicant must attach form PTO/SB/15A or B or equivalent.					
TOTAL NATIONAL FEE =				$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property. +				$	
TOTAL FEES ENCLOSED =				$ 3280.00	
				Amount to be refunded:	$
				Amount to be charged:	$

a. ☐ A check in the amount of $_____ to cover the above fees is enclosed.

b. ☑ Please charge my Deposit Account No. 19-3880 in the amount of $3280.00 to cover the above fees.

c. ☑ The Director is hereby authorized to charge additional fees which may be required, or credit any overpayment, to Deposit Account No. 19-3880 as follows:

i. ☑ any required fee.

ii. ☐ any required fee except for excess claims fees required under 37 CFR 1.492(d) and (e) and multiple dependent claim fee required under 37 CFR 1.492(f).

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038. The PTO-2038 should only be mailed or faxed to the USPTO. However, when paying the basic national fee, the PTO-2038 may NOT be faxed to the USPTO.

ADVISORY: If filing by EFS-Web, do **NOT** attach the PTO-2038 form as a PDF along with your EFS-Web submission. Please be advised that this is **not** recommended and by doing so your **credit card information may be displayed via PAIR**. To protect your information, it is recommended to pay fees online by using the electronic payment method.

NOTE: Where an appropriate time limit under 37 CFR 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the international Application to pending status.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File)Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013, and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.

NOTE 1: By providing this statement under 37 CFR 1.55 or 1.78, **this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA**.

NOTE 2: A U.S. national stage application may not claim priority to the international application of which it is the national phase. The filing date of a U.S. national stage application is the international filing date. See 35 U.S.C. 363.

Correspondence Address

☑ The address associated with Customer Number: 23914 OR ☐ Correspondence address below

Name					
Address					
City		State		Zip Code	
Country			Telephone		
Email					

Signature	Melissa Miller	Date	11/11/14
Name (Print/Type)	MELISSA MILLER	Registration No. (Attorney/Agent)	52,988

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.

Application Data Sheet 37 CFR 1.76

Application Information

Title of the Invention	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
Attorney Docket Number	11981-US-PCT
Large Entity Status Claimed	YES
Application Type	371 Entry Via PCT- US National Entry
Subject Matter	UTILITY
Total Number of Drawing Sheets (if any)	34
Sequence Submissions (if any)	YES

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Application Data Sheet 37 CFR 1.76

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Application Data Sheet 37 CFR 1.76

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Customer Number	23914

Domestic Benefit/National Stage Information: This 371 application claims the benefit of U.S. Provisional Patent Application No. 61/647,442, filed May 15, 2012, and U.S. Provisional Patent Application No. 61/790,747, file March 15, 2013.

Foreign Priority Information

This section allows for the applicant to claim benefit of foreign priority and to identify any prior foreign application for which priority is not claimed. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(a).

Application No	Country	Filing Date	Priority Claimed

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013

Authorization to Permit Access

☑ Authorization to Permit Access to the Instant Application by the Participating Offices

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO),the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO),and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent

Application Data Sheet 37 CFR 1.76

application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date of filing this Authorization.

Applicant Information

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Participating Office

Applicant 1: Bristol-Myers Squibb Company

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

[X] Assignee	[ ] Legal Representative under 35 U.S.C 117
[ ] Person to whom the inventor is obligated to assign	[ ] Person who shows sufficient proprietary interest

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

[ ] Deceased [ ] Legally incapacitated

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here [✓]

Organization Name: BRISTOL-MYERS SQUIBB COMPANY

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Application Data Sheet 37 CFR 1.76

Signature:

Signature	
Date	
Attorney/Agent Name	Melissa Miller
Registration Number	52,988
Telephone Number	609-252-4323

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF **JOHN COGSWELL ET AL**

FOR: **CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING**

APPLICATION NO: **TO BE ASSIGNED (35 U.S.C. 371 APPLICATION OF PCT/US2013/040764)**

APPLICATION DATE: **FILED HEREWITH**

EXAMINER:

ART UNIT: **TO BE ASSIGNED**

USPTO CUSTOMER NO: **23914**

CONFIRMATION NO: **TO BE ASSIGNED**

TRANSMITTED VIA EFS-WEB

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

Commissioner:

In advance of prosecution, Applicant submits the following amendments and remarks.

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

Remarks begin on page 8 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. 19-3880.

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 of treating a subject afflicted with a cancer comprising administering to the subject a therapeutically effective amount of an antibody or an antigen-binding portion thereof that disrupts the interaction between Programmed Death-1 (PD-1) and Programmed Death Ligand-1 (PD-L1), wherein the antibody or antigen-binding portion thereof binds specifically to PD-1 or to PD-L1.

2. (Original) The method of claim 1, wherein the cancer is selected from the group consisting of melanoma, renal cell carcinoma, squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, colorectal cancer, castration-resistant prostate cancer, ovarian cancer, gastric cancer, hepatocellular carcinoma, pancreatic carcinoma, squamous cell carcinoma of the head and neck, carcinomas of the esophagus, gastrointestinal tract and breast, and a hematological malignancy.

3. (Original) The method of claim 1, wherein the therapeutically effective amount of the antibody or antigen-binding portion thereof comprises a dose ranging from 0.1 to 10.0 mg/kg body weight which is administered at a dosing schedule of once per week, once every two weeks, or once a month.

4. (Original) A method for immunotherapy of a subject afflicted with cancer, which method comprises:

(a) selecting a subject that is a suitable candidate for immunotherapy, the selecting comprising:

(i) providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells;

(ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; and

(iii) selecting the subject as a suitable candidate based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and

(b) administering a composition comprising a therapeutically effective amount of an anti-PD-1 antibody to the selected subject.

5. (Original) The method of claim 4, wherein the proportion of cells 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.

6. (Original) The method of claim 5, wherein the test tissue sample is a formalin-fixed paraffin-embedded (FFPE) tissue sample.

7. (Original) The method of claim 6, wherein the presence of PD-L1 polypeptide is determined using an automated IHC assay.

8. (Currently Amended) The method of claim 7~~12~~, wherein the IHC assay is performed using an anti-PD-L1 monoclonal antibody to bind to the PD-L1 polypeptide, wherein the anti-PD-L1 monoclonal antibody is selected from 28-8, 28-1, 28-12, 29-8, and 5H1.

9. (Original) The method of claim 4, wherein the predetermined threshold level is 1% of tumor cells or a single tumor-infiltrating inflammatory cell expressing cell surface PD-L1 as determined by automated IHC using mAb 28-8.

10. (Original) A method for treatment of a subject afflicted with cancer, which method comprises:

(a) selecting a subject that is not suitable for anti-PD-1 antibody immunotherapy, the selecting comprising:

(i) providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells;

(ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; and

(iii) selecting the subject as not suitable for anti-PD-1 antibody immunotherapy based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is less than a predetermined threshold level; and

(b) administering a standard-of-care therapeutic other than an anti-PD-1 antibody to the selected subject.

11. (Currently Amended) ~~A~~The method of claim 10, wherein the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is determined with a monoclonal antibody or an antigen-binding portion thereof that binds specifically to a cell surface-expressed PD-L1 polypeptide in a formalin-fixed, paraffin-embedded (FFPE) tissue sample.

12. (Currently Amended) The method ~~monoclonal antibody or antigen binding portion thereof~~ of claim 116, wherein the monoclonal antibody or antigen binding ~~which antibody or~~ portion thereof comprises the CDR1, CDR2 and CDR3 regions in a heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 35, and the CDR1, CDR2 and CDR3 regions in a light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 36.

13. (Currently Amended) The method ~~monoclonal antibody or antigen binding portion thereof~~ of claim 116, wherein the monoclonal antibody or antigen binding ~~which antibody or~~ portion thereof comprises a heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 35, and a light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 36.

14. (Original) A method of treating a subject afflicted with a cancer comprising administering to the subject:

(a) an antibody or an antigen-binding portion thereof that specifically binds to and inhibits Programmed Death-1 (PD-1); and

(b) an antibody or an antigen-binding portion thereof that specifically binds to and inhibits Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4);

each antibody being administered at a dosage ranging from 0.1 to 20.0 mg/kg body weight in a concurrent regimen comprising:

(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 every 2, 3 or 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 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 once every 8, 12 or 16 weeks, or at least once a quarter, for at least 4, 6, 8, 10, 12 or 16 doses.

15. (Original) The method of claim 14, wherein 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.

16. (Original) A method of treating a subject afflicted with a cancer, the subject having previously been treated with an anti-CTLA-4 antibody, which method comprises administering to the subject in a sequenced regimen an antibody or an antigen-binding portion thereof that specifically binds to and inhibits PD-1 at a dosage ranging from 0.1 to 20.0 mg/kg body weight and at a dosing frequency of at least once every week, at least once every 2, 3 or 4 weeks, or at least once a month, for up to 6 to up to 72 doses.

17. (Currently Amended) The method of claim ~~15 or~~ 16, wherein the anti-PD-1 antibody is nivolumab.

18. (Currently Amended) The method of ~~15 or~~ 16, wherein the anti-CTLA-4 antibody is ipilimumab.

19. (Canceled)

20. (Original) A kit for treating a subject afflicted with a cancer, the kit comprising:

(a) a dosage ranging from 0.1 to 20.0 mg/kg body weight of an antibody or an antigen-binding portion thereof that specifically binds to and inhibits PD-1;

(b) a dosage ranging from 0.1 to 20.0 mg/kg body weight of an antibody or an antigen-binding portion thereof that specifically binds to and inhibits CTLA-4; and

(c) instructions for using the anti-PD-1 and anti-CTLA-4 antibodies the concurrent regimen method of claim 14.

21. (Original) A kit for treating a subject afflicted with a cancer, the kit comprising:

(a) a dosage ranging from 0.1 to 20.0 mg/kg body weight of an antibody or an antigen-binding portion thereof that specifically binds to and inhibits PD-1; and

(b) instructions for using the anti-PD-1 antibody in the sequenced regimen method of claim 16.

22. (New) The method of claim 15, wherein the anti-PD-1 antibody is nivolumab.

23. (New) The method of claim 15, wherein the anti-CTLA-4 antibody is ipilimumab.

Remarks

Upon entry of the foregoing amendment, claims 1-23 are pending in the application, with claims 1, 4, 10, 14, and 16 being the independent claims. Claims 1, 8, 11-13 and 17-18 are currently amended. Claim 19 is canceled. New claims 22 and 23 have been added. These changes are not believed to introduce new matter, and their entry is respectfully requested.

Claim 1 has been amended to correct a typographical error by adding a parenthetical. Claim 11 has been amended to make it a dependent claim. Claims 8, 12, 13 and 17-18 have been amended to correct their dependency. Support for new claims 22 and 23 is found, *inter alia*, in the claims as originally filed.

Conclusion

Prompt and favorable consideration of this Preliminary Amendment is respectfully requested. Applicant believes 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.

Respectfully submitted,

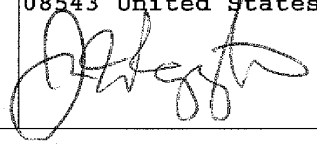
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VIII-4-1	**Declaration: Inventorship (only for the purposes of the designation of the United States of America)** Declaration of Inventorship (Rules 4.17(iv) and 51bis.1(a)(iv)) for the purposes of the designation of the United States of America:	I hereby declare that I believe I am the original inventor or an original joint inventor of a claimed invention in the application. This declaration is directed to the international application of which it forms a part PCT/US2013/040764 filed 13 May 2013. I hereby declare that the above-identified international application was made or authorized to be made by me. I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.
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VIII-4-1-3-4	Inventor's Signature: (The signature must be that of the inventor, not that of the agent)	Ashok Gupta
VIII-4-1-3-5	Date:	10 June 2013

11981-WO-PCT

VIII-4-1-4-1	Name (LAST, First)	JURE-KUNKEL, Maria
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VIII-4-1-4-5	Date:	June 19, 2013
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VIII-4-1-5-5	Date:	10 June 2013
VIII-4-1-6-1	Name (LAST, First)	WIGGINTON, Jon M.
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VIII-4-1-6-4	Inventor's Signature: (The signature must be that of the inventor, not that of the agent)	
VIII-4-1-6-5	Date:	17 July 2013

Electronic Patent Application Fee Transmittal	
Application Number:	
Filing Date:	
Title of Invention:	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
First Named Inventor/Applicant Name:	John P. Cogswell
Filer:	Melissa Miller/Nell Bartholomew
Attorney Docket Number:	11981 USPCT
Filed as Large Entity	

U.S. National Stage under 35 USC 371 Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
National Stage Fee	1631	1	280	280
Natl Stage Search Fee - Report provided	1642	1	480	480
National Stage Exam - all other cases	1633	1	720	720
Pages:				
Natl Stage Appl Sz fee per 50 pgs >100	1681	2	400	800
Claims:				
Claims in excess of 20	1615	2	80	160
Independent claims in excess of 3	1614	2	420	840

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**3280**

Electronic Acknowledgement Receipt	
EFS ID:	20675088
Application Number:	14400667
International Application Number:	PCT/US2013/040764
Confirmation Number:	8185
Title of Invention:	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
First Named Inventor/Applicant Name:	John P. Cogswell
Customer Number:	23914
Filer:	Melissa Miller/Nell Bartholomew
Filer Authorized By:	Melissa Miller
Attorney Docket Number:	11981 USPCT
Receipt Date:	12-NOV-2014
Filing Date:	
Time Stamp:	14:31:54
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$3280
RAM confirmation Number	754
Deposit Account	193880
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. 1.492 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)	
Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)	
Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal of New Application	20141112_TRNA_11981USPCT.pdf	3719072 a7a36ccae89110493da6423273a3fd2ac614f744	no	4
Warnings:					
Information:					
2	Application Data Sheet	20141112_ADS_11981USPCT.pdf	2844419 0413905b5eef32e23bfc74505092aef7663c709c	no	5
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
3	Preliminary Amendment	20141112_AMEN_11981USPCT.pdf	4198858 e74ecd0a065825cbd9f25b0af905ee5aa68c7ca2	no	8
Warnings:					
Information:					
4	Oath or Declaration filed	20141112_DEC_11981USPCT.pdf	62346 f9f2bfed87c73e65dbb3c23c0e7c0d4e90396e4e	no	2
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	40126 c6326d9db5ef86c1ec39499995d73aa87ae9ef0a	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	10864821		

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.

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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.

VIII-4-1	**Declaration: Inventorship (only for the purposes of the designation of the United States of America)** Declaration of Inventorship (Rules 4.17(iv) and 51bis.1(a)(iv)) for the purposes of the designation of the United States of America:	I hereby declare that I believe I am the original inventor or an original joint inventor of a claimed invention in the application. This declaration is directed to the international application of which it forms a part PCT/US2013/040764 filed 13 May 2013. I hereby declare that the above-identified international application was made or authorized to be made by me. I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.
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VIII-4-1-6-5	Date:	17 July 2013

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THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS *US61/647,442*

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CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING

The disclosures of publications cited throughout this application are hereby incorporated in their entireties by reference into this application. 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

The present invention relates to methods for cancer immunotherapy of a patient comprising administering to the patient antibodies that disrupt the PD-1/PD-L1 signaling pathway. A biomarker is used as part of this treatment for, *inter alia*, identifying suitable candidates for immunotherapy and for predicting the therapeutic efficacy of an anti-PD-1 antibody.

BACKGROUND OF THE INVENTION

Human cancers harbor numerous genetic and epigenetic alterations, generating neoantigens potentially recognizable by the immune system (Sjoblom *et al.* (2006) *Science* 314:268-74). Although an endogenous immune response to cancer is observed in preclinical models and patients, this response is ineffective, and established cancers are viewed as "self" and tolerated by the immune system. Contributing to this state of tolerance, tumors may exploit several distinct mechanisms to actively suppress the host immune response (Topalian *et al.* (2011) *J Clin Oncol* 29:4828-36; Mellman *et al.* (2011) *Nature* 480:480-489). Among these mechanisms, endogenous "immune checkpoints" that normally terminate immune responses, to mitigate collateral tissue damage, can be co-opted by tumors to evade immune destruction. Intensive efforts to develop specific immune checkpoint pathway inhibitors have begun to provide new immunotherapeutic approaches for treating cancer, including the development of the anti-CTLA-4 antibody, ipilimumab (Yervoy™), for the treatment of patients with advanced melanoma (Hodi *et al.* (2010) *N Engl J Med* 363:711-723).

Programmed Death-1 (PD-1) is a key immune checkpoint receptor expressed by activated T-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-1 (PD-L2), which 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). Unlike CTLA-4, PD-1 primarily functions in peripheral tissues where activated T-cells may encounter the immunosuppressive PD-L1 (B7-H1) and PD-L2 (B7-DC) ligands expressed by tumor and/or stromal cells (Flies *et al.* (2011) *Yale J Biol Med* 84:409-21; Topalian *et al.* (2012a) *Curr Opin Immunol* 24:1-6). Inhibition of the PD-1/PD-L1 interaction mediates potent antitumor activity in preclinical models (U.S. Patent No. 8,008,449; U.S. Patent No. 7,943,743), and the use of antibody inhibitors of the PD-1/PD-L1 interaction for treating cancer has entered clinical trials (Brahmer *et al.* (2010) *J Clin Oncol* 28:3167-75; Flies *et al.*, 2011; Topalian *et al.* (2012b) *N Engl J Med* (submitted); Brahmer *et al.* (2012) *N Engl J Med* (submitted)).

As we enter the age of personalized medicine, it is expected that advances in pharmacogenomics will increasing be used to coherently tailor particular 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 and commercialization of imatinib mesylate (Gleevac®), 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. Advances in validating PD-L1 expression as a biomarker for screening patients for anti-PD-1 immunotherapy are described herein.

SUMMARY OF THE INVENTION

The present invention provides a method for immunotherapy of a subject afflicted with cancer, which method comprises administering to the subject a composition comprising a therapeutically effective amount of an antibody that inhibits signaling from an inhibitory immunoregulator. In a preferred embodiment, the inhibitory immunoregulator is a component of the PD-1/PD-L1 signaling pathway. In another preferred embodiment, the antibody disrupts the interaction between PD-1 and PD-L1.

This antibody may be one that binds specifically to PD-1 or an antibody that binds specifically to PD-L1.

This invention also provides a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is a suitable candidate for immunotherapy, the selecting comprising (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells, (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface, and (iii) selecting the subject as a suitable candidate based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and (b) administering a composition comprising a therapeutically effective amount of an anti-PD-1 antibody to the selected subject.

The invention further provides a method for treatment of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is not suitable for anti-PD-1 antibody immunotherapy, the selecting comprising (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; and (iii) selecting the subject as not suitable for anti-PD-1 antibody immunotherapy based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is less than a predetermined threshold level; and (b) administering a standard-of-care therapeutic other than an anti-PD-1 antibody to the selected subject.

In addition, the invention provides a method for selecting a cancer patient for immunotherapy with an anti-PD-1 antibody, which method comprises: (a) providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold proportion; and (d) selecting the patient for immunotherapy based on an assessment that PD-L1 is expressed in cells of the test tissue sample.

This invention further provides a method for predicting the therapeutic effectiveness of an anti-PD-1 antibody for treating a cancer patient, which method comprises: (a) providing a test tissue sample obtained from a patient with cancer of the

tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold value; and (d) predicting the therapeutic effectiveness of the anti-PD-1 antibody, wherein if the proportion of cells that express PD-L1 on the cell surface exceeds the threshold proportion the antibody is predicted to be effective in treating the patient, and wherein if the proportion of cells that express PD-L1 on the cell surface is below the threshold proportion the antibody is predicted to not be effective in treating the patient.

The present invention also provides a method for determining an immunotherapeutic regimen comprising an anti-PD-1 antibody for treating a cancer patient, which method comprises: (a) providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold proportion; and (d) determining an immunotherapeutic regimen comprising an anti-PD-1 antibody based on the determination that the proportion of cells that express PD-L1 on the cell surface exceeds the predetermined threshold proportion.

In certain embodiments of the methods described herein, the test tissue sample is a formalin-fixed and paraffin-embedded (FFPE) sample. In certain other embodiments, assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is achieved by immunohistochemical (IHC) staining of the FFPE sample. In preferred embodiments, the mAb 28-8 is used in an automated IHC assay to bind to PD-L1 on the surface of cells in the test tissue sample. In preferred embodiments of any of the methods disclosed herein, the cancer is melanoma (MEL), renal cell carcinoma (RCC), squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, colorectal cancer (CRC), castration-resistant prostate cancer (CRPC), squamous cell carcinoma of the head and neck, or carcinomas of the esophagus, ovary, gastrointestinal tract and breast.

This invention additionally provides a monoclonal antibody or antigen-binding portion thereof that binds specifically to a cell surface-expressed PD-L1 antigen in a FFPE tissue sample. In preferred embodiments, the monoclonal antibody or antigen-binding portion thereof does not bind to a cytoplasmic PD-L1 antigen in the FFPE tissue

sample. In other preferred embodiments, the monoclonal antibody is the rabbit mAb designated 28-8, 28-1, 28-12, 29-8 or 20-12.

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 cited references, including scientific articles, newspaper reports, GenBank entries, patents and patent applications cited throughout this application are expressly incorporated herein by reference.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1C. Cross-competition between 5C4 and other HuMab anti-PD-1 mAbs for binding to hPD-1 expressed on CHO cells. A, the 5C4 Fab fragment substantially blocked the binding of mAbs 5C4 itself, as well as the binding of 2D3 and 7D3; B, the 5C4 Fab fragment substantially blocked the binding of mAb 4H1; C, the 5C4 mAb substantially blocked the binding of mAb 17D8.

Figures 2A-2F. Cross-competition of FITC-conjugated human anti-huPD-L1 mAbs for binding to hPD-L1 expressed on CHO cells. A, Binding of labeled 10H10 was partially blocked by 10A5, 11E6 and 13G4 and was significantly blocked by itself; B, Binding of labeled 3G10 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10; C, Binding of labeled 10A5 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10; D, Binding of labeled 11E6 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10; and E, Binding of labeled 12A4 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10; and F, Binding of labeled 13G4 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10.

Figure 3. Cross-competitive inhibition of binding of biotinylated mAb 12A4 to ES-2 cells by human anti-huPD-L1 mAbs. Fluorescence of bound biotin-12A4 plotted against the concentration of unlabeled hPD-L1 HuMabs.

Figure 4. Spider plot showing activity of anti-PD-1 mAb in patients with treatment-refractory melanoma (MEL). A representative plot of changes in tumor burden over time demonstrates the time course of change in the sum of the longest diameters of target lesions, compared with baseline, in 27 MEL patients treated with 5C4 at a dose of 1.0 mg/kg. In the majority of patients who achieved an objective response (OR), responses were durable and evident by the end of cycle 3 (6 months) of treatment (vertical dashed line). Tumor regressions followed conventional as well as "immune-

related" patterns of response, such as prolonged reduction in tumor burden in the presence of new lesions.

Figure 5. Activity of anti-PD-1 mAb in patient with metastatic RCC. Partial regression of metastatic RCC in a 57-year-old patient treated with 5C4 at 1 mg/kg is illustrated. This patient had previously undergone radical surgery and had developed progressive disease after receiving sunitinib, temsirolimus, sorafenib, and pazopanib. Arrows show regression of recurrent tumor in the operative field.

Figures 6. Activity of anti-PD-1 mAb in patient with metastatic MEL. A complete response of metastatic MEL is illustrated in a 62-year-old patient treated with 5C4 at 3 mg/kg, associated with vitiligo. (i) Pretreatment CT scan, inguinal lymph node metastasis (arrow); (ii) after 13 months of treatment. Numerous metastases in the subcutaneous tissue and retroperitoneum also regressed completely (not shown). Vitiligo developed after 6 months of treatment; photos taken at 9 months under visible light (iii) and ultraviolet light (iv). Skin biopsies with immunohistochemistry for microphthalmia-associated transcription factor (MITF) show melanocytes (arrows) at the epidermal-dermal junction in normal skin (v), and scarce (vi) or absent (vii) melanocytes in skin partially or fully affected by vitiligo.

Figure 7. Activity of anti-PD-1 mAb in patient with metastatic NSCLC. A partial response is illustrated in a patient with metastatic NSCLC (nonsquamous histology) treated with 5C4 at 10 mg/kg. Arrows show initial progression in pulmonary lesions followed by regression ("immune-related" pattern of response).

Figures 8A-8B. Correlation between tumor PD-L1 expression and anti-PD-1 clinical response. Pretreatment tumor cell surface expression of PD-L1, as determined by IHC on formalin-fixed paraffin-embedded specimens, correlates with objective response to PD-1 blockade. Forty-two subjects with advanced cancers including melanoma, non-small-cell lung cancer, colorectal cancer, renal-cell cancer, and castration-resistant prostate cancer (n=18, 10, 7, 5, and 2, respectively) were studied. A, There was a significant correlation of tumor cell surface PD-L1 expression with objective clinical response. No patients with PD-L1 negative tumors experienced an objective response. B, Examples of IHC analysis with the anti-PD-L1 monoclonal 5H1 are shown in a melanoma lymph node metastasis (top), a renal-cell cancer nephrectomy specimen (middle), and a lung adenocarcinoma brain metastasis (bottom). All 400X original magnification. Arrows indicate one of many tumor cells in each specimen with surface

membrane staining for PD-L1. Asterisk indicates a normal glomerulus in the nephrectomy specimen, which is negative for PD-L1 staining.

Figure 9. Graphical comparison of the binding of mAbs 28-8 and 5H1 to PD-L1 antigen in tumor tissues by histoscore analysis.The rabbit mAb 28-8 showed higher histoscores in 7 out of 10 samples tested.

Figures 10A-9D. Spider plot showing activity of anti-PD-L1 mAb in patients with treatment-refractory MEL and NSCLC. Representative plots demonstrate the time course of target lesion tumor burden over time in patients with MEL treated with BMS-936559 at doses of 1 (A), 3 (B), 10 mg/kg (C) and in patients with NSCLC treated at 10 mg/kg (D). In the majority of patients who achieved ORs, responses were durable and were evident by the end of cycle 2 (3 months) of treatment, irrespective of dose or tumor type. Tumor regressions followed conventional as well as "immune-related" patterns of response.

Figure 11. Complete response in a patient with melanoma treated with BMS-936559 at 3 mg/kg. Circles indicate an initial increase in the size of pulmonary nodules at 6 weeks and 3 months followed by complete regression at 10 months ("immune-related" pattern of response).

Figure 12. Complete response in a patient with melanoma treated with BMS-936559 at 1 mg/kg. This patient developed an isolated brain metastasis 3 months after initiation of treatment that was successfully treated with stereotactic radiosurgery. A partial response in abdominal disease (circled) was noted at 8 months, with no evidence of disease at 15 months.

Figure 13. Partial response in a patient with NSCLC (non-squamous histology) treated with BMS-936559 at 10 mg/kg. Note the response in disease in right lung pleura and liver.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods for immunotherapy of a subject afflicted with diseases such as cancer or an infectious disease, which methods comprise administering to the subject a composition comprising a therapeutically effective amount of a compound or agent that potentiates an endogenous immune response, either stimulating the activation of the endogenous response or inhibiting the suppression of the endogenous response. More specifically, the invention provides methods for potentiating an endogenous immune response in a subject afflicted with cancer so as to thereby treat

the patient, which method comprises administering to the subject a composition comprising a therapeutically effective amount of an antibody or an antigen-binding portion thereof that inhibits signaling from an inhibitory immunoregulator. In certain embodiment, the inhibitory immunoregulator is a component of the PD-1/PD-L1 signaling pathway. Accordingly, certain embodiments of the invention provide methods for immunotherapy of a subject afflicted with cancer, which methods comprise administering to the subject a composition comprising a therapeutically effective amount of an antibody or an antigen-binding portion thereof that disrupts the interaction between the PD-1 receptor and its ligand, PD-L1. In certain embodiments, the antibody or antigen-binding portion thereof binds specifically to PD-1. In other embodiments, the antibody or antigen-binding portion thereof binds specifically to PD-L1. In certain other embodiments, the subject is selected as suitable for immunotherapy in a method comprising determining the surface expression of PD-L1 in a test tissue sample obtained from a patient with cancer of the tissue, for example, determining the proportion of cells in the test tissue sample that express PD-L1 on the cell surface, and selecting the patient for immunotherapy based on an assessment that PD-L1 is expressed on the surface of cells in the test tissue sample.

Terms

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.

"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. Preferred routes of administration for antibodies of the invention 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. 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. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

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.

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 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 (CDRs), 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 (C1q) of the classical complement system.

Antibodies typically bind specifically to their cognate antigen with high affinity, reflected by a dissociation constant (K_D) of 10^{-5} to 10^{-11} M^{-1} or less. Any K_D greater than about 10^{-4} M^{-1} is generally considered to indicate nonspecific binding. As used herein, an

antibody that "binds specifically" to an antigen refers to an antibody that binds to the antigen and substantially identical antigens with high affinity, which means having a K_D of 10^{-7} M or less, preferably 10^{-8} M or less, even more preferably 5×10^{-9} M or less, and most preferably between 10^{-8} M and 10^{-10} M or less, but does not bind with high affinity to unrelated antigens. An antigen is "substantially identical" to a given antigen if it exhibits a high degree of sequence identity to the given antigen, for example, if it exhibits at least 80%, at least 90%, preferably at least 95%, more preferably at least 97%, or even more preferably at least 99 sequence identity to the sequence of the given antigen. By way of example, an antibody that binds specifically to human PD-1 may also have cross-reactivity with PD-1 antigens from certain primate species but may not cross-react with PD-1 antigens from certain rodent species or with an antigen other than PD-1, *e.g.*, a human PD-L1 antigen.

The 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 (*e.g.*, IgM or IgG1) that is encoded by the heavy chain constant region genes. "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.

An "isolated antibody" refers to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that binds specifically to PD-1 is substantially free of antibodies that bind specifically to antigens other than PD-1). An isolated antibody that binds specifically to PD-1 may, however, have cross-reactivity to other antigens, such as PD-1 molecules from different species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals. By comparison, an "isolated" nucleic acid refers to a nucleic acid composition of matter that is markedly different, *i.e.*, has a distinctive chemical identity, nature and

utility, from nucleic acids as they exist in nature. For example, an isolated DNA, unlike native DNA, is a free-standing portion of a native DNA and not an integral part of a larger structural complex, the chromosome, found in nature. Further, an isolated DNA, unlike native DNA, can be used as a PCR primer or a hybridization probe for, among other things, measuring gene expression and detecting biomarker genes or mutations for diagnosing disease or assessing the efficacy of a therapeutic. An isolated nucleic acid may be purified so as to be substantially free of other cellular components or other contaminants, *e.g.*, other cellular nucleic acids or proteins, using standard techniques well known in the art.

The term "monoclonal antibody" ("mAb") refers to a 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. Monoclonal antibodies may be produced by hybridoma, recombinant, transgenic or other techniques known to those skilled in the art.

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 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 terms "human" antibodies and "fully human" antibodies and are used synonymously.

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.

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.

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.

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 divide and grow 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.

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.

An "immunoregulator" refers to a substance, an agent, a signaling pathway or a component thereof that regulates an immune response. "Regulating," "modifying" or "modulating" an immune response refers to any alteration in a cell of the immune system or in the activity of such cell. Such regulation includes stimulation or suppression of the immune system which may be manifested by an increase or decrease in the number of various cell types, an increase or decrease in the activity of these cells, or any other changes which can occur within the immune system. Both inhibitory and stimulatory immunoregulators have been identified, some of which may have enhanced function in the cancer microenvironment.

The term "immunotherapy" refers to the treatment of a subject afflicted with, or at risk of contracting or suffering a recurrence of, a disease by a method comprising

inducing, enhancing, suppressing or otherwise modifying an immune response. "Treatment" or "therapy" of a subject refers to any type of intervention or process performed on, or administering an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, slowing down or prevent the onset, progression, development, severity or recurrence of a symptom, complication, condition or biochemical indicia associated with a disease.

A "predetermined threshold value," relating to cell surface PD-L1 expression, refers to the proportion of cells in a test tissue sample comprising tumor cells and tumor infiltrating leucocytes above which the sample is scored as being positive for cell surface PD-L1 expression. For cell surface expression assayed by IHC with the mAb 28-8, the predetermined threshold value for cells expressing PD-L1 on the cell surface ranges from at least about 0.01 to at least about 20% of the total number of cells. In preferred embodiments, the predetermined threshold value for cells expressing PD-L1 on the cell surface ranges from at least about 0.1 to at least about 10% of the total number of cells. More preferably, the predetermined threshold value is at least about 5%. Even more preferably, the predetermined threshold value is at least about 1%.

A "signal transduction pathway" or "signaling 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 the cell. The phrase "cell surface receptor" includes, for example, molecules and complexes of molecules located on the surface of a cell that are capable of receiving a signal and transmitting 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 located on the surface of activated B cells, T cells and myeloid cells, and transmits a signal that results in a decrease in tumor infiltrating lymphocytes, a decrease in T-cell receptor-mediated proliferation. An "inhibitor" of signaling refers to a compound or agent that antagonizes or reduces the initiation, reception or transmission of a signal, be that signal stimulatory or inhibitory, by any component of a signaling pathway such as a receptor or it ligand.

A "subject" includes any human or nonhuman animal. The term "nonhuman animal" includes, but is not limited to, vertebrates such as nonhuman primates, sheep, dogs, cats, rabbits, ferrets, rodents such as mice, rats and guinea pigs, avian species such as chickens, amphibians, and reptiles. In preferred embodiments, the subject is a mammal such as a nonhuman primate, sheep, dog, cat, rabbit, ferret or rodent. In more preferred

embodiments, the subject is a human. The terms, "subject," "patient" and "individual" are used interchangeably herein.

A "therapeutically effective amount" or "therapeutically effective dosage" of a drug or therapeutic agent, such as an antibody of the invention, is any amount of the drug that, when used alone or in combination with another therapeutic agent, 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.

By way of example, an anti-cancer agent promotes cancer regression in a subject. In preferred embodiments, a therapeutically effective amount of the drug promotes cancer regression to the point of eliminating the cancer. "Promoting cancer regression" means that administering an effective amount of the drug, alone or in combination with an anti-neoplastic agent, results in a reduction in tumor growth or size, necrosis of the tumor, a decrease in severity of at least one disease symptom, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. In addition, the terms "effective" and "effectiveness" with regard to a treatment includes both pharmacological effectiveness and physiological safety. Pharmacological effectiveness refers to the ability of the drug to promote cancer regression in the patient. Physiological safety refers to the level of toxicity, or other adverse physiological effects at the cellular, organ and/or organism level (adverse effects) resulting from administration of the drug.

By way of example for the treatment of tumors, a therapeutically effective amount of the drug 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. In other preferred embodiments of the invention, tumor regression may be observed and continue for a period of at least about 20 days, more preferably at least about 40 days, or even more preferably at least about 60 days. Notwithstanding these ultimate measurements of therapeutic effectiveness, evaluation of immunotherapeutic drugs must

also make allowance for "immune-related" response patterns.

An "immune-related" response pattern refers to a clinical response pattern often observed in cancer patients treated with immunotherapeutic agents that produce antitumor effects by inducing cancer-specific immune responses or by modifying native immune processes. This response pattern is characterized by a beneficial therapeutic effect that follows an initial increase in tumor burden or the appearance of new lesions, which in the evaluation of traditional chemotherapeutic agents would be classified as disease progression and would be synonymous with drug failure. Accordingly, proper evaluation of immunotherapeutic agents may require long-term monitoring of the effects of these agents on the target disease.

A therapeutically effective amount of a drug includes a "prophylactically effective amount," which is any amount of the drug that, when administered alone or in combination with an anti-neoplastic agent to a subject at risk of developing a cancer or of suffering a recurrence of cancer, inhibits the development or recurrence of the cancer. In preferred embodiments, the prophylactically effective amount prevents the development or recurrence of the cancer entirely. "Inhibiting" the development or recurrence of a cancer means either lessening the likelihood of the cancer's development or recurrence, or preventing the development or recurrence of the cancer entirely.

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.

"Potentiating an endogenous immune response" means increasing the effectiveness or potency of an existing immune response in a subject. This increase in effectiveness and potency may be achieved, for example, by overcoming mechanisms that suppress the endogenous host immune response or by stimulating mechanisms that enhance the endogenous host immune response.

The "Programmed Death-1 (PD-1)" receptor 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.

"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 analogs having at least one common epitope with hPD-L1. The complete hPD-L1 sequence can be found under GenBank Accession No. Q9NZQ7.

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.

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 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.

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.

Various aspects of the invention are described in further detail in the following subsections.

Antibodies of the Invention

Antibodies of the present invention include a variety of antibodies that bind to PD-1 and antibodies that bind to PD-L1. These antibodies may be used, for example, as therapeutic antibodies to treat subjects afflicted with disease or as reagents in diagnostic assays to detect their cognate antigens. Human mAbs (HuMAbs) that 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) with high affinity have been disclosed in U.S. Patent No. 8,008,449, whereas HuMAbs that bind specifically to PD-L1 with high affinity have been disclosed in U.S. Patent No. 7,943,743. The antibodies of the invention include, but are not limited to, all of the anti-PD-1 and anti-PD-L1 antibodies disclosed in U.S. Patent Nos. 8,008,449 and 7,943,743, respectively.

Anti-PD-1 Antibodies of the Invention

Each of the anti-PD-1 human mAbs (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 an 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*.

U.S. Patent No. 8,008,449 exemplifies seven anti-PD-1 HuMAbs: 17D8, 2D3, 4H1, 5C4 (also referred to herein as BMS-936558), 4A11, 7D3 and 5F4, which are each included herein as an antibody of the invention. Isolated DNA molecules encoding the heavy and light chain variable regions of these antibodies have been sequenced, from which the amino acid sequences of the variable regions were deduced. The V_H amino acid sequences of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are provided herein as 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 provided herein as SEQ ID NOs. 8, 9, 10, 11, 12, 13 and 14, respectively.

Preferred antibodies of the present invention include the anti-PD-1 HuMAbs 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4. These preferred antibodies bind specifically to human PD-1 and comprise: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 1 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 8; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID

NO: 2 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 9; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 3 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 10; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 5 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 12; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 6 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 13; or (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 7 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 14.

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 antibodies of the invention. PD-1 binding of such "mixed and matched" antibodies can be tested using binding assays, *e.g.*, enzyme-linked immunosorbent assays (ELISAs), western blots, radioimmunoassays and Biacore analysis that are well known in the art (*see, e.g.*, U.S. Patent No. 8,008,449). 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, anti-PD-1 antibodies of the invention include 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.

The CDR domains of the above antibodies have been delineated using the Kabat

system, and these antibodies may also be defined by combinations of their 3 heavy chain and 3 light chain CDRs (*see* U.S. Patent No. 8,008,449). Since each of these antibodies can bind to PD-1 and antigen-binding specificity is provided primarily by the CDR1, CDR2, and CDR3 regions, the V_H CDR1, CDR2, and CDR3 sequences and V_κ 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_κ CDR1, CDR2, and CDR3) to create other anti-PD-1 antibodies that also constitute antibodies of the invention. PD-1 binding of such "mixed and matched" antibodies can be tested using the binding assays described above (*e.g.*, ELISAs, western blots, radioimmunoassays and Biacore analysis).

Antibodies of the invention also include antibodies that bind specifically to PD-1 and comprise a heavy chain variable region derived from a particular germline heavy chain immunoglobulin and/or a light chain variable region derived from a particular germline light chain immunoglobulin. Specifically, in certain embodiments, antibodies of the invention include antibodies comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-33 or 4-39 germline sequence, and/or a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6, or L15 germline sequence. The amino acid sequences of the V_H and V_κ regions encoded by the V_H 3-33, V_H 4-39, V_κ L6 and V_κ L15 germline genes are provided in U.S. Patent No. 8,008,449.

As used herein, a human antibody can be identified as comprising a heavy or a light chain variable region that is "derived from" a particular human germline immunoglobulin sequence by comparing the amino acid sequence of the human antibody to the amino acid sequences encoded by human germline immunoglobulin genes, and selecting the human germline immunoglobulin sequence that is closest in sequence (*i.e.*, greatest percentage of sequence identity) to the sequence of the human antibody. A human antibody that is "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, the sequence of 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 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.

Preferred antibodies of the invention include antibodies or antigen-binding portions thereof comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-33 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6 germline sequence; or (b) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 4-39 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L15 germline sequence. Examples of antibodies having a V_H and a V_κ derived from V_H 3-33 and V_κ L6 germline sequences, respectively, are 17D8, 2D3, 4H1, 5C4, and 7D3. Examples of antibodies having V_H and V_κ regions derived from V_H 4-39 and V_κ L15 germline sequences, respectively, are 4A11 and 5F4.

In yet another embodiment, an anti-PD-1 antibody of the invention comprises heavy and light chain variable regions having amino acid sequences that are highly similar or homologous to the amino acid sequences of the preferred anti-PD-1 antibodies described herein, wherein the antibody retains the functional properties of the preferred anti-PD-1 antibodies of the invention. For example, antibodies of the invention include monoclonal antibodies comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises consecutively linked amino acids having a sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs. 1, 2, 3, 4, 5, 6 and 7, and the light chain variable region comprises consecutively linked amino acids having a sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of

SEQ ID NOs. 8, 9, 10, 11, 12, 13 and 14. In other embodiments, the V_H and/or V_L amino acid sequences may exhibit at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the sequences set forth above.

As used herein, the percent sequence identity (also referred to as the percent sequence homology) between two sequences (amino acid or nucleotide sequences) is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = number of identical positions/total number of positions being compared x 100), taking into account the number of any gaps, and the length of each such gap, introduced to maximize the degree of sequence identity between the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using mathematical algorithms that are well know to those of ordinary skill in the art (*see, e.g.*, U.S. Patent No. 8,008,449).

Antibodies having homologous amino acid sequences are likely to have essentially the same functional properties where the sequence differences are conservative modifications. As used herein, "conservative sequence modifications" refer to amino acid modifications that do not significantly affect the binding characteristics of the antibody containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Thus, for example, 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 using functional assays that are well known in the art. Accordingly, in certain embodiments of anti-PD-1 antibodies of the invention, the antibodies comprise heavy and light chain variable region each comprising CDR1, CDR2 and CDR3 domains, wherein one or more of these CDR domains comprise consecutively linked amino acid having sequences based on the preferred anti-PD-1 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.

Further, it is well known in the art that the heavy chain CDR3 is the primary determinant that defines the binding specificity and affinity of an antibody, and that multiple antibodies can predictably be generated having the same binding characteristics based on a common CDR3 sequence (*see, e.g.*, Klimka *et al.* (2000) *Br J Cancer*

83(2):252-60; Beiboer *et al.* (2000) *J Mol Biol* 296:833-49; Rader *et al.* (1998) *Proc Natl Acad Sci USA* 95:8910-15; Barbas *et al.* (1994) *J Am Chem Soc* 116:2161-62; Barbas *et al.* (1995) *Proc Natl Acad Sci USA* 92:2529-33; and Ditzel *et al.* (1996) *J Immunol* 157:739-49. The foregoing publications demonstrate that, in general, once the heavy chain CDR3 sequence of a given antibody is defined, variability in the other five CDR sequences will not greatly affect the binding specificity of that antibody. Thus, antibodies of the invention comprising 6 CDRs can be defined by specifying the sequence of the heavy chain CDR3 domain.

Anti-PD-1 antibodies of the invention also include isolated antibodies that bind specifically to human PD-1 and cross-compete for binding to human PD-1 with any of HuMAbs 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4. Thus, anti-PD-1 antibodies of the invention include antibodies or antigen-binding portions thereof that cross-compete for binding to PD-1 with a reference antibody or a reference antigen-binding portion thereof comprising: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 1 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 8; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 2 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 9; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 3 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 10; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 5 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 12; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 6 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 13; or (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set

forth in SEQ ID NO: 7 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 14.

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. Thus, the ability of a test antibody to competitively inhibit the binding of, for example, 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4, to human PD-1 demonstrates that the test antibody binds to the same epitope region of human PD-1 as 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 4A11. All antibodies that bind to the same epitope region of human PD-1 as does 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 4A11 are included among the antibodies of the invention. 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 invention. In a preferred embodiment, the antibody that binds to the same epitope region of 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 U.S. Patent No. 8,008,449. Data provided in Example 1 show that 5C4 or a Fab fragment thereof cross-competes with each of 2D3, 7D3, 4H1 or 17D8 for binding to hPD-1 expressed on the surface of a cell, indicating that all five anti-PD-1 mAbs cross-compete for binding to the same epitope region of hPD-1 (Figures 1A-1C).

An anti-PD-1 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. Specific modifications to antibodies include CDR grafting, site-specific mutation of amino acid residues within the V_H and/or V_κ CDR1, CDR2 and/or CDR3 regions to thereby improve one or more binding properties (*e.g.*, affinity) of the antibody, site-specific mutation of amino acid residues within the V_H and/or V_κ framework regions to decrease the immunogenicity of

the antibody, 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, and chemical modification such as pegylation or alteration in glycosylation patterns to increase or decrease the biological (*e.g.*, serum) half life of the antibody. Specific examples of such modifications and methods of engineering antibodies are described in detail in U.S. Patent No. 8,008,449. Anti-PD-1 antibodies of the invention include all such engineered antibodies that bind specifically to human PD-1 and are obtained by modification of any of the above-described anti-PD-1 antibodies.

Anti-PD-1 antibodies of the 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.

These fragments, obtained initially through proteolysis with enzymes such as papain and pepsin, have been subsequently engineered into monovalent and multivalent antigen-binding fragments. For example, 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 peptide 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 variable fragments (scFv). Divalent or bivalent scFvs (di-scFvs or bi-scFvs) can be engineered by linking two scFvs in within a single peptide chain known as a tandem scFv which contains two V_H and two V_L regions. ScFv dimers and higher multimers can also be created using linker peptides of fewer that 10 amino acids that are too short for the two variable regions to fold together, which forces the scFvs to dimerize and produce diabodies or form other multimers. Diabodies have been shown to bind to their cognate antigen with much higher affinity than the corresponding scFvs, having dissociation constants up to 40-fold lower than the K_D values for the scFvs. Very short linkers (≤ 3 amino acids) lead to the formation of trivalent triabodies or tetravalent tetrabodies that

exhibit even higher affinities for to their antigens than diabodies. Other variants include minibodies, which are scFv-C_{H3} dimers, and larger scFv-Fc fragments (scFv-C_{H2}-C_{H3} dimers), and even an isolated CDR may exhibit antigen-binding function. These antibody fragments are engineered using conventional recombinant techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies. All of the above proteolytic and engineered fragments of antibodies and related variants (*see* Hollinger and Hudson (2005) *Nature Biotech* 23(9):1126-36; Olafsen and Wu (2010) *Semin Nucl Med* 40(3):167-81, for further details) are intended to be encompassed within the term "antigen-binding portion" of an antibody.

Anti-PD-L1 Antibodies of the Invention

Each of the anti-PD-L1 HuMAbs disclosed in U.S. Patent No. 7,943,743 has been demonstrated to exhibit one or more of the following characteristics (a) binds to human PD-L1 with a K_D of 1 x 10^{-7} M or less; (b) increases T-cell proliferation in a Mixed Lymphocyte Reaction (MLR) assay; (c) increase interferon-γ production in an MLR assay; (d) increase IL-2 secretion in an MLR assay; (e) stimulates antibody responses; (f) inhibits the binding of PD-L1 to PD-1; and (g) reverses the suppressive effect of T regulatory cells on T cell effector cells and/or dendritic cells.

U.S. Patent No. 7,943,743 exemplifies ten anti-PD-1 HuMAbs: 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4. DNA molecules encoding the heavy and light chain variable regions of these antibodies have been sequenced, from which the amino acid sequences of the variable regions were deduced. The V_{II} amino acid sequences of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4 are shown in SEQ ID NOs. 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24, respectively, whereas their $V_{I.}$ amino acid sequences are shown in SEQ ID NOs. 25, 26, 27, 28, 29, 30, 31, 32, 33 and 34, respectively.

Preferred antibodies of the present invention include the anti-PD-L1 HuMAbs 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4. These preferred antibodies bind specifically to human PD-L1 and comprise: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 15 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 25; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region

comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 17 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 27; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 18 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 28; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 19 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 29; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 20 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 30; (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 21 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 31; (h) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 22 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 32; (i) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 23 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 33; or (j) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 24 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 34.

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 antibodies of the invention. PD-L1 binding of such "mixed and matched" antibodies can be tested using binding assays *e.g.*, ELISAs, western blots, radioimmunoassays and Biacore analysis that are well known in the art (*see, e.g.*, U.S. Patent No. 7,943,743). 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, antibodies of the invention also include a monoclonal antibody, or antigen binding portion thereof, comprising a heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in any of SEQ ID NOs. 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24, and a light chain variable region comprising consecutively linked amino acids having the sequence set forth in any of SEQ ID NOs. 25, 26, 27, 28, 29, 30, 31, 32, 33 or 34, wherein the antibody binds specifically to PD-L1, preferably human PD-L1.

The CDR domains of the above anti-PD-L1 HuMAbs have been delineated using the Kabat system, and these antibodies may also be defined by combinations of their 3 heavy chain and 3 light chain CDRs (*see* U.S. Patent No. 7.943,743). Since each of these antibodies can bind to PD-L1 and antigen-binding specificity is provided primarily by the CDR1, CDR2, and CDR3 regions, the V_H CDR1, CDR2, and CDR3 sequences and V_κ 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_κ CDR1, CDR2, and CDR3) to create other anti-PD-1 antibodies that also constitute antibodies of the invention. PD-L1 binding of such "mixed and matched" antibodies can be tested using the binding assays described above.

Antibodies of the invention also include antibodies that bind specifically to PD-L1 and comprise a heavy chain variable region derived from a particular germline heavy chain immunoglobulin and/or a light chain variable region derived from a particular germline light chain immunoglobulin. Specifically, in certain embodiments, antibodies of the invention include antibodies comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-18, 1-69, 1-3 or 3-9 germline sequence, and/or a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6, L15, A27 or L18 germline sequence. The amino acid sequences of the V_H and V_κ regions encoded by the V_H 1-18, V_H 1-3, V_H 1-69, V_H 3-9, V_κ L6, V_κ L15 and V_κ A27 germline genes are provided in U.S. Patent No. 7,943,743.

Preferred antibodies of the invention include antibodies or antigen-binding portions thereof comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-18

germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6 germline sequence; (b) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-69 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6 germline sequence; (c) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-3 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L15 germline sequence; (d) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-69 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ A27 germline sequence; (e) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-9 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L15germline sequence; or (f) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-9 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L18germline sequence.

An example of an antibody having a V_H and a V_κ derived from V_H 1-18 and V_κ L6 germline sequences, respectively, is 3G10. Examples of antibodies having V_H and V_κ regions derived from V_H 1-69 and V_κ L6 germline sequences, respectively, are 12A4, 1B12, 7H1 and 12B7. An example of an antibody having a V_H and a V_κ derived from V_H 1-3 and V_κ L15 germline sequences, respectively, is 10A5. Examples of antibodies having V_H and V_κ regions derived from V_H 1-69 and V_κ A27 germline sequences, respectively, are 5F8, 11E6 and 11E6a. An example of an antibody having a V_H and a V_K derived from V_H 3-9 and V_κ L15 germline sequences, respectively, is 10H10. An example of an antibody having a V_H and a V_κ derived from V_H 1-3 and V_κ L15 germline sequences, respectively, is 10A5. An example of an antibody having a V_H and a V_κ derived from V_H 3-9 and V_κ L18 germline sequences, respectively, is 13G4.

In yet another embodiment, an anti-PD-L1 antibody of the invention comprises heavy and light chain variable regions having amino acid sequences that are highly

similar or homologous to the amino acid sequences of the preferred anti-PD-L1 antibodies described herein, wherein the antibody retains the functional properties of the aforementioned anti-PD-L1 antibodies of the invention. For example, antibodies of the invention include monoclonal antibodies comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises consecutively linked amino acids having a sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs. 15, 16, 17, 18, 19, 20, 21, 22, 23, and 24, and the light chain variable region comprises consecutively linked amino acids having a sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs. 25, 26, 27, 28, 29, 30, 31, 32, 33, and 34. In other embodiments, the V_H and/or V_L amino acid sequences may exhibit at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the sequences set forth above.

In certain embodiments of anti-PD-L1 antibodies of the invention, the antibodies comprise heavy and light chain variable region each comprising CDR1, CDR2 and CDR3 domains, wherein one or more of these CDR domains comprise consecutively linked amino acid having sequences based on the preferred anti-PD-L1 antibodies described herein (*e.g.*, 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 and 13G4), or conservative modifications thereof, and wherein the antibodies retain the desired functional properties of the anti-PD-1 antibodies of the invention.

On the basis of the evidence that the heavy chain CDR3 is the primary determinant that defines the binding specificity and affinity of an antibody, it is generally true that once the heavy chain CDR3 sequence of a given antibody is defined, variability in the other five CDR sequences will not greatly affect the binding specificity of that antibody. Accordingly, anti-PD-L1 antibodies of the invention include antibodies comprising 6 CDRs, wherein the antibodies are defined by specifying the sequence of the heavy chain CDR3 domain.

Anti-PD-L1 antibodies of the invention also include isolated antibodies that bind specifically to human PD-L1 and cross-compete for binding to human PD-L1 with any of HuMAbs 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 and 13G4. Thus, anti-PD-L1 antibodies of the invention include antibodies or antigen-binding portions thereof that cross-compete for binding to PD-L1 with a reference antibody or a reference antigen-binding portion thereof comprising: (a) a human heavy chain variable region comprising

consecutively linked amino acids having the sequence set forth in SEQ ID NO: 15 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 25; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 17 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 27; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 18 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 28; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 19 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 29; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 20 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 30; (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 21 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 31; (h) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 22 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 32; (i) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 23 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 33; or (j) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 24 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 34.

The ability of these antibodies to cross-compete with each of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 and 13G4 for binding to human PD-L1

demonstrates that they bind to the same epitope region of each of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 and 13G4, respectively. All antibodies that bind to the same epitope region of human PD-L1 as does HuMAbs 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 or 13G4 are included among the antibodies of the invention. Such cross-competing antibodies can be identified in standard PD-L1 binding assays, *e.g.*, Biacore analysis, ELISA assays or flow cytometry, that are well known to persons skilled in the art. In a preferred embodiment, the antibody that binds to the same epitope region of 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 U.S. Patent No. 7,943,743.

Data provided in Example 2 show that each of the anti-PD-L1 HuMAbs 5F8, 7H1, 1B12, 3G10, 10A5, 11E6, 12A4, 12B7 and 13G4, *i.e.*, all of the HuMAbs tested except 10H10, substantially blocked binding of mAbs 3G10, 10A5, 11E6, 12A4 and 13G4 to Chinese Hamster Ovary (CHO) cells expressing PD-L1 cells. HuMAb 10H10 substantially blocked the binding only of itself to CHO/PD-L1 cells. These data show that 3G10, 10A5, 11E6, 12A4 and 13G4 cross-compete with all of the HuMAbs tested, except for 10H10, for binding to the same epitope region of human PD-L1 (Figures 2A-F). Data provided in Example 3 show that the binding of HuMAb 12A4 to ES-2 ovarian carcinoma cells expressing PD-L1 cells was substantially blocked by 12A4 itself and by 1B12, 12B7, 13G4 and 3G10, and was moderately blocked by mAbs 5F8 and 10A5, but was not blocked by mAb 10H10. These data, largely consistent with the data in Example 2, show that 12A4 itself, and 4 other HuMabs, 12B7, 3G10, 1B12 and 13G4, substantially cross-compete with 12A4 for binding to the same epitope region of human PD-L1; 5F8 and 10A5 exhibit a lower level of cross-competition with 12A4; and 10H10 does not cross-compete at all with 12A4 (Figure 3). Thus, at least 12B7, 3G10, 1B12 and 13G4 cross-compete with 12A4 for binding to hPD-L1 expressed on the surface of a cell, indicating that all these five anti-PD-1 mAbs bind to the same epitope region of hPD-L1. 5F8 and 10A5 appear to bind to the same or an overlapping epitope region as 12A4, whereas 10H10 binds to a different epitope region from 12A4.

Anti-PD-L1 antibodies of the invention also include antibodies engineered starting from an antibodies having one or more of the V_H and/or V_L sequences disclosed herein, which engineered antibodies may have altered properties from the starting antibodies. 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. Specific modifications to antibodies include CDR grafting, site-specific mutation of amino acid residues within the V_H and/or V_κ CDR1, CDR2 and/or CDR3 regions to thereby improve one or more binding properties (*e.g.*, affinity) of the antibody, site-specific mutation of amino acid residues within the V_H and/or V_κ framework regions to decrease the immunogenicity of the antibody, 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, and chemical modification such as pegylation or alteration in glycosylation patterns to increase or decrease the biological (*e.g.*, serum) half life of the antibody. Specific examples of such modifications and methods of engineering antibodies are described in detail in U.S. Patent No. 7,943,743.

Anti-PD-L1 antibodies of the invention also include 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. Yet, studies on measuring PD-L1 expression have often been conducted on frozen specimens because of the difficulty in isolating anti-human PD-L1 Abs that can be used to stain PD-L1 in FFPE specimens by IHC in general (Hamanishi *et al.*, 2007) and, in particular, antibodies that bind specifically to membranous PD-L1 in these tissues. The use of different Abs to stain PD-L1 in frozen versus FFPE tissues and the ability of certain Abs 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.*, 2007; Gadiot et al., 2010). This invention provides several rabbit antibodies that bind with high affinity specifically to membranous human PD-L1 in FFPE tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells.

Rabbit and mouse anti-hPD-L1 mAbs were produced as described in the Examples. Out of a total of one hundred and eight-five rabbit antibody multiclones and purified mouse subclones screened, only ten rabbit multiclone antibodies specifically detected the membranous form of PD-L1, and the top five multiclones (designated Nos. 13, 20, 28, 29 and 49) were subsequently subcloned. The lead 15 rabbit subclones were

purified for confirmatory screening. The clone with most robust detection specifically of membranous PD-L1, rabbit clone 28-8, was selected for the IHC assays. The sequences of the variable regions of mAb 28-8 are set forth in SEQ ID NOs. 35 and 36, respectively. Rabbit clones 28-1, 28-12, 29-8 and 20-12 were the next best Abs in terms of robust detection of membranous PD-L1 in FFPE tissues.

Anti-PD-L1 antibodies of the invention also include antigen-binding portions of the above antibodies, such as Fab, $F(ab')_2$ Fd, Fv, and scFv, di-scFv or bi-scFv, and scFv-Fc fragments, diabodies, triabodies, tetrabodies, an isolated CDRs (Hollinger and Hudson (2005) *Nature Biotech* 23(9):1126-36; Olafsen and Wu (2010) *Semin Nucl Med* 40(3):167-81, for further details).

Nucleic Acid Molecules Encoding Antibodies of the Invention

Another aspect of the present disclosure pertains to isolated nucleic acid molecules that encode any of the antibodies of the invention. These nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. 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. Nucleic acids encoding antibodies obtained from an immunoglobulin gene library (*e.g.*, using phage display techniques) can be recovered from the library.

Preferred nucleic acids molecules of the invention are those encoding the V_H and V_κ sequences of the anti-PD-1 HuMAbs, 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 (disclosed in U.S. Patent No. 8,008,449), and those encoding the V_H and V_κ sequences of the anti-PD-L1 HuMAbs, 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4 (disclosed in U.S. Patent No. 7,943,743). An isolated DNA encoding the V_H region can be converted to a full-length heavy chain gene by operatively linking the V_H-encoding DNA to another DNA molecule encoding heavy chain constant regions (C_{H1}, C_{H2} and C_{H3}), the sequences of which are known in the art and 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. Similarly, an isolated DNA encoding the V_L region can be converted to a full-length light chain gene by operatively linking the V_L-encoding DNA to another DNA molecule encoding the light chain constant region (C_L), the sequence of which is known in the art and 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.

Pharmaceutical Compositions

Antibodies of the present invention may be constituted in a composition, *e.g.*, a pharmaceutical composition, containing one or a combination of antibodies, or an antigen-binding portion(s) thereof, and a pharmaceutically acceptable carrier. As used herein, a "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). A pharmaceutical composition of the invention may include one or more pharmaceutically acceptable salts, anti-oxidant, aqueous and nonaqueous carriers, and/or adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents.

Dosage regimens are adjusted to provide the optimum desired response, *e.g.*, a therapeutic response or minimal adverse effects. For administration of an anti-PD-1 or anti-PD-L1 antibody, the dosage ranges from about 0.0001 to about 100 mg/kg, usually from about about 0.001 to about 20 mg/kg, and more usually from about 0.01 to about 10 mg/kg, of the subject's body weight. Preferably, the dosage is within the range of 0.1-10 mg/kg body weight. For example, dosages can be 0.1, 0.3, 1, 3, 5 or 10 mg/kg body weight, and more preferably, 0.3, 1, 3, or 10 mg/kg body weight. The dosing schedule is typically designed to achieve exposures that result in sustained receptor occupancy based on typical pharmacokinetic properties of an antibody. 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. The dosage and scheduling may change during a course of treatment. For example, dosing schedule may comprise administering the antibody: (i) every two weeks in 6-week cycles; (ii) every four weeks for six dosages, then every three months; (iii)

every three weeks; (iv) 3-10 mg/kg body weight once followed by 1 mg/kg body weight every 2-3 weeks. Considering that an IgG4 antibody typically has a half-life of 2-3 weeks, a preferred dosage regimen for an anti-PD-1 or anti-PD-L1 antibody of the invention comprises 0.3-10 mg/kg body weight, preferably 3-10 mg/kg body weight, more preferably 3 mg/kg body weight via intravenous administration, with the antibody being given every 14 days in up to 6-week or 12-week cycles until complete response or confirmed progressive disease.

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, every 2 weeks, every 3 weeks, 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, the 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 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. One of ordinary skill in the art would be able to determine appropriate dosages 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 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.

Uses and Methods of the Invention

The antibodies, antibody compositions, nucleic acids and methods of the present invention have numerous *in vitro* and *in vivo* utilities including, for example, methods to determine and quantify the expression of PD-1 or PD-L1 comprising binding of the antibodies to the target polypeptides or measuring the amount of nucleic acid encoding these polypeptides, methods for immunotherapy of a subject afflicted with a disease comprising administering to the subject a composition comprising a therapeutically effective amount of a therapeutic agent that inhibits signaling from an inhibitory immunoregulator. In preferred embodiments of the latter methods, the inhibitory immunoregulator is a component of the PD-1/PD-L1 signaling pathway, more preferably an antibody disrupts the interaction between PD-1 and PD-L1. This antibody may be one that binds specifically to PD-1 or an antibody that binds specifically to PD-L1. Thus, the invention provides methods for potentiating an immune response in a subject comprising administering an antibody directed against PD-1 and/or PD-L1 in order to disrupt the interaction between PD-1 and PD-L1, and methods of treating diseases mediated by such a potentiation of the immune response. When antibodies to PD-1 and PD-L1 are administered together, the two can be administered sequentially in either order or simultaneously. In certain aspects, the invention provides methods of modifying an immune response in a subject comprising administering to the subject an anti-PD-1 and/or an anti-PD-L1 antibody of the invention, or antigen-binding portion thereof, such that the immune response in the subject is modified. Preferably, the immune response is potentiated, enhanced, stimulated or up-regulated. In preferred embodiments, the

antibodies of the present invention are human antibodies.

Preferred subjects include human patients in need of enhancement of an immune response. The immunotherapeutic methods are particularly suitable for treating human patients having a disorder that can be treated by augmenting a T-cell mediated immune response. In certain embodiments, the methods are employed for treatment of subjects afflicted with a disease caused by an infectious agent. In preferred embodiments, the methods are employed for treatment of subjects afflicted with a cancer.

Cancer Immunotherapy

Disruption of the PD-1/PD-L1 interaction by antagonistic 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) *Nature Med* 8:787-89). The interaction between PD-1 and PD-L1 impairs T cell responses as manifested by a decrease in tumor infiltrating lymphocytes (TILs) and a decrease in T-cell receptor mediated proliferation, resulting in T cell anergy, exhaustion or apoptosis, and immune evasion by the cancerous cells (Zou and Chen (2008) *Nat Rev Immunol* 8:467-77; Blank *et al.* (2005) *Cancer Immunol Immunother* 54:307-14; Konishi *et al.* (2004) *Clin. Cancer Res.* 10:5094-100; Dong *et al.* (2003) *J Mol Med* 81:281-7; Iwai *et al.* (2002) *Proc Natl Acad Sci USA* 99:12293-97). Immune suppression can be reversed by inhibiting the local interaction between PD-L1 and PD-1 using an anti-PD-1 or an anti-PD-L1 antibody. These antibodies may be used alone or in combination to inhibit the growth of cancerous tumors. In addition, either or both of these antibodies may be used in conjunction with other immunogenic agents such as cytokines, standard cancer treatments, or other antibodies.

Immunotherapy of Cancer Patients using an Anti-PD-1 Antibody

This invention provides a method for immunotherapy of a subject afflicted with cancer, which method comprises administering to the subject a composition comprising a therapeutically effective amount of an antibody or an antigen-binding portion thereof that disrupts the interaction between PD-1 and PD-L1. The invention also provides a method of inhibiting growth of tumor cells in a subject, comprising administering to the subject an antibody or an antigen-binding portion thereof that disrupts the interaction between PD-1 and PD-L1 in an amount effective to inhibit growth of the tumor cells. In preferred embodiments, the subject is a human. In other preferred embodiments, the antibody or antigen-binding portion thereof binds specifically to PD-1. In certain embodiments, the

antibody or antigen-binding portion thereof is of an IgG1 or IgG4 isotype. In certain embodiments, the antibody or antigen-binding portion thereof is a monoclonal antibody or an antigen-binding portion thereof. In certain other embodiments, the antibody or antigen-binding portion thereof is a chimeric, humanized or human antibody or an antigen-binding portion thereof. In preferred embodiments for treating human subjects, the antibody or antigen-binding portion thereof is a human antibody or antigen-binding portion thereof.

The clinical trials described in the Examples employed the anti-PD-1 HuMAb BMS-936558 (designated 5C4 in U.S. Patent No. 8,008,449) to treat cancer. While 5C4 was selected as the lead Ab for entering the clinic, it is notable that several anti-PD-1 antibodies of the invention share with 5C4 functional properties that are important to the therapeutic activity of 5C4, including high affinity binding specifically to human PD-1, increasing T-cell proliferation, IL-2 secretion and interferon-γ production in an MLR assay, inhibiting the binding of PD-L1 and/or PD-L2 to PD-1, and inhibiting tumor cell growth *in vivo*. Moreover, certain of the anti-PD-1 antibodies of the invention, 17D8, 2D3, 4H1 and 7D3 are structurally related to 5C4 in having a V_H and a V_κ derived from V_H 3-33 and V_κ L6 germline sequences, respectively. In addition, 5C4, 2D3, 7D3, 4H1 and 17D8 all cross-compete for binding to the same epitope region of hPD-1 (Example 1). Thus, the preclinical characterization of 5C4 and other anti-PD-1 HuMabs indicate that the methods of treating cancer provided herein may be performed using a broad genus of anti-PD-1 antibodies including, but not limited to, any anti-PD-1 antibody of the invention.

Accordingly, certain embodiments of the immunotherapy methods disclosed herein comprise administering to a patient an anti-PD-1 antibody or antigen-binding portion thereof comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-33 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6 germline sequence, or (b) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 4-39 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L15 germline sequence.

In certain other embodiments, the antibody or antigen-binding portion thereof to

be administered to the patient cross-competes for binding to PD-1 with a reference antibody or a reference antigen-binding portion thereof comprising: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 1 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 8; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 2 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 9; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 3 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 10; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 5 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 12; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 6 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 13; or (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 7 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 14. In preferred embodiments, the antibody or antigen-binding portion thereof cross-competes for binding to PD-1 with a reference antibody or reference antigen-binding portion thereof comprising a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11.

In certain preferred embodiments of the immunotherapy methods disclosed herein, the anti-PD-1 antibody or antigen-binding portion thereof administered to the patient comprises: (a) a human heavy chain variable region comprising consecutively linked

amino acids having the sequence set forth in SEQ ID NO: 1 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 8; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 2 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 9; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 3 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 10; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 5 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 12; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 6 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 13; or (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 7 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 14. In more preferred embodiments, the anti-PD-1 antibody or antigen-binding portion comprises a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11.

In the clinical trials of anti-PD-1 immunotherapy described in the Examples below, intriguing objective responses (ORRs) with durable clinical responses, even in heavily pretreated patients, were observed across multiple tumor types including a substantial proportion of NSCLC, MEL, and RCC patients and in various sites of metastasis including liver, lung, lymph nodes, and bone. MEL and RCC are considered to be immunogenic neoplasms, having previously been demonstrated to be responsive to cancer immunotherapy, *i.e.*, IL-2 and/or anti-CTLA-4 antibody. In contrast, NSCLC has been considered "non-immunogenic" and poorly responsive to immune-based therapies

(Holt *et al.* (2011) *Therapy* 8:43-54). Thus, the results with NSCLC are particularly striking, unexpected and surprising. In NSCLC patients, 14 objective responses (ORs) were observed at BMS-936558 doses of 1, 3, or 10 mg/kg with response rates of 6%, 32%, and 18%, respectively. ORs were observed across NSCLC histologies: 6 responders of 18 squamous (33%), 7 of 56 nonsquamous, (13%), and 1 of 2 unknown. This level of activity seen with anti-PD-1 in NSCLC patients with significant prior therapy (47% with 3 lines of previous therapy) and across histologies is unique, particularly in the squamous histology patients (Gridelli *et al.* (2008) *J Thorac Onco*l 3:430-40; Miller (2006) *Semin Oncol* 33:S25-S31).

The durability of ORs across multiple cancer types in patients treated with the anti-PD-1 antibody was also notable. Although not compared directly in this study, this contrasts with the relatively modest durability of ORs observed in many NSCLC, MEL, and RCC patients treated with conventional chemotherapy and/or tyrosine-kinase inhibitor (TKI) treatments. The ORR in heavily pretreated NSCLC patients receiving anti-PD-1 antibody, including patients with squamous histology, is particularly surprising and unexpected, as standard salvage therapies historically show modest benefit in these patients (Scagliotti *et al.* (2011) *J Thorac Oncol* 6:64-70). As measured by standard RECIST in this study, ORs were long-lasting, with response durations $\geq$ 1 year in 20 of 31 responders. In addition, patterns of tumor regression consistent with immune-related patterns of response were observed.

It has become evident that conventional response criteria may not adequately assess the activity of immunotherapeutic agents because progressive disease (by initial radiographic evaluation) does not necessarily reflect therapeutic failure. For example, treatment with the anti-CTLA-4 antibody, ipilimumab (Yervoy™), have been shown to produce four distinct response patterns, all of which were associated with favorable survival: (a) shrinkage in baseline lesions, without new lesions; (b) durable stable disease (in some patients followed by a slow, steady decline in total tumor burden); (c) response after an increase in total tumor burden; and (d) response in the presence of new lesions. Accordingly, to properly evaluate immunotherapeutic agents, long-term effects on the target disease must also be captured. In this regard, systematic immune-related response criteria (irRC) that make allowances for an early increase in tumor burden and/or the appearance of new lesions, and which seek to enhance the characterization of immune-related response patterns, have been proposed (Wolchok *et al.* (2009) *Clin Cancer Res*

15:7412-7420). While the full impact of these unconventional response patterns remains to be defined in randomized trials of BMS-936558 with survival endpoints, the present observations are reminiscent of findings with ipilimumab in which a significant extension of overall survival was observed in treated patients (Hodi *et al.* (2010) *N Engl J Med* 363:711-23; Robert *et al.* (2011) *N Engl J Med* 364:2517-26).

The overall risk/benefit profile of anti-PD-1 immunotherapy is also favorable, with a low incidence of more severe drug-related adverse events (AEs; > grade 3), the specific events observed to date being consistent with other immunotherapeutic agents. This suggests that that anti-PD-1 immunotherapy can be delivered in an outpatient setting with minimal supportive care.

The clinical data presented herein demonstrate that immunotherapy based on PD-1 blockade is not limited to only "immunogenic" tumor types, such as MEL and RCC, but extends to tumor types not generally considered to be immune-responsive, including NSCLC. The unexpected successes with treatment-refractory metastatic NSCLC underscore the possibility that any neoplasm can be "immunogenic" in the context of proper immune modulation, and suggest that PD-1 blockade as an immunotherapeutic approach is broadly applicable across a very diverse range of tumor types. Preferred cancers that may be treated using the anti-PD-1 antibodies of the invention include cancers typically responsive to immunotherapy. Non-limiting examples of preferred cancers for treatment include MEL, RCC, colorectal cancer (CRC), castration-resistant prostate cancer (CRPC), squamous cell carcinoma of the head and neck, and carcinomas of the esophagus, ovary, gastrointestinal tract and breast. Although NSCLC is not generally considered responsive to immunotherapy, data disclosed herein unexpectedly demonstrate that both squamous and non-squamous NSCLC are preferred cancers for treatment with an anti-PD-1 antibody. Additionally, the invention includes refractory or recurrent malignancies whose growth may be inhibited using the anti-PD-1 antibodies of the invention.

Examples of other cancers that may be treated using the methods of the invention, based on the indications of very broad applicability of anti-PD-1 immunotherapy provided herein, include 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 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, multiple myeloma, environmentally induced cancers including those induced by asbestos, metastatic cancers, and any combinations of said cancers. The present invention is also applicable to treatment of metastatic cancers.

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. PD-1 blockade may also be effectively combined with standard cancer treatments, including chemotherapeutic regimes, radiation, surgery, hormone deprivation and angiogenesis inhibitors, as well as another immunotherapeutic antibody (*e.g.*, an anti-PD-L1 or anti-CTLA-4 antibody.

One aspect of this invention is the use of any anti-PD-1 antibody or antigen-binding portion thereof of the invention for the preparation of a medicament for inhibiting signaling from the PD-1/PD-L1 pathway to thereby potentiate an endogenous immune response in a subject afflicted with cancer. Another aspect is the use of any anti-PD-1 antibody or an antigen-binding portion thereof of the invention for the preparation of a medicament for immunotherapy of a subject afflicted with cancer comprising disrupting the interaction between PD-1 and PD-L1. These uses for the preparation of medicaments are broadly applicable to the full range of cancers disclosed herein. In preferred embodiments of these uses, the cancers include squamous NSCLC, non-squamous NSCLC, MEL, RCC, CRC, CRPC, squamous cell carcinoma of the head and neck, and

carcinomas of the esophagus, ovary, gastrointestinal tract and breast. This invention also provides medical uses of any anti-PD-1 antibody or antigen-binding portion thereof of the invention corresponding to all the embodiments of the methods of treatment employing an anti-PD-1 antibody described herein.

The invention also provides an anti-PD-1 antibody or an antigen-binding portion thereof of the invention for use in potentiating an endogenous immune response in a subject afflicted with cancer by inhibiting signaling from the PD-1/PD-L1 pathway. The invention further provides an anti-PD-1 antibody or an antigen-binding portion thereof of the invention for use in immunotherapy of a subject afflicted with cancer comprising disrupting the interaction between PD-1 and PD-L1. These antibodies may be used in potentiating an endogenous immune response against, or in immunotherapy of, the full range of cancers disclosed herein. In preferred embodiments, the cancers include squamous NSCLC, non-squamous NSCLC, MEL, RCC, CRC, CRPC, squamous cell carcinoma of the head and neck, and carcinomas of the esophagus, ovary, gastrointestinal tract and breast.

Immunotherapy of Cancer Patients using an Anti-PD-L1 Antibody

Blockade of PD-1/PD-L1 interaction has been shown to potentiate immune responses *in vitro* (Fife *et al.* (2009) *Nat Immunol* 10:1185-92) and mediate preclinical antitumor activity (Dong *et al.* (2002) *Nat Med* 8:793-800; Iwai *et al.* (2002) *Proc Natl Acad Sci USA* 99:12293-97). PD-L1 is the primary PD-1 ligand up-regulated within solid tumors, where it can inhibit cytokine production and the cytolytic activity of PD-1-positive, tumor-infiltrating CD4-positive and CD8-positive T-cells, respectively (Dong *et al.* (2002) *Nat Med* 8:793-800; Hino *et al.* (2010) *Cancer* 116:1757-66; Taube *et al.* (2012) *Sci Transl Med* 4:127ra37). These properties make PD-L1 a potentially promising target for cancer immunotherapy.

The clinical trials of anti-PD-L1 immunotherapy described in the Examples demonstrate for the first time that mAb blockade of the immune inhibitory ligand, PD-L1, produces both durable tumor regression and prolonged (≥ 24 weeks) disease stabilization in patients with metastatic NSCLC, MEL, RCC and OV, including those with extensive prior therapy. BMS-936559 had a favorable safety profile overall at doses up to and including 10 mg/kg, as is evident from the low (9%) incidence of grade 3-4 drug-related AEs. These findings are consistent with the mild autoimmune phenotype seen in PD-L1$^{-/-}$ mice (Dong *et al.* (2004) *Immunity* 20:327-36) and the more severe hyperproliferation

seen in CTLA-4$^{-/-}$ mice relative to PD-1$^{-/-}$ mice (Phan *et al.* (2003) *Proc Natl Acad Sci USA* 100:8372-77; Tivol *et al.* (1995) *Immunity* 3:541-47; Nishimura *et al.* (1999) *Immunity* 11:141-51). Most of the toxicities associated with anti-PD-L1 administration in patients were immune-related, suggesting on-target effects. The spectrum and frequency of AEOSI is somewhat different between anti-PD-L1 and anti-CTLA-4, emphasizing the distinct biology of these pathways (Ribas *et al.* (2005) *J Clin Oncol* 23:8968-77). Infusion reactions were observed with BMS-936559, although they were mild in most patients. Severe colitis, a drug-related AE observed in ipilimumab-treated patients (Beck *et al.* (2006) *J Clin Oncol* 24:2283-89), was infrequently noted with anti-PD-L1.

Another important feature of anti-PD-L1 therapy is the durability of responses across multiple tumor types. This is particularly notable considering the advanced disease and prior treatment of patients on the current study. Although not compared directly, this durability appears greater than that observed with most chemotherapies and kinase inhibitors used in these diseases.

Because peripheral blood T-cells express PD-L1, it is possible to assess *in vivo* RO by BMS-963559 as a pharmacodynamic measure. Median RO was 65.8%, 66.2%, and 72.4% for the doses tested. Whereas these studies provide a direct assessment and evidence of target engagement in patients treated with BMS-936559, relationships between RO in peripheral blood and the tumor microenvironment remain poorly understood.

Based on the clinical data disclosed herein, this invention provides a method for immunotherapy of a subject afflicted with cancer, which method comprises administering to the subject a composition comprising a therapeutically effective amount of an antibody or an antigen-binding portion thereof that specifically binds to PD-L1. The invention also provides a method of inhibiting growth of tumor cells in a subject, comprising administering to the subject an antibody or an antigen-binding portion thereof that specifically binds to PD-L1. In preferred embodiments, the subject is a human. In certain embodiments, the antibody or antigen-binding portion thereof is of an IgG1 or IgG4 isotype. In certain embodiments, the antibody or antigen-binding portion thereof is a monoclonal antibody or an antigen-binding portion thereof. In certain other embodiments, the antibody or antigen-binding portion thereof is a chimeric, humanized or human antibody or an antigen-binding portion thereof. In preferred embodiments for treating human subjects, the antibody or antigen-binding portion thereof is a human antibody or

antigen-binding portion thereof.

Clinical trials described in the Examples employed the anti-PD-L1 HuMAb BMS-936559 to treat cancer. While BMS-936559 (designated HuMAb 12A4 in U.S. Patent No. 7,943,743) was selected as the lead anti-PD-L1 Ab for entering the clinic, it is notable that several anti-PD-L1 Abs of the invention share with 12A4 functional properties that are important to the therapeutic activity of 5C4, including high affinity binding specifically to human PD-L1, increasing T-cell proliferation, IL-2 secretion and interferon-γ production in an MLR assay, inhibiting the binding of PD-L1 to PD-1, and reversing the suppressive effect of T regulatory cells on T cell effector cells and /or dendritic cells. Moreover, certain of the anti-PD-L1 antibodies of the invention, namely 1B12, 7H1 and 12B7 are structurally related to 5C4 in having a V_H and a V_κ derived from V_H 1-69 and V_κ L6 germline sequences, respectively. In addition, at least 12B7, 3G10, 1B12 and 13G4 cross-compete with 12A4 for binding to the same epitope region of hPD-L1, whereas 5F8 and 10A5 may bind to the same or an overlapping epitope region as 12A4 (Examples 2 and 3). Thus, the preclinical characterization of 12A4 and other anti-PD-L1 HuMabs indicate that the methods of treating cancer provided herein may be performed using a broad genus of anti-PD-L1 of the invention.

Accordingly, this invention provides immunotherapy methods comprising administering to a patient an anti-PD-L1 antibody or antigen-binding portion thereof comprising (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-18 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6 germline sequence; (b) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-69 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6 germline sequence; (c) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-3 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L15 germline sequence; (d) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-69 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ A27 germline sequence; (e) a

heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-9 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L15germline sequence; or (f) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-9 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L18germline sequence.

In certain other embodiments, the anti-PD-L1 antibody or antigen-binding portion thereof administered to the patient cross-competes for binding to PD-L1 with a reference antibody or a reference antigen-binding portion thereof comprising: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 15 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 25; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 17 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 27; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 18 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 28; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 19 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 29; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 20 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 30; (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 21 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 31; (h) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set

forth in SEQ ID NO: 22 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 32; (i) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 23 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 33; or (j) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 24 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 34. In preferred embodiments, the antibody or antigen-binding portion thereof cross-competes for binding to PD-1 with a reference antibody or reference antigen-binding portion thereof comprising a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26.

In certain preferred embodiments of the immunotherapy methods disclosed herein, the anti-PD-L1 antibody or antigen-binding portion thereof administered to the subject comprises: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 15 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 25; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 17 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 27; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 18 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 28; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 19 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 29; (f) a human heavy chain variable region comprising consecutively linked amino acids having

the sequence set forth in SEQ ID NO: 20 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 30; (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 21 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 31; (h) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 22 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 32; (i) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 23 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 33; or (j) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 24 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 34. In more preferred embodiments, the anti-PD-L1 antibody or antigen-binding portion comprises a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26.

The clinical activity of anti-PD-L1 in patients with advanced NSCLC, similar to the activity of anti-PD-1 in these patients, was surprising and unexpected since NSCLC has been considered to be poorly responsive to immune-based therapies (Holt *et al.* (2011) *Therapy* 8:43-54). The present clinical data obtained with an anti-PD-L1 antibody substantiate and extend the evidence obtained using the anti-PD-1 antibody that immunotherapy based on PD-1 blockade is not applicable only to "immunogenic" tumor types, such as MEL and RCC, but is also effective with a broad range of cancers, including treatment-refractory metastatic NSCLC, that are generally not considered to be immune-responsive. Preferred cancers that may be treated using the anti-PD-L1 antibodies of the invention include MEL (*e.g.*, metastatic malignant melanoma), RCC, squamous NSCLC, non-squamous NSCLC, CRC, ovarian cancer (OV), gastric cancer (GC), breast cancer (BC), pancreatic carcinoma (PC) and carcinoma of the esophagus. Additionally, the invention includes refractory or recurrent malignancies whose growth may be inhibited using the anti-PD-L1 antibodies of the invention.

Examples of other cancers that may be treated using the methods of the invention, based on the indications of very broad applicability of anti-PD-L1 immunotherapy provided herein, include bone cancer, skin cancer, cancer of the head or neck, breast cancer, lung cancer, cutaneous or intraocular malignant melanoma, renal cancer, uterine cancer, castration-resistant prostate cancer, colon 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, carcinomas of the ovary, gastrointestinal tract and breast, 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, multiple myeloma, environmentally induced cancers including those induced by asbestos, metastatic cancers, and any combinations of said cancers. The present invention is also applicable to treatment of metastatic cancers.

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. PD-1 blockade may also be effectively combined with standard cancer treatments, including chemotherapeutic regimes, radiation, surgery, hormone deprivation and angiogenesis inhibitors, as well as another immunotherapeutic antibody (*e.g.*, an anti-PD-1 or anti-CTLA-4 antibody).

This invention provides the use of any anti-PD-L1 antibody or antigen-binding portion thereof of the invention for the preparation of a medicament for inhibiting signaling from the PD-1/PD-L1 pathway to thereby potentiate an endogenous immune

response in a subject afflicted with cancer. The invention also provides the use of any anti-PD-L1 antibody or antigen-binding portion thereof of the invention for the preparation of a medicament for immunotherapy of a subject afflicted with cancer comprising disrupting the interaction between PD-1 and PD-L1. The invention provides medical uses of any anti-PD-L1 antibody or antigen-binding portion thereof of the invention corresponding to all the embodiments of the methods of treatment employing an anti-PD-L1 antibody described herein.

This invention also provides an anti-PD-L1 antibody or an antigen-binding portion thereof of the invention for use in potentiating an endogenous immune response in a subject afflicted with cancer by inhibiting signaling from the PD-1/PD-L1 pathway. The invention further provides an anti-PD-L1 antibody or an antigen-binding portion thereof of the invention for use in immunotherapy of a subject afflicted with cancer comprising disrupting the interaction between PD-1 and PD-L1. These antibodies may be used in potentiating an endogenous immune response against, or in immunotherapy of, the full range of cancers disclosed herein. In preferred embodiments, the cancers include MEL (*e.g.*, metastatic malignant melanoma), RCC, squamous NSCLC, non-squamous NSCLC, CRC, ovarian cancer (OV), gastric cancer (GC), breast cancer (BC), pancreatic carcinoma (PC) and carcinoma of the esophagus.

Validation of Cancer Immunotherapy by Immune Checkpoint Blockade

A major implication of the clinical activity of immune checkpoint blockade is that significant endogenous immune responses to tumor antigens are generated and these responses may be harnessed therapeutically to mediate clinical tumor regression upon checkpoint inhibition. In fact, there is evidence that inhibitory ligands such as PD-L1 are induced in response to immune attack, a mechanism termed adaptive resistance (Taube *et al.* (2012) *Sci Transl Med* 4,127ra37; Gajewski *et al.* (2010) *Cancer J* 16,399-403). This potential mechanism of immune resistance by tumors suggests that PD-1/PD-L1-directed therapy might synergize with other treatments that enhance endogenous antitumor immunity. Follow-up studies are ongoing to verify whether patients continue to demonstrate tumor control after cessation of PD-1/PDL1 pathway blockade. Such tumor control could reflect a persistent antitumor immune response and the generation of effective immunologic memory, to enable sustained control of tumor growth.

The data disclosed herein on the clinical testing of antibodies that block the immune-regulatory receptor, PD-1, and also of antibodies that block one of its cognate

ligands, PD-L1, are unprecedented. These data constitute the largest clinical experience to date with PD-1 pathway-directed cancer immunotherapy, and the first report specifically describing the safety, tolerability, and initial clinical activity of an anti-PD-L1-directed agent. These findings show that anti-PD-L1 has a favorable overall safety profile and intriguing evidence of clinical activity across diverse cancers, including NSCLC, a tumor not historically considered responsive to immunotherapy, as well as tumors known to respond to immunotherapy, including MEL and RCC. These findings, consistent with the data provided herein on the results of treatment with anti-PD-1, provide strong validation of the PD-1/PD-L1 pathway as an important target for therapeutic intervention in cancer.

The remarkable similarities observed between the patterns of clinical activity obtained with the anti-PD-1 and anti-PD-L1 mAbs, and among the tumor types analyzed to date, validate the general importance of the PD-1/PD-L1 signaling pathway in tumor immune resistance and as a target for therapeutic intervention. However, the molecular interactions potentially blocked by these two antibodies are not identical: anti-PD-1 antibodies of the invention disrupt PD-1/PD-L1 and potentially PD-1/PD-L2 interactions; in contrast, whereas anti-PD-L1 antibodies of the invention also disrupt PD-1/PD-L1 interactions, they do not block PD-1/PD-L2 interactions but instead potentially disrupt the PD-1-independent PD-L1/CD80 interaction, which has also been shown to down-modulate T-cell responses *in vitro* and *in vivo* (Park *et al.* (2010) *Blood* 116:1291-98; Paterson *et al.* (2011) *J Immunol* 187:1097-105; Yang *et al.* (2011) *J Immunol* 187:1113-19; Butte *et al.* (2007) *Immunity* 27:111-22; Butte et al. (2008) *Mol Immunol* 45:3567-72). Thus, it is possible that among these varied ligand-receptor pairings, different interactions may dominate in different cancer types, contributing to dissimilar activity profiles for the two antibodies. Nevertheless, irrespective of mechanistic details, what has been clearly demonstrated herein is that both anti-PD-1 and anti-PD-L1 antibodies of the invention are effective in treating patients afflicted with a wide variety of cancers, including "immunogenic" cancers such as MEL and RCC as well as treatment-refractory metastatic NSCLC, a tumor that is generally not considered to be immune-responsive. In certain embodiments of the invention, either or both of these antibodies can be administered in combination with another therapeutic agent such as a cytokine.

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-PD1 or an anti-PD-L1 antibody, or antigen-binding portion thereof, of the invention such that the subject is treated for the infectious disease. Preferably, the antibody is a human anti-human PD-1 or PD-L1 antibody (such as any of the human 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, and 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.

In the above methods, PD-1 or PD-L1 blockade can be combined with other forms of immunotherapy such as cytokine treatment (*e.g.*, administration of interferons, GM-CSF, G-CSF or IL-2).

Kits

Also within the scope of the present invention are kits, including pharmaceutical kits comprising an anti-PD-1 and/or an anti-PD-L1 antibody of the invention for therapeutic uses, and diagnostic kits comprising an anti-PD-L1 antibody of the invention for assaying membranous PD-L1 expression as a biomarker for screening patients for immunotherapy or for predicting the efficacy of an immunotherapeutic agent. 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 and/or anti-PD-L1 antibodies may be co-packaged with other therapeutic agents in unit dosage form. In certain embodiments of a diagnostic kit,

the anti-PD-L1 antibody may be co-packaged with other reagents for performing an assay to detect and/or quantify PD-L1 expression.

In certain preferred embodiments, the pharmaceutical kit comprises the anti-human PD-1 HuMAb, BMS-936558. In other preferred embodiments, the pharmaceutical kit comprises the anti-human PD-L1 HuMAb, BMS-936559. In certain preferred embodiments, the diagnostic kit comprises the rabbit anti-human PD-L1 mAb, 28-8, comprising the V_H and V_κ regions whose amino acid sequences are set forth in SEQ ID NOs. 35 and 36, respectively. In other preferred embodiments, the diagnostic kit comprises the murine anti-human PD-L1 mAb, 5H1 (Dong *et al.* (2002) *Nat Med* 8:793-800).

PD-L1 Biomarker for Predicting anti-PD-1 Efficacy

A particular challenge in cancer immunotherapy has been the identification of mechanism-based predictive biomarkers to enable patient selection and guide on-treatment management. Data disclosed in the Examples below indicate that cell surface PD-L1 expression in tumors is a useful molecular marker for predicting the efficacy of, and selecting patients for, anti-PD-1 immunotherapy.

There are conflicting reports in the literature about the clinical implications of PD-L1 being expressed in tumors. Several studies have concluded that PD-L1 expression in tumors correlates with a poor prognosis for the patient. *See, e.g.*, Hino *et al.* (2010) Cancer 116:1757-66 (MEL); Hamanishi *et al.* (2007) *Proc Natl Acad Sci USA* 104:3360-65 (ovarian cancer); Thompson *et al.* (2006) *Cancer Res* 66:3381-85 (RCC). These findings may be rationalized on the basis that the interaction of PD-L1 on tumor cells and PD-1 on T cells helps abrogate immune responses directed against the tumor, resulting in immune evasion from tumor-specific T cells. However, in contrast to the foregoing studies, Gadiot *et al.* ((2011) *Cancer* 117:2192-201) and Taube *et al.* (2012) have recently reported that PD-L1 expression in melanoma tumors correlates with a trend toward better survival. These seemingly contradictory data may reflect the relatively small numbers of patients analyzed, different histologic subtypes studied, or different methodologies used, *e.g.*, the use of different antibodies to stain PD-L1, the use of frozen versus paraffin-embedded material for IHC, and the detection of membranous and/or cytoplasmic staining of PD-L1. Taube *et al.* (2012) note that PD-L1 is a type I transmembrane molecule, and hypothesize that while the cytoplasmic presence of PD-L1 may represent intracellular stores of this polypeptide that may be deployed to the cell surface upon

appropriate stimulation, it is cell surface expression of that is biologically relevant as a potential biomarker for predicting clinical response to PD-1 blockade. *See, also*, Brahmer *et al.* (2010) *J Clin Oncol* 28:3167-75. The data described in the Examples below on the use of membranous PD-L1 expression as a biomarker for anti-PD-1 efficacy support this hypothesis.

Specifically, membranous PD-L1 expression was assayed using an automated IHC protocol and a rabbit anti-hPD-L1 antibody. Strikingly, no patients with cell surface PD-L1-negative tumors (MEL, NSCLC, CRC, RCC and CRPC) experienced an objective response (OR) following treatment with the anti-PD-1 antibody, BMS-936558. In contrast, cell surface expression of PD-L1 on tumor cells in pretreatment biopsies may be associated with an increased rate of OR among patients treated with BMS-936558. While tumor cell expression of PD-L1 may be driven by constitutive oncogenic pathways, it may also reflect "adaptive immune resistance" in response to an endogenous antitumor immune response, part of a host inflammatory response, which may remain in check unless unleashed via PD-1/PD-L1 pathway blockade (Taube *et al.*, 2012). This emerging concept of adaptive immune resistance in cancer immunology suggests that inhibitory ligands such as PD-L1 are induced in response to immune attack (Taube *et al.* (2012) *Sci Transl Med* 4,127ra37; Gajewski *et al.* (2010) *Cancer J* 16,399-403). A major implication of the clinical activity of immune checkpoint blockade as described herein is that significant endogenous immune responses to tumor antigens are generated and these responses may be harnessed therapeutically to mediate clinical tumor regression upon checkpoint inhibition. This potential mechanism of immune resistance by tumors suggests that PD-1/PD-L1-directed therapy might synergize with other treatments that enhance endogenous antitumor immunity. It also suggests that cell surface expression of PD-L1 in tumors and/or inflammatory cells in the tumor microenvironment may be a marker not just for treatment of cancer patients with an anti-PD-1 Ab, but also for treatment with an anti-PD-L1 Ab as well as treatments targeting inhibitory immunoregulatory pathways other than the PD-1/PD-L1 pathway.

Assaying Cell-Surface PD-L1 Expression by Automated IHC

As described in the Examples, an automated IHC method was developed for assaying the expression of PD-L1 on the surface of cells in FFPE tissue specimens. The invention 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 control sample, with a monoclonal antibody 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. Preferably, 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 control sample is indicative of the presence of human PD-L1 antigen in the sample. Various methods are used to quantify PD-L1 expression.

For assessing PD-L1 expression in tumors, a pathologist examines the number of membrane PD-L1$^+$ tumor cells in each field 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 the intermediate 1+ and 2+ intensities are considered. For highly heterogeneous, 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%.

Staining is also assessed in tumor-infiltrating inflammatory cells such as macrophages and lymphocytes. In most cases macrophages serve as internal positive control since the staining is observed in a large number of cases. While not required to stain with 3+ intensity, an absence of staining 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 cells and/or on the bounds 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.

In certain embodiments of these scoring methods, the samples are scored 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.

A histoscore is used as a more quantitative measure of the IHC data. The histoscore is calculated as follows:

Histoscore (range 0-300) = [(% tumor x 1 (low intensity)) + (% tumor x 2 (medium intensity)) + (% tumor X 3 (high intensity)]

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).

An alternative means of quantifying PD-L1 expression by 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.*, 2012).

Cancer Immunotherapy with Anti-PD-1 Comprising a Patient Selection Step

This invention also provides a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is a suitable candidate for immunotherapy, the selecting comprising (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells, (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface, and (iii) selecting the subject as a suitable candidate based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and (b) administering to the selected subject a composition comprising a therapeutically effective amount of an antibody or an antigen-binding portion thereof that inhibits signaling from an inhibitory immunoregulator.

There is evidence that PD-L1 expression is a surrogate for an endogenous antitumor immune response that is part of a host inflammatory response (Taube *et al.* (2012) *Sci Transl Med* 4,127ra37; Gajewski *et al.* (2010) *Cancer J* 16,399-403). Accordingly, cell surface expression of PD-L1 in tumors and/or tumor-infiltrating inflammatory cells is a marker not just for selecting cancer patients who would benefit from treatment with an anti-PD-1 Ab, but also for treatment with an anti-PD-L1 Ab as well as treatments targeting inhibitory immunoregulatory pathways other than the PD-

1/PD-L1 pathway. For example, cell surface expression of PD-L1 in tumors and/or tumor-infiltrating inflammatory cells may be used as a marker for identifying or selecting suitable cancer patients who would benefit from immunotherapy with agents, including antibodies, that target, and disrupt signaling from, inhibitory immunoregulators such as PD-L1, Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4), B and T Lymphocyte Attenuator (BTLA), T cell Immunoglobulin and Mucin domain-3 (TIM-3), Lymphocyte Activation Gene-3 (LAG-3), Killer cell Lectin-like Receptor G1 (KLRG-1), Natural Killer Cell Receptor 2B4 (CD244), and CD160 (Baitsch et al. (2012) PloS One 7(2):e30852).

Although many of the immunotherapy methods below comprising assaying PD-L1 expression, *i.e.*, employing a PD-L1 expression biomarker, are described as comprising the administration of an anti-PD-1 antibody for immunotherapeutic purposes, it should be understood that these methods apply generally to the administration of an inhibitor of an inhibitory immunoregulator or a component or ligand thereof. Further, in any the methods comprising the measurement of PD-L1 expression in a test tissue sample, 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 "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 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 medical practitioner for use in selecting a suitable candidate for immunotherapy and/or administering an immunotherapeutic agent to the patient. In certain embodiments, the steps that provide the intermediate result may be performed by a medical practitioner or someone acting under the direction of a medical practitioner. In other embodiment, these steps are performed by an independent person or laboratory.

The invention further provides a method for treatment of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is not suitable for anti-PD-1

antibody immunotherapy, the selecting comprising (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; and (iii) selecting the subject as not suitable for anti-PD-1 antibody immunotherapy based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is less than a predetermined threshold level; and (b) administering a standard-of-care therapeutic other than an anti-PD-1 antibody to the selected subject.

As used herein, a "standard-of-care therapeutic" is a treatment process, including a drug or combination of drugs, radiation therapy, surgery or other medical intervention that is recognized by medical practitioners as appropriate, accepted, and/or widely used for a certain type of patient, disease or clinical circumstance. Standard-of-care therapeutics for treating different types of cancer are well known by persons of skill in the art.

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 preferred embodiments, PD-L1 expression is assayed by IHC. Flow cytometry may be particularly suitable for assaying PD-L1 expression in cells of liquid tumors. In preferred embodiments of all of these methods, cell surface expression of PD-L1 is assayed.

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 will 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 (2010) *Cold Spring Harb Perspect Biol* 2010;2:a003848). 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 (2010) *Cancer Biother Radiopharm* 25(3):253-61; Olafsen *et al.*, 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 certain preferred 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 further embodiments, the IHC assay is performed using an anti-PD-L1 monoclonal antibody to bind to the PD-L1 polypeptide.

An antibody may bind to an antigen in fresh tissues but completely fail to recognize the antigen in an FFPE tissue sample. This phenomenon, well known in the art, is thought to be due primarily to intra- and intermolecular cross-linking of polypeptides induced by formalin fixation, which alters the epitope recognized by the antibody (Sompuram *et al.* (2006) *Am J Clin Pathol* 125:82-90). In addition, several factors known to influence staining in FFPE tissue, including variable time to fixation, inadequate fixation period, differences in fixative used, tissue processing, antibody clone and dilution, antigen retrieval, detection system, and interpretation of results using different threshold points are important variables that can affect tissue antigenicity and IHC measurements (Bordeaux *et al.* (2010) *BioTechniques* 48:197-209). Specifically, a lack of anti-human PD-L1 Abs that stain PD-L1 in FFPE specimens has been noted in the art (Hamanishi *et al.*, 2007). Further, our analysis of several commercially available anti-hPD-L1 Abs shows that these antibodies do not stain PD-L1 in cells expressing this antigen (see Examples). Monoclonal antibodies that detect hPD-L1 on the surface of cells in FFPE tissues are provided in the present invention.

Specifically, one aspect of the present invention is a monoclonal antibody or antigen-binding portion thereof that binds specifically to a cell surface-expressed PD-L1 antigen in a FFPE tissue sample. In preferred embodiments, the monoclonal antibody or

antigen-binding portion thereof does not bind to a cytoplasmic PD-L1 polypeptide in the FFPE tissue sample or exhibits a very low level of background binding. In certain other embodiments, the presence or absence of binding specifically to a cell surface-expressed or a cytoplasmic PD-L1 polypeptide is detected by immunohistochemical staining. In certain preferred aspects of the invention, the monoclonal antibody or antigen-binding portion is a rabbit antibody or portion thereof. In further aspects, the monoclonal antibody or antigen-binding portion thereof is of an IgG1 or IgG4 isotype. In other preferred embodiments, the monoclonal antibody is the rabbit mAb designated 28-8, 28-1, 28-12, 29-8 or 20-12. In more preferred embodiments, the monoclonal antibody is the rabbit mAb designated 28-8 or an antigen-binding portion thereof comprising a heavy chain variable region (V_H) comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 35 and a light chain variable region (V_κ) comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 36. In certain aspects, the monoclonal antibody or antigen-binding portion thereof does not cross-compete with mouse mAb 5H1 for binding to PD-L1. In certain other aspects, the monoclonal antibody or antigen-binding portion cross-competes with mouse mAb 5H1 for binding to PD-L1.

The invention also provides nucleic acids encoding all of the rabbit anti-hPD-L1 antibodies or portions thereof disclosed herein.

The availability of antibodies that bind with high affinity specifically to membranous PD-L1 in FFPE tissue specimens facilitates methods comprising detecting PD-L1 polypeptide on the surface if cells in tissue FFPE samples. Accordingly, this invention also provides a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is a suitable candidate for immunotherapy, the selecting comprising: (i) optionally providing a FFPE test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface by IHC using mAb 28-8 to bind to the PD-L1; and (iii) selecting the subject as a suitable candidate based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and (b) administering a composition comprising a therapeutically effective amount of an anti-PD-1 antibody to the selected subject.

In certain embodiments of methods employing IHC to assay PD-L1 expression in FFPE tissues, an automated IHC assay is used. The automated IHC process is performed on an autostainer and comprises: (a) de-paraffinizing the FFPE sample with xylene and rehydrating the sample; (b) retrieving the antigen using a decloaking chamber; (c) blocking nonspecifc protein binding sites by incubation with a Protein Block; (d) incubating the sample with a primary anti-PD-L1 antibody; (e) adding a polymeric horseradish peroxidase (HRP)-conjugated secondary antibody; (f) detecting the bound secondary antibody comprising staining with a 3,3'-diaminobenzidine (DAB) chromogen; and/or (g) counterstaining with hematoxylin. This automated IHC process has been optimized by minimization of the number of steps, optimization of incubation times, and selection of primary antibodies, blocking and detection reagents that produce strong specific staining with a low level of background staining. In preferred embodiments of this automated IHC assay, the primary anti-PD-L1 antibody is mAb 28-8 or 5H1. In certain embodiments of this invention, this IHC assay, and any other IHC assay described herein to measure PD-L1 expression, may be used as part of a method of immunotherapy. In other embodiments, any of the IHC methods described herein is used independently of any therapeutic process requiring the administration of a therapeutic, *i.e.*, solely as a diagnostic method to assay PD-L1 expression.

In certain embodiments any of the immunotherapy methods described herein, the antibody to the selected subject is any anti-PD-1 or anti-PD-L1 antibody or antigen-binding portion thereof of the invention. In certain preferred embodiments, the subject is a human. In other preferred embodiments, the antibody is a human antibody or antigen-binding portion thereof. In more preferred embodiments, the anti-PD-1 antibody is BMS-936558 and the anti-PD-L1 antibody is BMS-936559. In certain other embodiments, the anti-PD-1 antibody is an antibody or antigen-binding portion thereof that cross-competes for binding to PD-1 with BMS-936558, and the anti-PD-L1 antibody is an antibody or antigen-binding portion thereof that cross-competes for binding to PD-L1 with BMS-936559. In certain preferred embodiments, the cancer to be treated is selected from the group consisting of melanoma (MEL), renal cell carcinoma (RCC), squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, colorectal cancer (CRC), castration-resistant prostate cancer (CRPC), squamous cell carcinoma of the head and neck, or carcinomas of the esophagus, ovary, gastrointestinal tract and breast.

In certain embodiments of the disclosed methods, the predetermined threshold is based on a proportion of (a) tumor cells, (b) tumor-infiltrating inflammatory cells, (c) particular tumor-infiltrating inflammatory cells, *e.g.*, TILs or macrophages, or (d) a combination of tumor cells and tumor-infiltrating inflammatory cells in a test tissue sample that expresses PD-L1 on the cell surface. In certain embodiments, the predetermined threshold is at least 0.001% of tumor cells expressing membranous PD-L1 as determined by IHC. In other embodiments, the predetermined threshold is at least 0.01%, preferably at least 0.1%, more preferably at least 1% of tumor cells expressing membranous PD-L1, as determined by IHC. In certain embodiments, the predetermined threshold is at least 5% of tumor cells expressing membranous PD-L1 as determined by IHC. In certain embodiments, the predetermined threshold is at least 0.01%, at least 0.1%, at least 1%, or at least 5% of tumor cells expressing membranous PD-L1 as determined by IHC, and/or a single tumor-infiltrating inflammatory cell expressing membranous PD-L1 as determined by IHC. In certain other embodiments, the predetermined threshold is at least 0.01%, at least 0.1%, at least 1%, or at least 5% of a tumor-infiltrating inflammatory cell expressing membranous PD-L1 as determined by IHC. In certain other embodiments, the predetermined threshold is at least 0.01%, at least 0.1%, at least 1%, or at least 5% of a tumor-infiltrating lymphocyte expressing membranous PD-L1 as determined by IHC. In certain other embodiments, the predetermined threshold is at least 0.01%, at least 0.1%, at least 1%, or at least 5% of a tumor-infiltrating macrophage expressing membranous PD-L1 as determined by IHC. In yet other embodiments, the predetermined threshold is at least a single tumor cell or a single tumor-infiltrating inflammatory cell expressing membranous PD-L1 as determined by IHC. Preferably, PD-L1 expression is assayed by automated IHC using mAb 28-8 or 5H1 as the primary antibody.

This invention provides a method for treatment of a subject afflicted with cancer, which method comprises: (a) screening a plurality of subjects to identify a subject that is not a suitable candidate for immunotherapy comprising the administration of an anti-PD-1 antibody to the subject, the screening comprising: (i) optionally providing test tissue samples from the plurality of subjects, the test tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue samples that express on the surface of the cells; and (iii) selecting the subject as a candidate that is not suitable for anti-PD-1 antibody immunotherapy based on an assessment that the proportion of cells that express PD-L1 on the surface of cells in the

subject's test tissue sample is below a predetermined threshold level; and (b) administering a standard-of-care therapeutic other than an anti-PD-1 antibody to the selected subject.

This invention also provides a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) screening a plurality of subjects to identify a subject that is a suitable candidate for immunotherapy, the screening comprising: (i) optionally providing test tissue samples from the plurality of subjects, the test tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue samples that express on the surface of the cells; and (iii) selecting the subject as a candidate that is suitable for anti-PD-1 antibody immunotherapy based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and (b) administering a composition comprising a therapeutically effective amount of an anti-PD-1 antibody to the selected subject.

This invention further provides a method for treatment of a subject afflicted with cancer, which method comprises: (a) screening a plurality of subjects to identify a subject that is a suitable candidate for the treatment, the screening comprising: (i) optionally providing test tissue samples from the plurality of subjects, the test tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue samples that express on the surface of the cells, wherein the subject is identified as a suitable candidate for anti-PD-1 antibody immunotherapy if cells in the tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level, and the subject is identified as a candidate that is not a suitable candidate for anti-PD-1 antibody immunotherapy if cells in the tissue sample that express PD-L1 on the cell surface is below a predetermined threshold level; and (b) administering a composition comprising a therapeutically effective amount of an anti-PD-1 antibody to the subject identified as a suitable candidate for anti-PD-1 antibody immunotherapy, or (c) administering a standard-of-care therapeutic other than an anti-PD-1 antibody to the subject identified as not a suitable candidate for anti-PD-1 antibody immunotherapy.

One aspect of this invention is a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor

cells and tumor-infiltrating inflammatory cells; (b) determining that cells in the test tissue sample express PD-L1 on the cell surface; and (c) based on that determination administering a composition comprising a therapeutically effective amount of an anti-PD-1 antibody to the subject. Another aspect of the invention is a method for treatment of a subject afflicted with cancer, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) determining that cells in the test tissue sample do not express PD-L1 on the cell surface; and (c) based on that determination administering a standard-of-care therapeutic other than an anti-PD-1 antibody to the subject.

Yet another aspect of the invention is a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) determining that cells in the test tissue sample express PD-L1 on the cell surface; (c) selecting an anti-PD-1 antibody as a treatment for the subject based on the recognition that an anti-PD-1 antibody is effective in patients whose test tissue sample contains a proportion of cells above a predetermined threshold level that express PD-L1 on the cell surface; and (d) administering a composition comprising a therapeutically effective amount of an anti-PD-1 antibody to the subject. In a further aspect, the invention provides a method for treatment of a subject afflicted with cancer, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) determining that cells in the test tissue sample do not express PD-L1 on the cell surface; (c) selecting a standard-of-care therapeutic other than an anti-PD-1 antibody as a treatment for the subject based on the recognition that an anti-PD-1 antibody is ineffective in patients whose test tissue sample contains a proportion of cells below a predetermined threshold level that express PD-L1 on the cell surface; and (d) administering the standard-of-care therapeutic to the subject.

This invention also provides a method of selecting an immunotherapy for a subject afflicted with cancer, which method comprises: (a) assaying cells of a test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells to assess the proportion of cells in the test tissue sample that express PD-L1; and (b) based on an assessment that the proportion of cells that express membranous PD-L1 is above a

predetermined threshold level, selecting an immunotherapy comprising a therapeutically effective amount of an anti-PD-1 antibody for the subject. The invention further provides a method of selecting a treatment for a subject afflicted with cancer, which method comprises: (a) assaying cells of a test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells to assess the proportion of cells in the test tissue sample that express PD-L1; and (b) based on an assessment that the proportion of cells that express membranous PD-L1 is above a predetermined threshold level, selecting a standard-of-care treatment other than an anti-PD-1 antibody for the subject.

In addition, the invention provides a method for treatment of a subject afflicted with cancer, which method comprises administering to the subject a composition comprising a therapeutically effective amount of an anti-PD-1 antibody, the subject having been selected on the basis that the proportion of cells in a test tissue sample from the subject that express PD-L1 is determined to exceed a predetermined threshold level, wherein the test tissue sample comprises tumor cells and tumor-infiltrating inflammatory cells. This invention also provides a method for treatment of a subject afflicted with cancer, which method comprises administering to the subject a standard-of-care treatment other than an anti-PD-1 antibody, the subject having been selected on the basis that the proportion of cells in a test tissue sample from the subject that express PD-L1 is determined to be below a predetermined threshold level, wherein the test tissue sample comprises tumor cells and tumor-infiltrating inflammatory cells.

This invention further provides a method for selecting a cancer patient for immunotherapy with an anti-PD-1 antibody, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold proportion; and (d) selecting the patient for immunotherapy based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 is above the predetermined threshold level. In any of the methods described herein comprising a step for assessing PD-L1 expression, the test tissue sample may be a FFPE tissue sample.

In addition, in any method where an immunotherapy is selected or administered based on an assessment that the proportion of cells in a test tissue sample from the subject

expresses PD-L1 at a level above a predetermined threshold level, it follows that a complementary method of treatment may be performed wherein a standard-of-care treatment other than the immunotherapy is selected or administered based on an assessment that the proportion of cells in a test tissue sample from the subject expresses PD-L1 at a level below a predetermined threshold level.

This invention further provides a method for predicting the therapeutic effectiveness of an anti-PD-1 antibody for treating a cancer patient, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold value; and (d) predicting the therapeutic effectiveness of the anti-PD-1 antibody, wherein if the proportion of cells that express PD-L1 on the cell surface exceeds the threshold proportion the antibody is predicted to be effective in treating the patient, and wherein if the proportion of cells that express PD-L1 on the cell surface is below the threshold proportion the antibody is predicted to not be effective in treating the patient.

This invention also provides a method for determining an immunotherapeutic regimen comprising an anti-PD-1 antibody for treating a cancer patient, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold proportion; and (d) determining an immunotherapeutic regimen comprising an anti-PD-1 antibody based on the determination that the proportion of cells that express PD-L1 on the cell surface exceeds the predetermined threshold proportion.

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.

EXAMPLE 1

Cross-Competition between Anti-PD-1 HuMAbs for Binding to CHO Cells Expressing Human PD-1

Chinese Hamster Ovary (CHO) cells transfected to express human PD-1 (CHO/PD-1 cells) were incubated with 10 μg/ml of Fab fragment of the anti-PD-1 HuMAb 5C4 or human IgG1 (hIgG1) isotype control Ab for 30 minutes at 4°C before addition of anti-PD-1 HuMAbs 4H1, 2D3 or 7D3 at a concentration of 0.2 μg /ml. Binding of 4H1, 2D3 or 7D3 to CHO/PD-1 cells were detected by fluorescein isothiocyanate (FITC)-conjugated goat anti-hIgG, Fc-gamma specific antibody. In the case of cross-competition assay with 5C4 and 17D8, CHO/PD-1 cells were incubated with the whole molecule of 5C4 before addition of FITC-labeled 17D8. Binding of 2D3, 7D3, 4H1 or 17D8 to the CHO/PD-1 cells was measured by flow cytometric analysis using a FACScalibur flow cytometer (Becton Dickinson, San Jose, CA). The results are depicted in Figure 1. The data show that the 5C4 Fab fragment substantially blocked the binding of mAbs 5C4 itself, as well as the binding of 4H1, 2D3 and 7D3, while the 5C4 whole mAb substantially blocked the binding of 17D8, to CHO/PD-1 cells as measured by mean fluorescent intensity (MFI) of staining.

EXAMPLE 2

Cross-Competition between Anti-PD-L1 HuMAbs for Binding to CHO Cells Expressing Human PD-L1

CHO cells transfected to express hPD-L1 (CHO/PD-L1 cells) were incubated with 10 μg/ml of each of ten unconjugated human anti-PD-L1 mAbs (5F8, 7H1, 10H10, 1B12, 3G10, 10A5, 11E6, 12A4, 12B7, and 13G4) or human IgG1 (huIgG1) isotype control Ab for 20 min at 4°C. FITC-conjugated 10H10 (A), 3G10 (B), 10A5 (C), 11E6 (D), 12A4 (E) or 13G4 (F) was added to the cells to a final concentration of 0.09 μg/ml (B,D), 0.27 μg/ml (A,C), 0.91 μg/ml (F), or 2.73 μg/ml (E) for an additional 20 min at 4°C without prior washout of unbound, unconjugated Ab. Different quantities of the various FITC-conjugated HuMAbs were used due to differences in binding efficiency following labeling, and the optimal amounts of these FITC-conjugated HuMAbs were previously determined by dose-titration analysis of binding to CHO/PD-L1 cells. Binding of FITC-conjugated 10H10, 3G10, 10A5, 11E6, 12A4 or 13G4 to the CHO/PD-L1 cells was measured by flow cytometry. The results are depicted in Figure 2. Each of anti-PD-L1 HuMAbs 5F8, 7H1, 1B12, 3G10, 10A5, 11E6, 12A4, 12B7 and 13G4 substantially

blocked binding of mAbs 3G10, 10A5, 11E6, 12A4 and 13G4 to CHO/PD-L1 cells as measured by MFI.

Binding of labeled 10H10 was partially blocked by 10A5, 11E6 and 13G4, but was substantially blocked only by itself. Conversely, 10H10 substantially blocked the binding only of itself to CHO/PD-L1 cells.

EXAMPLE 3

Cross-Competition between Anti-PD-L1 mAbs for Binding to Ovarian Carcinoma Cells Expressing Human PD-L1

Anti-PD-L1 HuMAbs 5F8, 12B7, 3G10, 1B12, 13G4, 10H10, 10A5 and 12A4, and a huIgG1 isotype control antibody were serially diluted from 10 μg/ml and incubated with hPD-L1-expressing ES-2 ovarian carcinoma cells for 20 minutes at 4°C. Without washing, biotinylated-12A4 antibody was added to a final concentration of 0.4 μg/ml for an additional 20 minutes at 4°C. After washing, bound biotin-12A4 was detected using fluorescent streptavidin-PE secondary reagent and measured by flow cytometry. Figure 3 shows the fluorescence of bound biotin-12A4 plotted against the concentration of unlabeled hPD-L1 HuMAbs. Binding of biotin-12A4 to ES-2 cells was substantially blocked by 12A4 itself and by 1B12, 12B7, 13G4 and 3G10, and was moderately blocked by mAbs 5F8 and 10A5, but was not blocked by mAb 10H10.

EXAMPLE 4

Design of Phase I Clinical Study on Anti-PD-1 Antibody Study Design

A Phase I study was conducted to assess the safety, antitumor activity, and pharmacokinetics of an anti-PD-1 in patients with selected advanced solid tumors. The human anti-PD-1 mAb, BMS-936558 (also referred to herein and in U.S. Patent No. 8,008,449 as 5C4), was administered as an intravenous infusion every 2 weeks of each 8-week treatment cycle. Patients continued treatment 2 years (12 cycles), unless they experienced complete remission, unacceptable toxicity, disease progression, or withdrew consent. In patients who were otherwise clinically stable, study treatment was continued beyond apparent initial disease progression until further progression was noted as recommended by proposed immune response criteria (Wolchok *et al.* (2009) *Clin Cancer Res* 15:7412-20). Patients with stable disease (SD) or an ongoing objective response (OR: complete [CR] or partial response [PR]) at the end of treatment were followed for 1 year and were offered retreatment for 1 additional year in the event of progression.

Dose Escalation

Patients with advanced melanoma (MEL), non-small-cell lung (NSCLC), renal cell carcinoma (RCC), castration-resistant prostate (CRPC) and colorectal cancer (CRC) were eligible to enroll. Cohorts of 3-6 patients per dose level were enrolled sequentially at 1.0, 3.0, and 10.0 mg/kg. Dose escalation proceeded when a minimum of 3 patients had completed the safety evaluation period (56 days) at the given dose level, with dose-limiting toxicity in less than one-third of patients. Intra-patient dose escalation was not permitted.

Cohort Expansion

A maximum tolerated dose (MTD) was not reached. Initially, 5 expansion cohorts of approximately 16 patients each were enrolled at 10 mg/kg for MEL, NSCLC, RCC, CRPC and CRC. Based on initial signals of activity, additional expansion cohorts of approximately 16 patients each were enrolled for MEL (at 1.0 and 3.0 mg/kg, followed by cohorts randomized to 0.1, 0.3, or 1.0 mg/kg), NSCLC (squamous or nonsquamous histology cohorts randomized to 1, 3, or 10 mg/kg), and RCC (at 1.0 mg/kg).

Patients

Eligible patients had documented advanced solid tumors; age >18 years; life expectancy >12 weeks; Eastern Cooperative Oncology Group performance status of ≤2; measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST), v1.0 with modification (see protocol, supplemental data); adequate hematologic, hepatic, and renal function; and received 1-5 prior systemic treatment regimens. Patients with stable treated brain metastases were enrolled. Exclusion criteria included a history of chronic autoimmune disease, prior therapy with T-cell modulating antibodies (e.g., anti-CTLA-4), conditions requiring immunosuppressive medications, and chronic infections (HIV, hepatitis B or C).

A total of 296 patients with advanced solid tumors including MEL (n=104), NSCLC (n=122), RCC (n=34), CRPC (n=17), and CRC (n=19) were treated with BMS-936558 for 40 months. The majority of patients were heavily pretreated, with 49% having received at least 3 prior regimens. Notable prior therapies included immunotherapy (64%) and B-RAF inhibitor (8%) in MEL patients; platinum-based chemotherapy (94%) and tyrosine kinase inhibitors (TKI, 34%) in NSCLC patients; and nephrectomy (94%), immunotherapy (59%), and anti-angiogenic therapy (74%) in RCC patients. Baseline characteristics of the total treated population (N=296) were similar to those of the

efficacy population (response evaluable patients, N=236). Details on the patient pre-treatments are provided in Topalian *et al*. (2012b) *N Engl J Med* (submitted).

Statistical Analysis

All patients (N=296) treated as of the date of analysis were used for summaries of baseline characteristics and AEs. Pharmacokinetic and molecular-marker populations consisted of treated patients with available data as of the date of analysis. The efficacy population consisted of 236 response-evaluable patients commencing treatment at least 8 months before the date of analysis. AEs were coded using Medical Dictionary for Regulatory Activities (MedDRA), version 14.1. AEs of special interest (AEOSI), with a potential immune-related etiology, were identified using a pre-defined list of MeDRA terms. Individual best ORs were derived from investigator-reported data per modified RECIST v1.0. OR was confirmed by at least one sequential tumor assessment and OR rate (ORR={[CR + PR] ÷ n} × 100) was calculated.

EXAMPLE 5

Safety Evaluations on Patients Treated with Anti-PD-1 Antibody

Safety evaluations, including clinical examination and laboratory assessments, were conducted in all treated patients at baseline and regular intervals. The severity of AEs was graded based on the NCI Common Terminology Criteria for Adverse Events (NCI CTCAE), v3.0. Computed tomography (CT) or magnetic resonance imaging was performed for tumor assessment at baseline and following each treatment cycle.

A MTD was not defined across the doses of BMS-936558 tested on this study. A relative BMS-936558 dose intensity of 90% or higher was achieved in 87% of patients (*see* Topalian *et al*. (2012b) *N Engl J Med* (submitted) for details). AEs were coded using Medical Dictionary for Regulatory Activities (MedDRA), version 14.1. Adverse events of special interest (AEOSI) were identified using a pre-defined list of MeDRA terms. Fifteen of 296 (5%) patients discontinued treatment due to BMS-936558-related AEs. As of the date of analysis, 62 (21%) patients had died, with disease progression being the most common cause of death (Topalian *et al*. (2012b) *N Engl J Med* (submitted)).

The most common adverse events, regardless of causality included fatigue, decreased appetite, diarrhea, nausea, cough, dyspnea, constipation, vomiting, rash, pyrexia and pruritus (Topalian *et al*. (2012b) *N Engl J Med* (submitted)). Common BMS-936558-related AEs included fatigue, rash, diarrhea, decreased appetite, and nausea. The

majority of the events were low grade, with grade 3-4 drug-related AEs observed in 41 of 296 (14%) patients. Drug-related serious AEs occurred in 32 of 296 (11%) patients (Topalian *et al*. (2012b) *N Engl J Med* (submitted)). The spectrum, frequency, and severity of BMS-936558-related AEs were generally similar across the dose levels tested. Drug-related AEOSI, with potential immune-related etiologies, included pneumonitis, vitiligo, colitis, hepatitis, hypophysitis, and thyroiditis among others. Hepatic or gastrointestinal AEOSIs were managed with treatment interruption and, as necessary, with administration of corticosteroids. Among patients treated to-date, these AEs (in 13 patients with diarrhea; 11 patients with hepatic AEs) were reversible in all cases. Endocrine AEOSIs were managed with replacement therapy. At the discretion of the treating physician, patients successfully reinitiated treatment with BMS-936558. Drug-related pneumonitis occurred in 9 of 296 (3%) patients. Grade 3-4 pneumonitis developed in 3 patients (1%). No clear relationship between the occurrence of pneumonitis and tumor type, dose level, or the number of doses received was noted. Early grade pneumonitis was generally reversible with treatment discontinuation and corticosteroid administration. In 3 patients with pneumonitis, infliximab and/or mycophenolate were utilized for additional immunosuppression; however, given the small number of patients and variable outcomes, the effectiveness of this treatment is unclear. There were 3 (1%) drug-related deaths due to pneumonitis (2 NSCLC patients, 1 CRC).

EXAMPLE 6

Pharmacokinetics/Pharmacodynamics Analyses on Anti-PD-1 Antibody

For pharmacokinetic (PK) analyses, serial blood samples were collected and serum concentrations of BMS-936558 were quantified using by ELISA. For pharmacodynamic (PD) analysis, peripheral blood mononuclear cells were isolated from patients at baseline and following cycle 1 to estimate PD-1 receptor occupancy (RO) by BMS-936558 on circulating CD3+ T-cells via flow cytometry (Brahmer *et al*. (2010) *J Clin Oncol* 28:3167-75).

The maximum concentration of BMS-936558 was observed at a median T_{max} of 1-4 hours after the start of infusion. The PK of BMS-936558 was linear with a dose proportional increase in C_{max} and $AUC_{(0\text{-}14\ d)}$ in the dose range of 0.1-10 mg/kg (n=35). BMS-936558 PD was assessed by PD-1 RO on circulating T-cells. PBMCs from 65 MEL patients treated with one cycle of BMS-936558 at 0.1-10 mg/kg biweekly demonstrated median occupancy of PD-1 molecules on circulating CD3+ T-cells by BMS-936558

ranging from 64%-70% (*see* Topalian *et al.* (2012b) *N Engl J Med* (submitted) for details).

EXAMPLE 7

Antitumor Efficacy Exhibited by Anti-PD-1 Antibody

Clinical antitumor activity was observed at all BMS-936558 doses tested. ORs (confirmed CR or PR) were observed in a substantial portion of patients with NSCLC, MEL, and RCC (Tables 1 and 2; Figure 4), and in various sites of metastatic disease including liver, lung, lymph nodes, and bone (Figures 5-7 and not shown). Tumor regressions followed conventional as well as "immune-related" patterns of response, such as prolonged reduction in tumor burden in the presence of new lesions. Individual best overall responses were derived from investigator-reported data according to modified RECIST v1.0. OR was confirmed by at least one sequential tumor assessment. At the time of data analysis, 2 patients with NSCLC who were treated with 10 mg/kg had unconfirmed responses, and 8 additional patients (with MEL, NSCLC, or RCC) had a persistent reduction in baseline target lesions in the presence of new lesions, (i.e., an "immune-related" response pattern). None of these patients were categorized as responders for the purpose of calculating OR rates. Antitumor responses and/or prolonged disease stabilization were observed in patients irrespective of prior therapies received (see summary of progression free interval for patients with OR and SD in Supplementary Appendix 4).

In NSCLC patients, 14 ORs were observed at BMS-936558 doses of 1, 3, or 10 mg/kg with response rates of 6%, 32%, and 18%, respectively. ORs were observed across NSCLC histologies: 6 responders of 18 squamous (33%), 7 responders of 56 nonsquamous (13%), and 1 of 2 unknown. All 14 patients with OR started treatment ≥24 weeks before data analysis, and of these, 8 had response duration ≥ 24 weeks (Table 1). Stable disease (SD) lasting ≥ 24 weeks was observed in 5 (7%) NSCLC patients, all with nonsquamous histology. Among MEL patients, 26 ORs were observed at doses ranging from 0.1-10 mg/kg, with response rates ranging from 19%-41% per dose level. At the 3 mg/kg dose level, ORs were noted in 7 of 17 (41%) patients. Of 26 MEL patients who achieved an OR, 17 started treatment ≥ 1 year before data analysis, and of these, 13 patients had an OR duration ≥ 1 yr. The remaining 8 patients with OR were on study < 1 year and 6 had responses ranging from 1.9-5.6 months. SD lasting ≥ 24 weeks was observed in 6 (6%) patients. In RCC patients, ORs occurred in 4 of 17 (24%) patients

treated with a BMS-936558 dose of 1 mg/kg and 5 of 16 (31%) patients treated with 10 mg/kg. Among 8 RCC patients with OR who started treatment ≥ 1 year prior to data analysis, 5 had OR duration ≥ 1 yr. SD lasting ≥ 24 weeks was observed in an additional 9 (27%) patients.

Table 1. Clinical Activity of BMS-936558 in the Efficacy Population* (N=236)†

Tumor Type	Dose (mg/kg)	n	ORR‡ No. Patients (%) [95% CI]†	SD ≥ 24 wk No. Patients (%) [95% CI]	PFSR§ at 24 wk (%) [95% CI]
MEL	0.1	14	4 (29) [8-58]	1 (7) [0.2-34]	40 [13-66]
	1.0	27	8 (30) [14-50]	3 (11) [2-29]	45 [26-65]
	3.0	17	7 (41) [18-67]	1 (6) [0.1-29]	55 [30-80]
	10.0	20	4 (20) [6-44]	0	30 [9-51]
All MEL		94	26 (28) [19-38]	6 (6) [2-13]	41 [30-51]
NSCLC**					
All	1	18	1 (6) [0.1-27]	1 (6) [0.1-27]	16 [0-34]
Squamous	1	5	0	0	0
Nonsquamous	1	12	0	1 (8) [0.2-39]	14 [0-37]
Unknown	1	1	1(100) [3-100]	0	1
All	3	19	6 (32) [13-57]	2 (11) [1-33]	41 [18-64]
Squamous	3	6	3 (50) [12-88]	0	50 [10-90]
Nonsquamous	3	13	3 (23) [5-54]	2 (15) [2-45]	37 [10-64]
All	10	39	7 (18) [8-34]	2 (5) [0.6-17]	24 [11-38]
Squamous	10	7	3 (43) [10-82]	0	43 [6-80]
Nonsquamous	10	31	4 (13) [4-30]	2 (7) [0.8-21]	21 [6-36]
Unknown	10	1	0	0	0
ALL NSCLC		**76**	**14 (18) [11-29]**	**5 (7) [2-15]**	**26 [16-36]**
All Squamous		**18**	**6 (33) [13-59]**	**0**	**33 [12-55]**
All Nonsquamous		**56**	**7 (13) [5-24]**	**5 (9) [3-20]**	**22 [11-34]**
RCC	1	17	4 (24) [7-50]	4 (24) [7-50]	47 [23-71]
	10	16	5 (31) [11-59]‡	5 (31) [11-59]¶	67 [43-91]
ALL RCC		**33**	**9 (27) [13-46]**	**9 (27) [13-46]**	**56 [39-73**

*The efficacy population consists of response-evaluable patients whose treatment was initiated at least 8 months before data analysis, and had measurable disease at baseline and one of the following: at least 1 on-treatment scan or clinical evidence of disease progression or death.

†CR denotes complete response, MEL melanoma, NSCLC non-small-cell lung cancer, ORR objective response rate, PFSR progression-free survival rate, PR partial response, RCC renal-cell cancer, SD stable disease, n number of patients.

‡Objective response rates ({[CR + PR] ÷ n} × 100) have been calculated based on confirmed responses with confidence intervals calculated using the Clopper-Pearson method. Individual patient responses were adjudicated per RECIST v1.0 with

modification (*see* Topalian *et al*. (2012b) *N Engl J Med* (submitted)).

[§]Progression-free survival rate was the proportion of patients who did not progress and were alive at 24 weeks calculated by the Kaplan-Meier methodology with confidence intervals using the Greenwood method.

[¶]One CR.

**One NSCLC patient who was treated at the 3 mg/kg dose level had an initial evaluation of progressive disease, subsequently had a PR, and was classified as a responder.

Table 2. Duration of Objective Responses to BMS-936558*

Tumor Type	Dose (mg/kg)	No. Patients with OR	Duration of Response (months)[†]
MEL	0.1	4	7.5+, 5.6+, 5.6, 5.6
	0.3	3	3.8+, 2.1+, 1.9+
	1	8	24.9+, 22.9, 20.3+, 19.3+, 18.4+, 7.6+, 5.6+, 5.3+
	3	7	22.4+, 18.3+, 15.2+, 12.9, 11.1, 9.3, 9.2+
	10	4	24.6+, 23.9+, 18.0+, 17.0
NSCLC[§]	1	1	9.2+
	3	6	30.8+, 7.6+, 5.5+, 3.7+, 1.9+, NA[‡]
	10	7	14.8+, 7.6+, 7.3+, 6.7, 4.2, 3.7+, 3.7
RCC	1	4	17.5+, 9.2+, 9.2, 5.6+
	10	5	22.3+. 21.7+, 12.9, 12.0, 8.4

*MEL denotes melanoma, NA not applicable, NSCLC non-small-cell lung cancer, RCC renal-cell cancer.

[†]Time from first response to time of documented progression, death, or for censored data, time to last tumor assessment.

[‡]One patient was treated beyond an initial evaluation of progressive disease and subsequently had a PR; this patient was classified as a responder for the purposes of calculating response rates by RECIST v1.0 but was not eligible for calculation of duration of response.

EXAMPLE 8

Measurement of Membranous PD-L1 Expression by Standard IHC Assay

IHC staining of PD-L1 was performed on pretreatment formalin-fixed paraffin-embedded (FFPE) tumor specimens using the murine anti-human PD-L1 mAb 5H1 (Dong *et al*. (2002) *Nat Med* 8:793-800) in a standard IHC protocol (Taube *et al*. (2012) *Sci Transl Med* 4,127ra37; Supp. Materials). Briefly, 5μm-FFPE sections mounted on glass slides were deparaffinized in xylene, and antigen retrieval was performed using a Tris-EDTA buffer, pH 9.0 at 120°C for 10 min in a Decloaking Chamber (Biocare Medical). Endogenous peroxidase, biotin and proteins were blocked (CAS system K1500, Dako; Avidin/biotin Blocking Kit, SP-2001, Vector Laboratories; Serotec Block ACE).

The primary 5H1 antibody was applied at a concentration of 2 μg/ml and allowed to incubate at 4°C for 20 h. Secondary antibody (biotinylated anti-mouse IgG1, 553441 BD) was applied at a concentration of 1 μg/ml for 30 min at room temperature (RT). The signal was then developed with amplification according to the manufacturer's protocol (CAS system K1500, Dako). Sections were counterstained with hematoxylin, dehydrated in graded ethanol and cleared in xylene, and a coverslip was applied.

The percentage of tumor cells exhibiting cell surface staining for PD-L1 was scored by two independent pathologists who were blinded to treatment outcomes. PD-L1 positivity was defined per specimen by a 5% expression threshold (Taube *et al.* (2012) *Sci Transl Med* 4,127ra37; Thompson *et al.* (2006) *Cancer Res* 66:3381-85), and in cases with multiple specimens, if any specimen met this criterion. A Fisher's exact test was applied to assess the association between PD-L1 expression and OR, noting, however, that this analysis was based in part on optional biopsies from a non-random subset of the population and testing of a statistical hypothesis was not pre-specified.

Sixty-one pretreatment tumor specimens from 42 patients (18 MEL, 10 NSCLC, 7 CRC, 5 RCC, and 2 CRPC) were analyzed for tumor cell surface PD-L1 expression (Figure 8). Biopsy specimens from 25 of 42 patients were positive for PD-L1 expression by IHC. A Fisher's exact test was applied to assess the association between PD-L1 expression and OR in a post-hoc analysis. Among the 42 surface-PD-L1$^+$ patients, 9 (36%) achieved an OR. Importantly, among 17 patients with PD-L1$^-$ tumors, none achieved an OR. Thus, in a subset of patients cell surface expression of PD-L1 on tumor cells in pretreatment biopsies is associated with an increased rate of OR among patients treated with BMS-936558, while no patients with documented PD-L1-negative tumors experienced an OR. These data indicate that tumor PD-L1 expression is a molecular marker that can enable patient selection for anti-PD-1 immunotherapy.

EXAMPLE 9

Isolation of Rabbit mAbs that Detect Membranous hPD-L1 Antigen in FFPE Tissues

Rabbit antibodies against human PD-L1 polypeptide were prepared by Epitomics, Inc. (Burlingame, CA) by immunization of rabbits using a recombinant human PD-L1 fusion protein. Antiserum titers were evaluated using standard direct ELISA with the hPD-L1 antigen and cell ELISA using transfected cells overexpressing hPD-L1. These Abs were also screened for their ability to bind to PD-L1 by IHC assay of FFPE tissue sections. The rabbit with the highest Ab titer was selected for splenectomy. Lymphocytes

isolated from the spleen were fused to myeloma cells in 40 x 96-well plates, and screened by ELISA against the immunizing PD-L1 antigen and by cell ELISA against cells overexpressing hPD-L1. Positive clones were expanded into 24-well plates, and confirmatory screens were conducted by direct ELISA and cell ELISA. The supernatants (sups) of clones that were specific to the screening antigen were re-screened by IHC.

A set of mouse anti-hPD-L1 mAbs were also produced by immunization of mice using a protocol similar to that described above for the rabbit mAbs.

Out of a total of 185 multiclones from both rabbit and mouse immunizations screened, only ten rabbit multiclone antibodies specifically detected the membranous form of hPD-L1. None of the purified mouse subclones were found to specifically detect cell surface hPD-L1. Sixty subclones from the top five rabbit multiclones (designated Nos. 13, 20, 28, 29 and 49, each comprising 12 subclones) were screened initially by IHC on FFPE low density tissue microarrays (TMAs), followed by confirmation and specificity verification in narrowed 25 subclones. Rabbit IgG was used as a negative isotype control, and mAb 5H1 (Dong *et al.* (2002) *Nat Med* 8:793-800) was used as the positive control. Specificity was further verified by antigen preabsorption assay. Through two rounds of IHC, the following 15 purified subclones were selected as the most promising antibodies in terms of specificity and intensity of staining: 13-1, 13-3, 13-7, 13-8; 20-5, 20-7, 20-12, 20-6; 28-1, 28-8, 28-12; 49-5, 49-7, 49-9; and 29-8. Immunoreactivity data on these selected Abs are summarized in Table 3.

Additional assays were performed to further characterize the purified antibody clones, including determining binding affinity and cross-competition among the Abs (to identify overlapping versus different epitope regions) by surface plasmon resonance. All the antibodies exhibited high binding affinity ($K_D < 10^{-9}$ M). These 15 purified clones were also re-screened by IHC on FFPE low density TMA or regular sections against various cell and tissue types known to be positive or negative for cell surface expression of PD-L1. Rabbit IgG was used as the isotype control, and mAb 5H1 was used as the positive control. At high concentration (10 μg/ml), clones 28-x and 49-x displayed low to moderate levels of background staining in tissues, while clones 13-x exhibited no background staining, which suggests that the 13-x clones have a wider dynamic range. The 20-x clones displayed various degree of background staining which was primarily cytoplasmic and diffuse. The clone with most robust detection specifically of membranous PD-L1, rabbit clone 28-8 ($K_D = 2.4 \times 10^{-10}$ M, as determined by SPR), was

selected as the lead antibody for subsequent IHC assays. The sequences of the heavy chain variable (V_H) and light (kappa) chain variable (V_κ) regions of mAb 28-8 are set forth in SEQ ID NOs. 35 and 36, respectively. The 28-8 Ab was shown to recognize a different epitope from mouse mAb 5H1, based on SPR analysis. Clones 28-1, 28-12, 29-8 and 20-12 were the next best Abs in terms of robust detection of membranous PD-L1 in FFPE tissues. Although mAb 13-1 had the best specificity in terms of detection of membranous PD-L1, the maximal detection level was lower than that of the other lead antibodies. Western blotting was also performed with plus/minus antigen competition to verify the specificity of the top selected antibodies for PD-L1.

Table 3. Immunoreactivity of rabbit anti-hPD-L1 mAbs

	Specific Staining		Non-Specific staining
Antibody Name	Pos. vs Neg. Staining on PD-L1 Cells*	Intensity Range on Tissues† (Very High, High, Medium, Low)	Background Staining on Tissues (High, Medium, Low)
13-1	Pos	Low to Very High	None
13-3	Pos	Low to High	None
13-7	Pos	Low to High	None
13-8	Pos	Low to High	None
20-5	Pos	Low to Very High	High
20-6	Pos	Low to High	Medium
20-7	Pos	Low to High	Medium
20-12	Pos	Low to High	Medium
28-1	Pos	Low to Very High	None
28-8	Pos	Medium to Very High	None
28-10	Pos	Medium to Very High	Low
28-12	Pos	Low to Very High	None
49-5	Pos	Low to High	None
49-7	Pos	Low to High	Low
49-9	Pos	Low to High	None
5H1	Pos	High to Very High	None
Rb IgG	Neg	Neg	None

*PD-L1 stably transfected CHO cells vs. CHO-S control;
†PD-L1 positive tissues included placenta and one non-small cell lung cancer; Detection up to "very high" expression suggests better sensitivity at detecting membranous PD-L1.

The binding of mAbs 5H1 and 28-8 to membranous PD-L1 in FFPE test tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells from different

types of tumors was compared. Membrane PD-L1 expression was evaluated using the histoscore method performed by 2 independent pathologists. Four NSCLC, 2 MEL, and 2 RCC tumors were stained with 28-8 at 2 mg/ml and 5H1 at 5 mg/ml. The data are tabulated in Table 4, and shown graphically in Figure 9. The rabbit mAb 28-8 showed better detection (higher histoscores) for 7 out of 10 samples using 2.5-fold less antibody.

Table 4. Comparison of mAbs 28-8 and 5H1 by Histoscore analysis

Tissue	Sample I.D.	Histoscore Average (5H1)	Histoscore Average (28-8)
NSCLC	1080754B	245	261
NSCLC	1080766B	103	130
NSCLC	1080790	40	37
NSCLC	1080993B	12	16
Mel	T030668	98	113
Mel	T980744	98	123
Mel	1168657B	0	0
Mel	T980747	1	9
RCC	1164619B	4	4
RCC	1167809B	108	125

Taube *et al*. ((2012) *Sci Transl Med* 4,127ra37, Supp. Materials) reported that cell surface PD-L1 expression on cultured cells was confirmed with flow cytometry, and the specificity of 5H1 for PD-L1 was confirmed using a PD-L1 fusion protein to competitively block binding of the 5H1 mAb to tissue sections. These authors also tested a rabbit polyclonal anti-PD-L1 Ab, 4059, previously described by Gadiot *et al*. (2011) *Cancer* 117, 2192-201. Taube *et al*. found that 5H1 staining showed a cell surface staining pattern on FFPE samples, whereas Ab 4059 demonstrated diffuse cytoplasmic staining. When 5H1 was compared to Ab 4059 by western blot analysis, Ab 4059 bound to multiple proteins in lysates of melanoma cells in addition to a 50 kDa protein corresponding to the expected mass of glycosylated PD-L1, whereas 5H1 specifically detected the 50 kDa band of glycosylated PD-L1 (Taube *et al*. (2012) *Sci Transl Med* 4,127ra37, Supp. Materials).

We also compared by automated IHC assay the binding of mAb 28-8, several commercially anti-PD-L1 antibodies and 5H1 to PPPE tissue samples containing various cells expressing PD-L1. The results are summarized in Table 5. These data show in the

IHC assay used that none of the commercially available antibodies tested specifically recognized membranous PD-L1 expression in human tissues known to express PD-L1, or to clearly distinguish CHO cells expressing PD-L1 versus the untransfected parent CHO cells that did not express PD-L1. The inability of the pAb 4059 to bind specifically recognized membranous PD-L1 is consistent with the findings of Taube *et al.* ((2012) *Sci Transl Med* 4,127ra37, Supp. Materials). The binding of 28-8 was similar to that of 5H1 in this assay, though histoscore analysis suggests that 28-8 performs better than 5H1.

Table 5. Binding of mAbs to FFPE Samples Containing PD-L1-expressing cells

Source	Antibody Types	Clone No. (mAb) /Catalog No. (pAb)	Pos. vs. Neg. Staining on PD-L1 Cells*	Human Positive Tissues†
MBL	mAb	27A2	Failed	Failed
BioLegend	mAb	29E.2A3	Failed	Failed
eBiosciences	mAb	M1H1	No Staining	No staining
Collaborator	mAb	5H1	Passed	Passed
ProSci	pAb	4059	Failed	Failed
LifeSpan BioSciences	pAb	LS-B480/0604	Failed	Failed

*PD-L1 stably transfected CHO cells vs. parent CHO-S negative control;
†PD-L1 positive tissues included tonsil and/or thymus;
mAb, mouse monoclonal antibody; pAb, rabbit polyclonal antibody.

It is known in the art that rabbit Abs have certain advantages over murine Abs. For example, rabbit generally Abs exhibit more diverse epitope recognition, improved immune response to small-size epitopes, and higher specificity and affinity compared to murine antibodies (*e.g.*, Fischer *et al.* (2008) *PloS One* 3(12):e4069; Cheang et al. (2006) *J Clin Oncol* 24:5637-44); Rossi *et al.* (2005) *Am J Clin Pathol* 124:295-302). Further, many protein targets relevant to signal transduction and disease are highly conserved between mice, rats and humans, and can therefore be recognized as self-antigens by a mouse or rat host, making them less immunogenic. This problem is avoided by generating antibodies in rabbits. In addition, in applications in which two antigen-specific antibodies are required, it is more convenient to have the antibodies come from two different species. Thus, for example, it is easier to multiplex a rabbit antibody such as 28-8 with other antibodies (likely to be murine Abs since the best immune marking antibodies are

murine Abs) that can mark immune cells that also express PD-L1 (*e.g.*, macrophages and lymphocytes). Thus, the rabbit anti-hPD-L1 mAb, 28-8, has distinct advantages over the murine Ab, 5H1.

EXAMPLE 10

Development of Automated IHC Protocol for Assessing PD-L1 Expression

An automated IHC protocol was developed to assay PD-L1 expression in FFPE specimens. Tissue sections (4 μm) were mounted on slides, deparaffinized in an autostainer (Leica) by soaking twice for 5 min in xylene, and re-hydrated through soaking twice for 2 min each time in 100% EtOH, twice in 95% (v/v) EtOH, once in 70% (v/v) EtOH, and once in de-ionized water (dH_2O). Antigen retrieval was performed using a decloaking chamber (Biocare Medical Decloaking Chamber Plus) and Dako pH 6 buffer, heated to 110°C (P1) for 10 min, then moved to the next step (P2 FAN ON at 98°C; FAN OFF at 90 °C). The slides were cooled at room temperature (RT) for 15 min and rinsed with water for ~ 1 min.

Reagents were set up on an autostainer (BioGenex i6000), using a pap pen to define the tissue area. The IHC assay, was run using the autostainer in research mode, comprised the following steps: neutralizing endogenous peroxidase using the Peroxidase Block (Leica) for 10 min, followed by rinsing 3 times with IHC wash buffer (Dako); applying Protein Block (Leica) to the slides, and incubating for 10 min at RT, followed by washing 3 times with wash buffer; applying the primary antibody to the slides (2 μg/ml) and incubating for 1 h at RT, followed by washing 6 times with wash buffer; adding Post Primary Block (NovoLink Kit) to the slides and incubating for 30 min, followed by washing 6 times with wash buffer; adding NovoLink Polymer (NovoLink Kit) to the slides and incubating for 30 min, followed by washing 6 times with wash buffer; adding the DAB chromogen substrates (NovoLink Kit) and developing for 3 min, followed by rinsing 5 times with dH_2O at RT; counterstaining with hematoxylin (NovoLink Kit) for 1 min at RT, followed by washing 3 times with dH_2O for 5 times at RT. The primary antibody was selected from the rabbit anti-PD-L1 antibodies shown in Table 3; mAb 28-8 was the preferred antibody. As a negative control, rabbit IgG (Dako) was used. The tissue sections were dehydrated using a Leica autostainer by washing once for 2 min in 70% EtOH, twice for 2 min in 95% EtOH, and three times for 2 min in 70% EtOH, then cleared by washing three times for 5 min in xylene. The sections were permanently mounted with permount to the slide, covered with a coverslip, and

transferred to a chemical hood to dry.

EXAMPLE 11

Design of Phase I Clinical Study on Anti-PD-L1 Antibody

Study Design

A Phase I study was conducted to assess the safety and tolerability of BMS-936559 (also referred to herein and in U.S. Patent No. 7,943,743 as 12A4) in patients with selected advanced solid tumors. Secondary objectives included initial assessment of the antitumor activity of BMS-936559 and pharmacokinetic evaluation. Pharmacodynamic measures were included under exploratory objectives. Patients were treated in 6-week cycles of BMS-936559 administered as a 60-minute intravenous infusion every 2 weeks on days 1, 15, and 29 of each cycle. Patients continued treatment for up to 16 cycles unless they experienced unacceptable toxicity, disease progression, or withdrew consent. In some patients who were clinically stable, treatment beyond initial disease progression was permitted until further progression was confirmed.

Dose Escalation

Patients with advanced NSCLC, MEL, CRC, RCC, ovarian (OV), gastric (GC), breast (BC), and pancreatic (PC) carcinoma were eligible to enroll. Using an accelerated titration design, safety was assessed at doses of 0.3, 1, 3, and 10 mg/kg. One patient was enrolled in each successive cohort until there was a $\geq$ grade 2 drug-related AE during cycle 1. Two additional patients were then enrolled at that dose level and the study was transitioned to a standard 3+3 design. Intra-patient dose escalation or de-escalation was not permitted. The maximum tolerated dose (MTD) was defined as the highest dose where less than one-third of patients had a dose-limiting toxicity.

Cohort Expansion

Initially, 5 expansion cohorts (n=16/cohort) were enrolled at 10 mg/kg for patients with NSCLC, MEL, RCC, OV, and CRC. Based on initial signals of activity, additional expansion cohorts (up to n=16/cohort) were enrolled for MEL (at 1.0 and 3.0 mg/kg), NSCLC (squamous or nonsquamous histology cohorts randomized to 1, 3, or 10 mg/kg), and at 10 mg/kg for PC, BC, and GC.

Patients

Patients were required to have documented advanced NSCLC, MEL, RCC, OV, CRC, PC, GC, or BC, and have failed at least one prior tumor-appropriate therapy for

advanced/metastatic disease (except for PC or GC patients who may be treatment-naïve). Other inclusion criteria included age ≥ 18 years, life expectancy ≥ 12 weeks, Eastern Cooperative Oncology Group performance status of ≤2, measurable disease as defined by RECIST v1.0, and adequate hematologic, hepatic, and renal function. Patients with treated brain metastases were allowed, if stable for at least 8 weeks. Major exclusion criteria included a history of autoimmune disease or other diseases requiring systemic steroids or immunosuppressive medication, prior therapy with T cell- modulating antibodies (including anti-PD-1, anti-PD-L1 and anti-CTLA-4), history of HIV, or active hepatitis B or C.

In this ongoing study, 207 patients with NSCLC (n=75), MEL (n=55), CRC (n=18), RCC (n=17), OV (n=17), PC (n=14), GC (n=7), or BC (n=4) were treated with BMS-936559 during a 34-month period and are included in the safety data. Efficacy was characterized in 160 response-evaluable patients. The baseline demographic characteristics of the total and response-evaluable patient populations were very similar (Brahmer *et al.* (2012) *N Engl J Med* (submitted)). Among treated patients, 86% had received prior chemotherapy and 28% immunologic or biological therapy. Prior therapies by tumor type included immunotherapy (56%) and B-RAF inhibitor (9%) in patients with MEL; platinum-based chemotherapy (95%) and tyrosine kinase inhibitors (TKI; 41%) in patients with NSCLC; and nephrectomy (94%), anti-angiogenic therapy (82%), and immunotherapy (41%) in patients with RCC (*see* Brahmer *et al.* (2012) *N Engl J Med* (submitted) for more details on patient pre-treatments).

Statistical Analysis

All 207 patients commencing treatment as of the date of analysis were used for summaries of baseline characteristics and AEs. The efficacy population consisted of 160 response-evaluable patients who initiated treatment at least 7 months before the date of analysis. AEs were coded using MedDRA v14.1. Individual best overall responses were derived from radiographic scan measurements according to modified RECIST v1.0. ORs were confirmed by at least one sequential tumor assessment. Additional details regarding statistical methods are provided in Brahmer *et al.* (2012) *N Engl J Med* (submitted), Supplementary Materials and the statistical analysis plan.

EXAMPLE 12

Safety Evaluations on Patients Treated with Anti-PD-L1 Antibody

Safety evaluations (clinical examination and laboratory assessments) were conducted in all treated patients at baseline and regular intervals (weekly during cycle 1 and biweekly thereafter). AE severity was graded based on the NCI CTCAE, v3.0. Disease assessment via computed tomography (CT) scans or magnetic resonance imaging was performed at baseline and prior to each treatment cycle.

A MTD was not reached up to the highest tested dose of 10 mg/kg of BMS-936558. The median duration of therapy was 12 weeks (range 2.0-111.1 weeks). A relative dose intensity of ≥ 90% was achieved in 86% of patients. Twelve of 207 patients (6%) discontinued treatment due to a BMS-936559-related AE (*see* Brahmer *et al.* (2012) *N Engl J Med* (submitted) for details).

AEs regardless of causality (any grade) were reported in 188 of 207 patients. Investigator-assessed BMS-936559-related AEs were noted in 126 of 207 (61%) patients. The most common drug-related AEs were fatigue, infusion reactions, diarrhea, arthralgia, rash, nausea, pruritus, and headache. Most events were low grade with BMS-936559-related grade 3-4 events noted in 19 of 207 (9%) patients (Brahmer *et al.* (2012) *N Engl J Med* (submitted)). The spectrum, frequency, and severity of BMS-936559-related AEs were similar across the dose levels, with the exception of infusion reactions. Drug-related AEOSI, with potential immune-related etiologies, were observed in 81 of 207 (39%) of the patients and included rash, hypothyroidism, hepatitis, and single cases each of sarcoidosis, endophthalmitis, diabetes mellitus, and myasthenia gravis (Brahmer *et al.* (2012) *N Engl J Med* (submitted)). These AEs were predominantly grade 1-2 and generally reversible with treatment interruption or discontinuation. Notably, 9 patients were treated with corticosteroids for the management of AEs. AEs improved or resolved in all patients. Furthermore, 4 of these 9 patients maintained disease control despite treatment with corticosteroids. Endocrine AEs were managed with replacement therapy and patients reinitiated treatment with BMS-936559 at the discretion of the treating physician. Infusion reactions were observed in 21 of 207 (10%) patients, predominantly at 10 mg/kg. They were grade 1-2 with the exception of one grade 3 event at 10 mg/kg. Infusion reactions were generally rapidly reversible with antihistamines and antipyretics and, in some cases, corticosteroids. A prophylactic regimen with antihistamines and antipyretics was implemented during the study. Patients with grade 1-2 infusion reactions were able to continue treatment with BMS-936559 with prophylactic antihistamines and antipyretics and at a reduced infusion rate. BMS-936559-related serious AEs occurred in

11 of 207 (5%) patients. As of the data analysis date, 45 patients (22%) had died (Brahmer *et al.* (2012) *N Engl J Med* (submitted)); no drug-related deaths were observed.

EXAMPLE 13

Pharmacokinetics/Pharmacodynamics Analyses on Anti-PD-L1 Antibody

For PK analyses, serial blood samples were collected and serum concentrations of BMS-936559 quantified by ELISA. Peripheral blood mononuclear cells were isolated from patients at baseline and following one treatment cycle to assay PD-L1 receptor occupancy (RO) by BMS-936559 on circulating CD3-positive T-cells via flow cytometry (Brahmer *et al.* (2010) *J Clin Oncol* 28:3167-75).

Serum concentrations of BMS-936559 increased in a dose-dependent manner from 1-10 mg/kg (n=131). Geometric mean area under the curve (0-14 days) for the 1, 3, and 10 mg/kg dose levels were 2210, 7750, and 36620 μg/mL•hr (coefficient of variation [CV] 34-59%), respectively; geometric mean peak concentrations at these dose levels were 27, 83, and 272 μg/mL (CV 30−34%), respectively, after the first dose. The half-life of BMS-936559 was estimated from population pharmacokinetic data as approximately 15 days. PD-L1 RO on CD3-positive peripheral blood lymphocytes was assessed in 29 MEL patients at the end of 1 cycle of treatment, at BMS-936559 doses from 1-10 mg/kg. Median RO exceeded 65% for all groups (Brahmer *et al.* (2012) *N Engl J Med* (submitted)).

EXAMPLE 14

Antitumor Efficacy Exhibited by Anti-PD-L1 Antibody

One-hundred and sixty patients out of the 207 treated were evaluable for response and included those with NSCLC, MEL, CRC, RCC, OV, and PC, but not patients with GC or BC. Clinical activity was observed at all doses ≥ 1 mg/kg (Brahmer *et al.* (2012) *N Engl J Med* (submitted)). ORs (confirmed complete [CR] or partial [PR] responses) were observed in patients with MEL, NSCLC, RCC, and OV (Table 6), as illustrated by representative spider plots and CT scans (Figures 10-13), and many OR were also durable (Table 7). Four additional patients had a persistent reduction in target lesions in the presence of new lesions, consistent with an "immune-related" pattern of response; however, for the purpose of calculating response rates, they were not categorized as responders. Antitumor responses and/or prolonged stable disease (SD) were observed in

patients with a variety of prior therapies received. ORs were observed even in patients with an extensive burden of metastatic disease.

In patients with MEL, there were 9 ORs across the 1, 3, and 10 mg/kg dose levels, with response rates of 6%, 29%, and 19%, respectively. Three MEL patients achieved a CR. All 9 MEL patients who experienced an OR started treatment ≥ 1 year prior to data analysis; of these 5 had a response duration ≥ 1 year. Additionally 14 MEL patients (27%) had SD lasting ≥ 24 weeks. In patients with NSCLC, there were 5 ORs amongst the 3 and 10 mg/kg dose levels, with response rates of 8% and 16%, respectively. There were ORs in patients with non-squamous (n=4) or squamous histology (n=1). All 5 NSCLC responders started treatment ≥ 24 weeks prior to data analysis; of these, 3 had responses lasting ≥ 24 weeks. Six additional NSCLC patients had SD lasting ≥ 24 weeks. There was 1 PR out of 17 patients with OV (6% response rate) and 3 patients (18%) with SD lasting ≥ 24 weeks, all at the 10 mg/kg dose. In patients with RCC, there were ORs in 2 of 17 (12%) patients treated at 10 mg/kg with responses lasting 4 and 18 months, respectively. Seven additional RCC patients had SD lasting ≥ 24 weeks.

Table 6 Clinical Activity of BMS-936559 in 160 Patients, Response Evaluable*

Tumor Type	Dose (mg/kg)	n	ORR[§] No. patients (%) [95% CI]	SD ≥ 24 wk No. patients (%) [95% CI]	PFSR** at 24 wk (%) [95% CI]
MEL	0.3	1	0 [0-98]	0 [0-98]	N/A
	1	18	1 (6) [0.1-27]	6 (33) [13-59]	39 [16-61]
	3	17	5 (29)[†] [10-56]	3 (18) [4-43]	47 [21-72]
	10	16	3 (19)[††] [4-46]	5 (31) [11-59]	44 [19-68]
ALL MEL		**52**	**9 (17) (8-30)**	**14 (27) [16-41]**	**42 [28-56]**
NSCLC[§]	1	11	0 [0-29]	0 [0-29]	N/A
Squamous	1	1	0 [0-98]	0 [0-98]	N/A
Non-Squamous	1	10	0 [0-31]	0 [0-31]	N/A
	3	13	1 (8) [0.2-36]	1 (8) [0.2-36]	34 [7-60]
Squamous	3	4	0 [0-60]	1 (25) [0.6-81]	50 [1-99]
Non-Squamous	3	9	1 (11) [0.3-48]	0 [0-34]	25 [0-55]
	10	25	4 (16) [5-36]	5 (20) [7-41]	46 [25-67]
Squamous	10	8	1 (13) [0.3-53]	2 (25) [3-65]	47 [10-83]
Non-Squamous	10	17	3 (18) [4-43]	3 (18) [4-43]	46 [20-72]
ALL NCSLC		**49**	**5 (10) [3-22]**	**6 (12) [5-25]**	**31 [17-45]**
ALL Squamous		13	1 (8) [0.2-36]	3 (23.1) [5-54]	43 [15-71]
ALL Non-Squamous		36	4 (11) [3-26]	3 (8) [2-23]	26 [10-42]
OV	3	1	0 [0-98]	0 [0-98]	N/A
	10	16	1 (6) [0.2-30]	3 (19) [4-46]	25 [4-46]
ALL OV		**17**	**1 (6) [0.1-29]**	**3 (18) [4-43]**	**22 [2-43]**
RCC	10	17	2 (12) [2-36]	7 (41) [18-67]	53 [29-77]

CI denotes Confidence intervals, MEL melanoma, RCC renal cell carcinoma, NSCLC non-small-cell lung cancer, OV ovarian cancer, RCC renal cell carcinoma, N/A not applicable, ORR objective response rate (complete response + partial response), SD stable disease, and PFSR progression-free survival rate.

*Efficacy population consists of response-evaluable patients who initiated treatment at least 7 months prior to the date of analysis and had measurable disease at a baseline tumor assessment and at least one of the following: an on-study tumor assessment, clinical progression, or death.

†Includes two CRs.

††Includes one CR.

§Objective response rates ({[CR + PR] ÷ n} × 100) are based on confirmed responses only, with confidence intervals calculated using the Clopper-Pearson method.

** Progression-free survival rate was the proportion of patients who did not progress, and were alive at 24 weeks, calculated by the Kaplan-Meier methodology, with confidence intervals using the Greenwood method

Individual patient responses were adjudicated per RECIST v1.0 with modification (see study protocol in Brahmer *et al.* (2012) *N Engl J Med* (submitted) for additional information).

Table 7. Duration of Objective Responses to BMS-936559*

Tumor Type	Dose (mg/kg)	No. Patients with OR	Duration of Response (months)†
MEL	1	1	6.9
	3	5	23.5+, 22.9+, 16.2+, 4.1+, 3.5
	10	4	24.6+, 23.9+, 18.0+, 17.0
NSCLC	1	0	9.2+
	3	1	2.3+
	10	4	16.6+, 12.6+, 9.8, 3.5
RCC	10	2	17, 4
OV	10	1	1.3+

*MEL denotes melanoma, NSCLC non-small-cell lung cancer, RCC renal-cell cancer, OV ovarian cancer.

†Time from first response to time of documented progression, death, or for censored data Denoted by +), time to last tumor assessment.

Sequence Listing Summary

SEQ ID NO:	Description
1	V_H amino acid sequence of 17D8
2	V_H amino acid sequence of 2D3
3	V_H amino acid sequence of 4H1
4	V_H amino acid sequence of 5C4
5	V_H amino acid sequence of 4A11
6	V_H amino acid sequence of 7D3
7	V_H amino acid sequence of 5F4
8	V_L amino acid sequence of 17D8
9	V_L amino acid sequence of 2D3
10	V_L amino acid sequence of 4H1
11	V_L amino acid sequence of 5C4
12	V_L amino acid sequence of 4A11
13	V_L amino acid sequence of 7D3
14	V_L amino acid sequence of 5F4
15	V_H amino acid sequence of 3G10
16	V_H amino acid sequence of 12A4
17	V_H amino acid sequence of 10A5
18	V_H amino acid sequence of 5F8

SEQ ID NO:	Description
19	V_H amino acid sequence of 10H10
20	V_H amino acid sequence of 1B12
21	V_H amino acid sequence of 7H1
22	V_H amino acid sequence of 11E6
23	V_H amino acid sequence of 12B7
24	V_H amino acid sequence of 13G4
25	V_L amino acid sequence of 3G10
26	V_L amino acid sequence of 12A4
27	V_L amino acid sequence of 10A5
28	V_L amino acid sequence of 5F8
29	V_L amino acid sequence of 10H10
30	V_L amino acid sequence of 1B12
31	V_L amino acid sequence of 7H1
32	V_L amino acid sequence of 11E6
33	V_L amino acid sequence of 12B7
34	V_L amino acid sequence of 13G4
35	V_H amino acid sequence of 28-8
36	V_L amino acid sequence of 28-8

CLAIMS

What is claimed is:

1. A method for potentiating an endogenous immune response in a subject afflicted with cancer so as to thereby treat the patient, which method comprises administering to the subject a composition comprising a therapeutically effective amount of an antibody or an antigen-binding portion thereof that inhibits signaling from an inhibitory immunoregulator.

CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING

ABSTRACT

The disclosed invention provides a method for immunotherapy of a subject afflicted with cancer, comprises administering to the subject a composition comprising a therapeutically effective amount of an antibody that inhibits signaling from the PD-1/PD-L1 signaling pathway. This invention also provides a method for immunotherapy of a subject afflicted with cancer comprising selecting a subject that is a suitable candidate for immunotherapy based on an assessment that the proportion of cells in a test tissue sample from the subject that express PD-L1 on the cell surface exceeds a predetermined threshold level, and administering a therapeutically effective amount of an anti-PD-1 antibody to the selected subject. The invention additionally provides rabbit monoclonal antibodies that bind specifically to a cell surface-expressed PD-L1 antigen in a FFPE tissue sample, and an automated IHC method for assessing cell surface expression in FFPE tissues using the provided anti-PD-L1 antibodies.

FIG. 1A

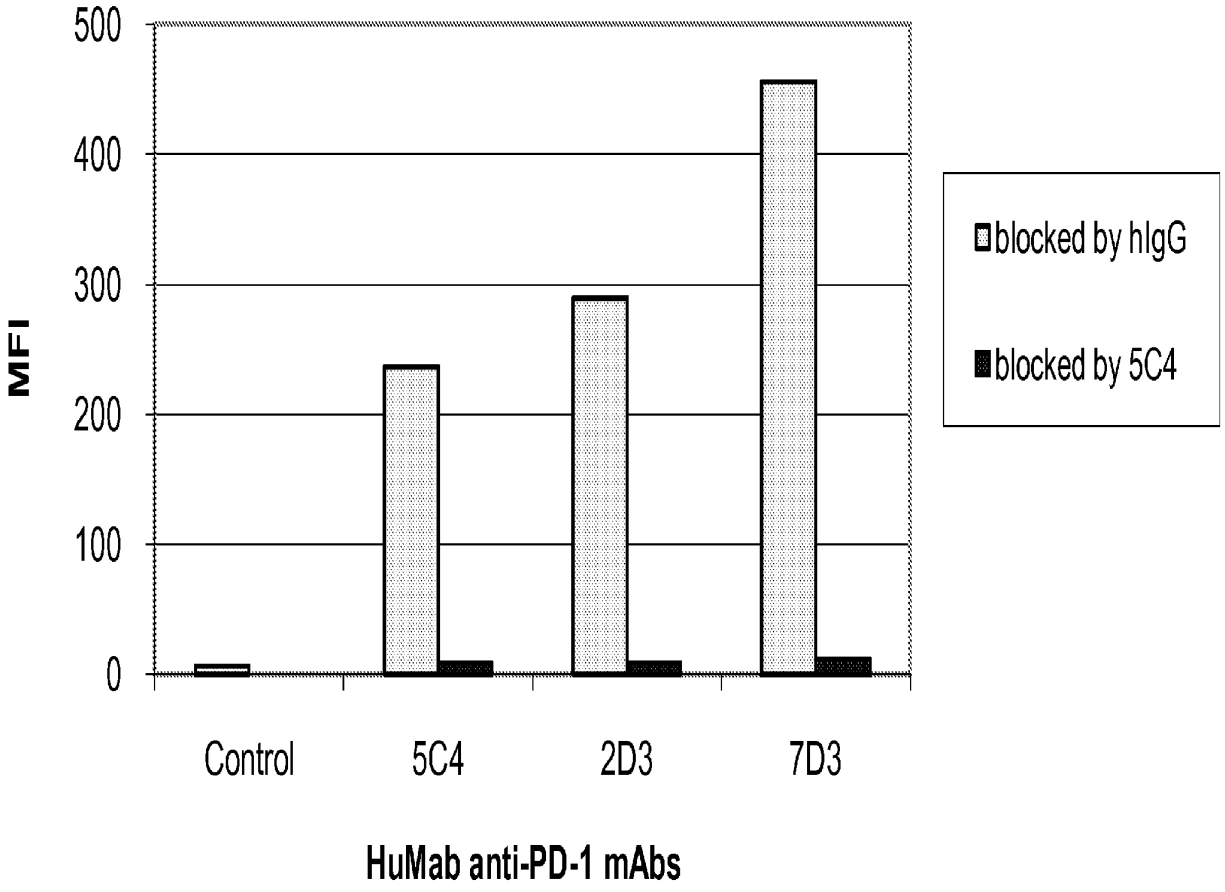

500
400
300
200
100
0
MFI
Control
5C4
2D3
7D3
HuMab anti-PD-1 mAbs
blocked by hIgG
blocked by 5C4

FIG. 1B

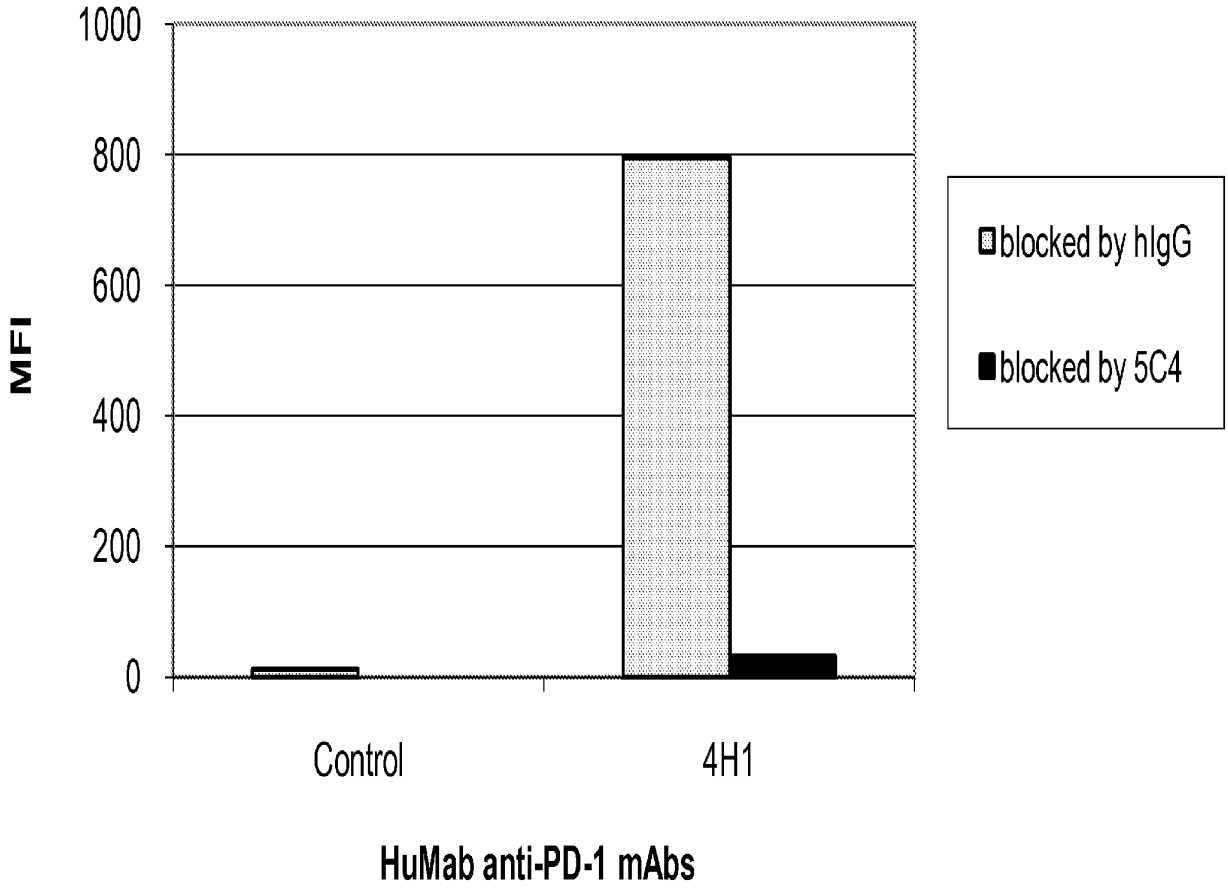

1000
800
600
400
200
0
MFI
blocked by hIgG
blocked by 5C4
Control
4H1
HuMab anti-PD-1 mAbs

FIG. 1C

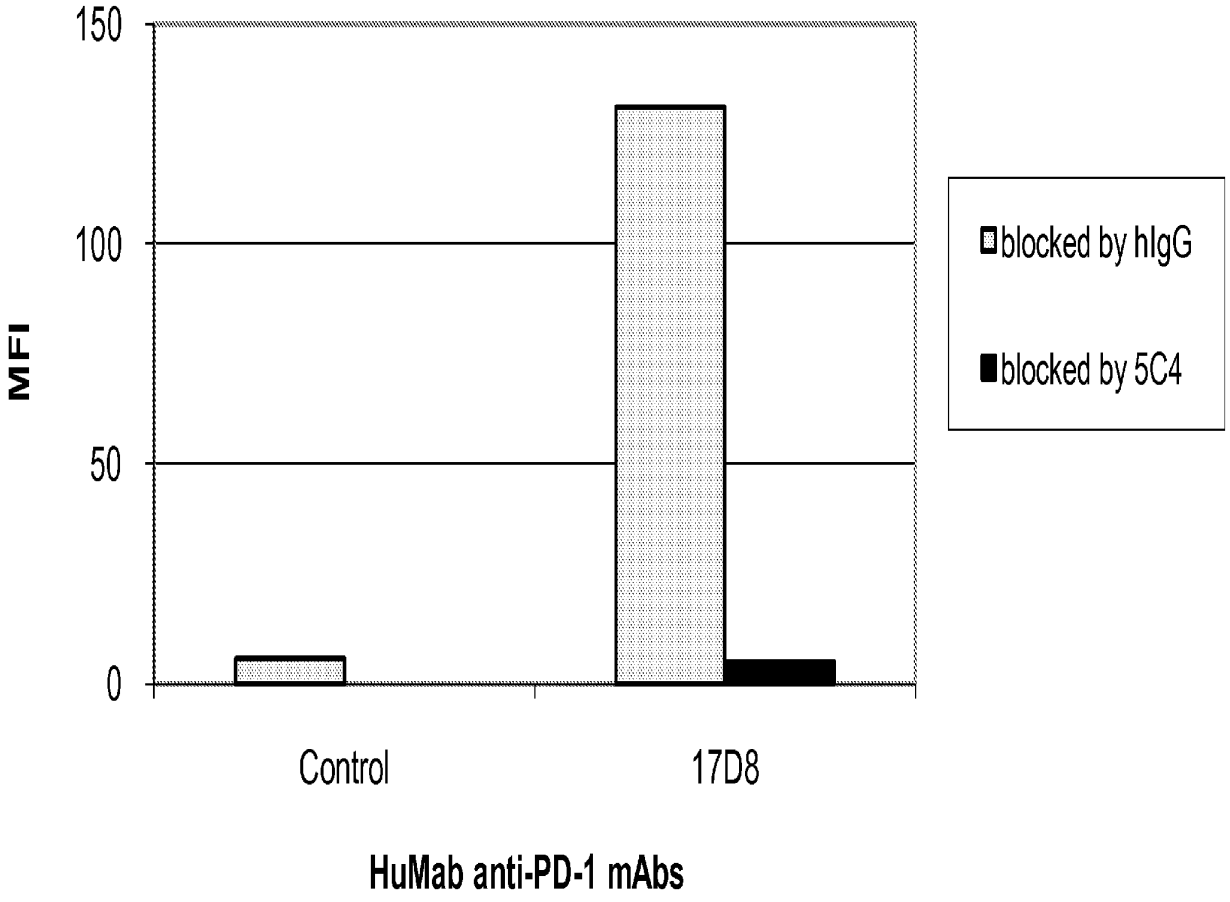

150
100
50
0
MFI
blocked by hIgG
blocked by 5C4
Control
17D8
HuMab anti-PD-1 mAbs

FIG. 2A

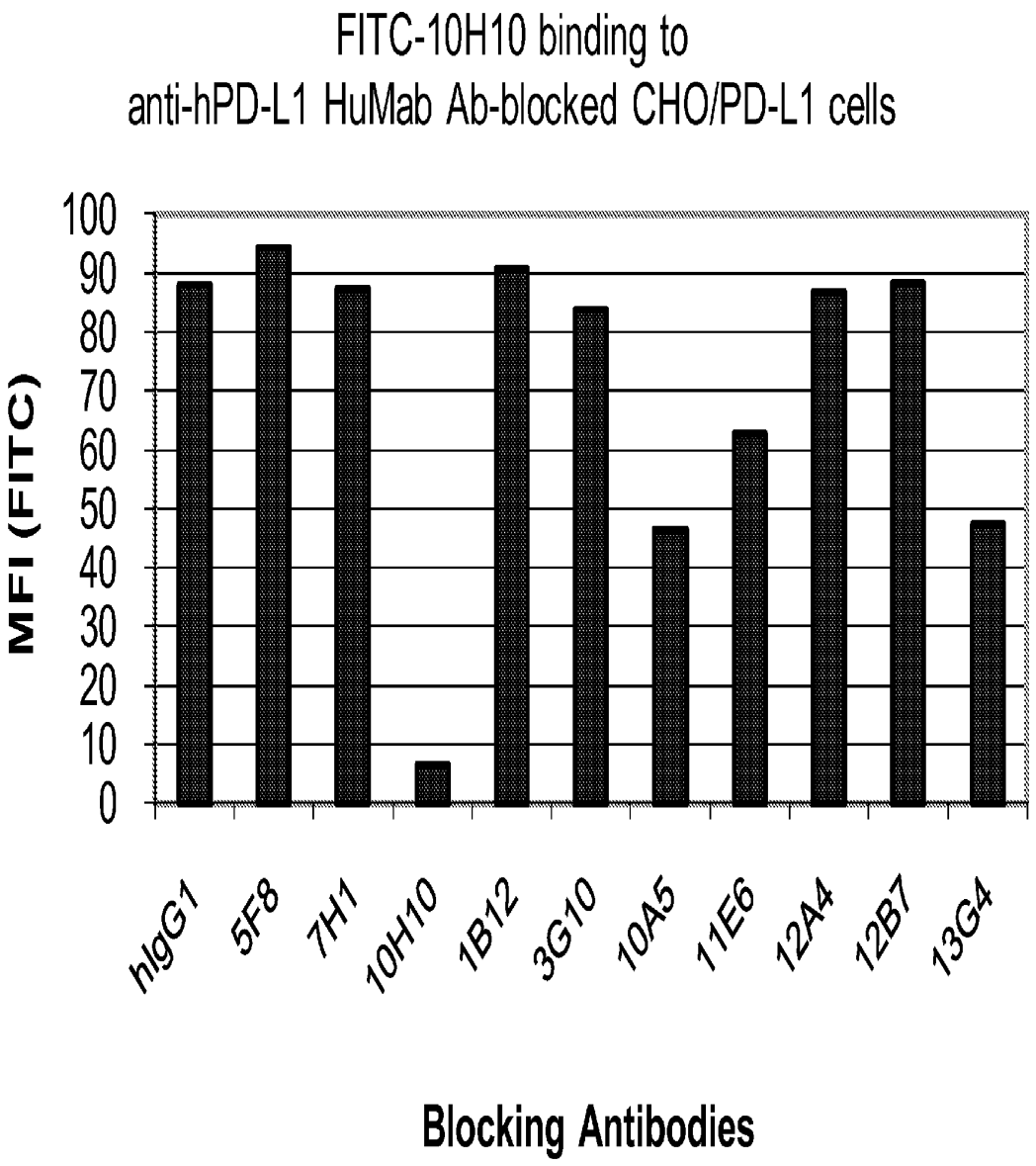

FITC-10H10 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
100
90
80
70
60
50
40
30
20
10
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2B

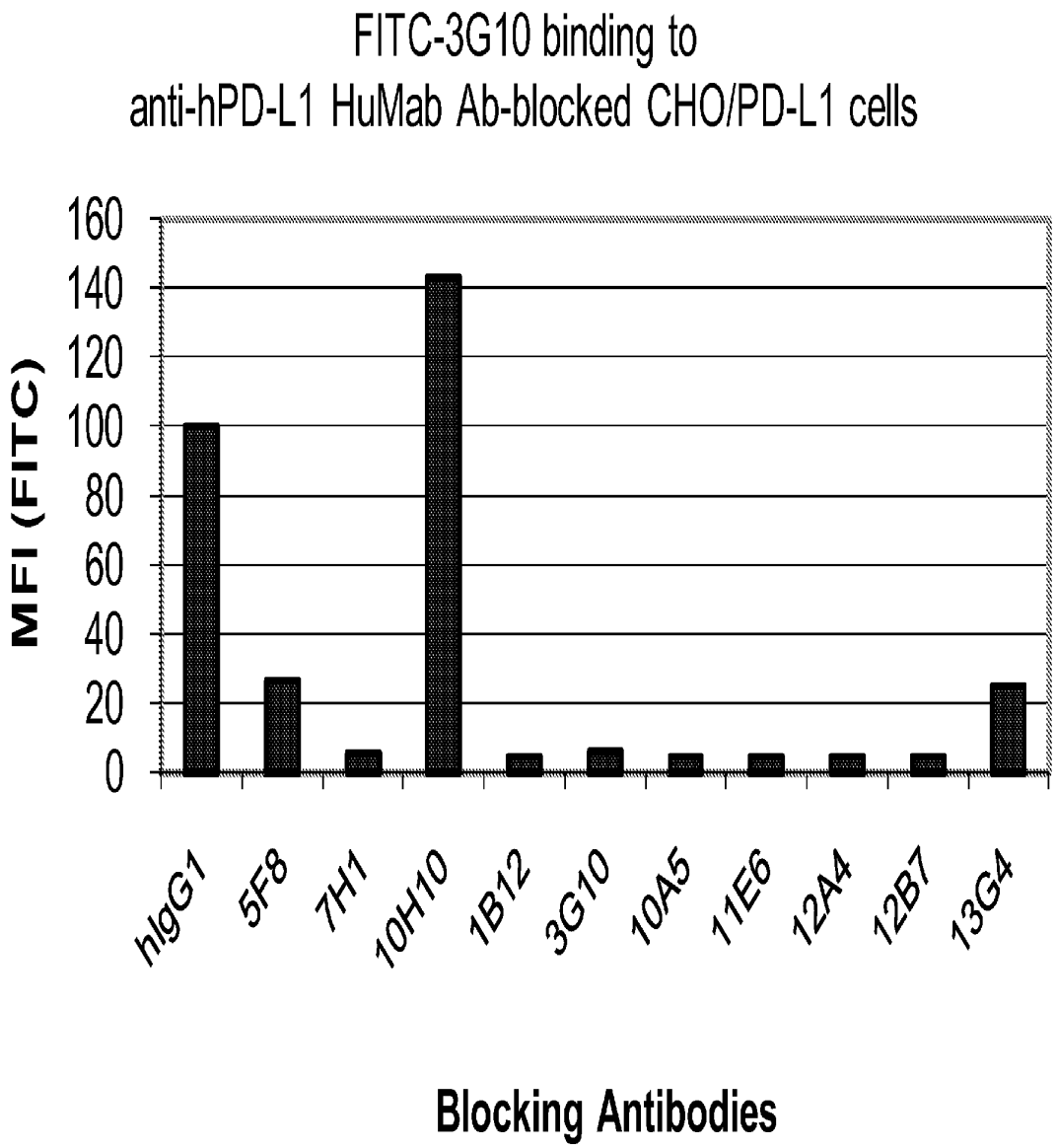

FITC-3G10 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
160
140
120
100
80
60
40
20
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2C

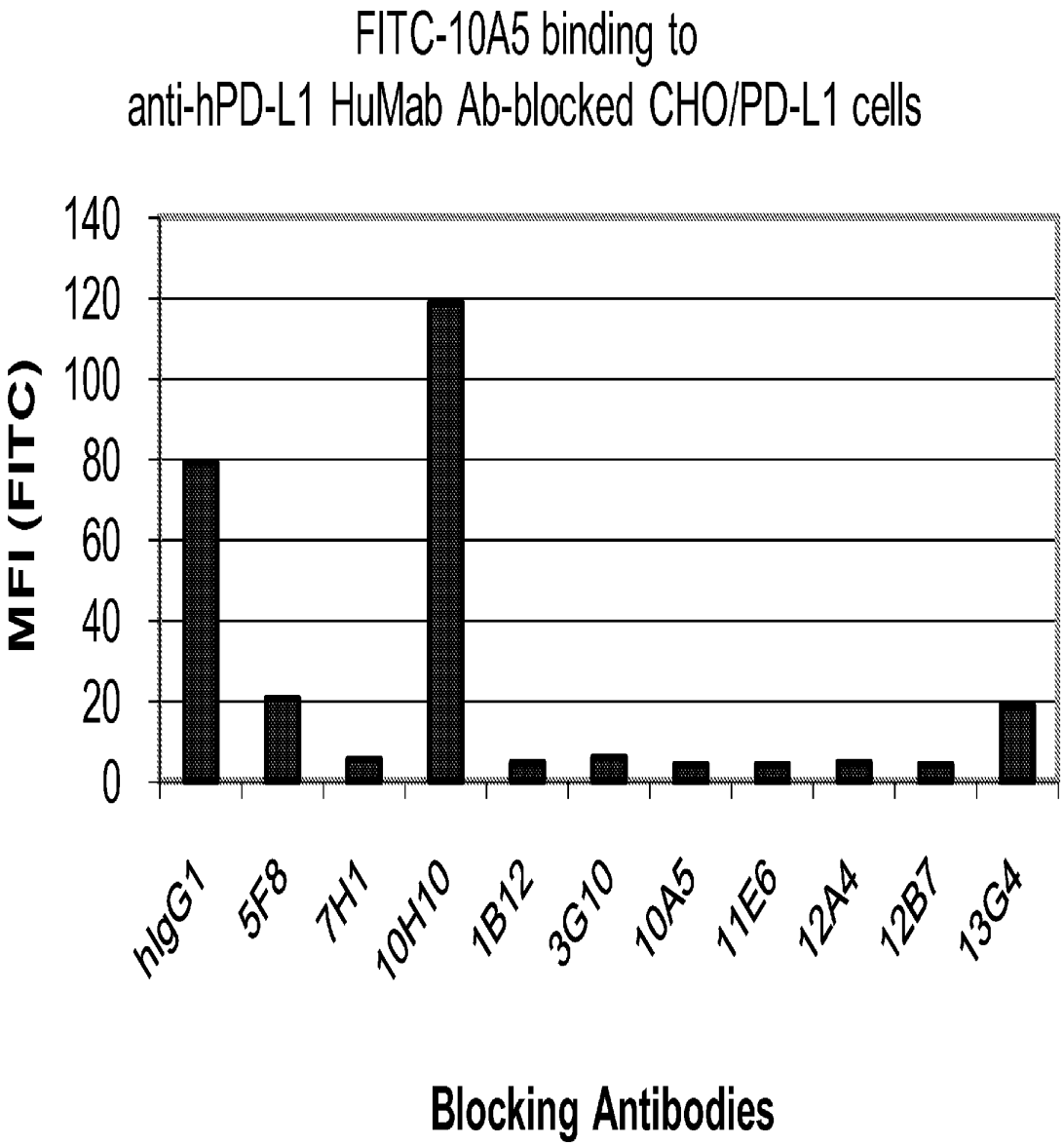

FITC-10A5 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
140
120
100
80
60
40
20
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2D

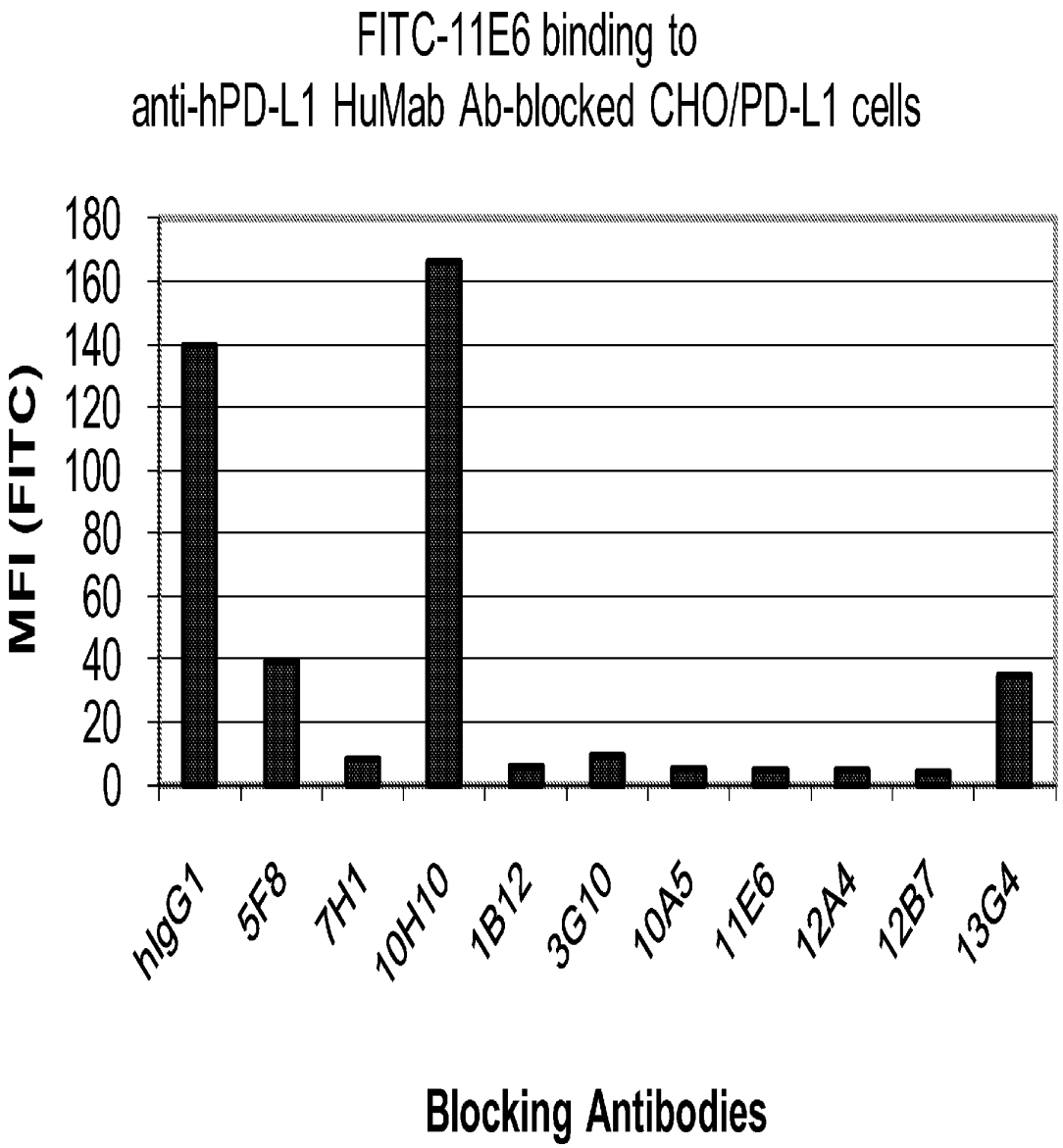

FITC-11E6 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
180
160
140
120
100
80
60
40
20
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2E

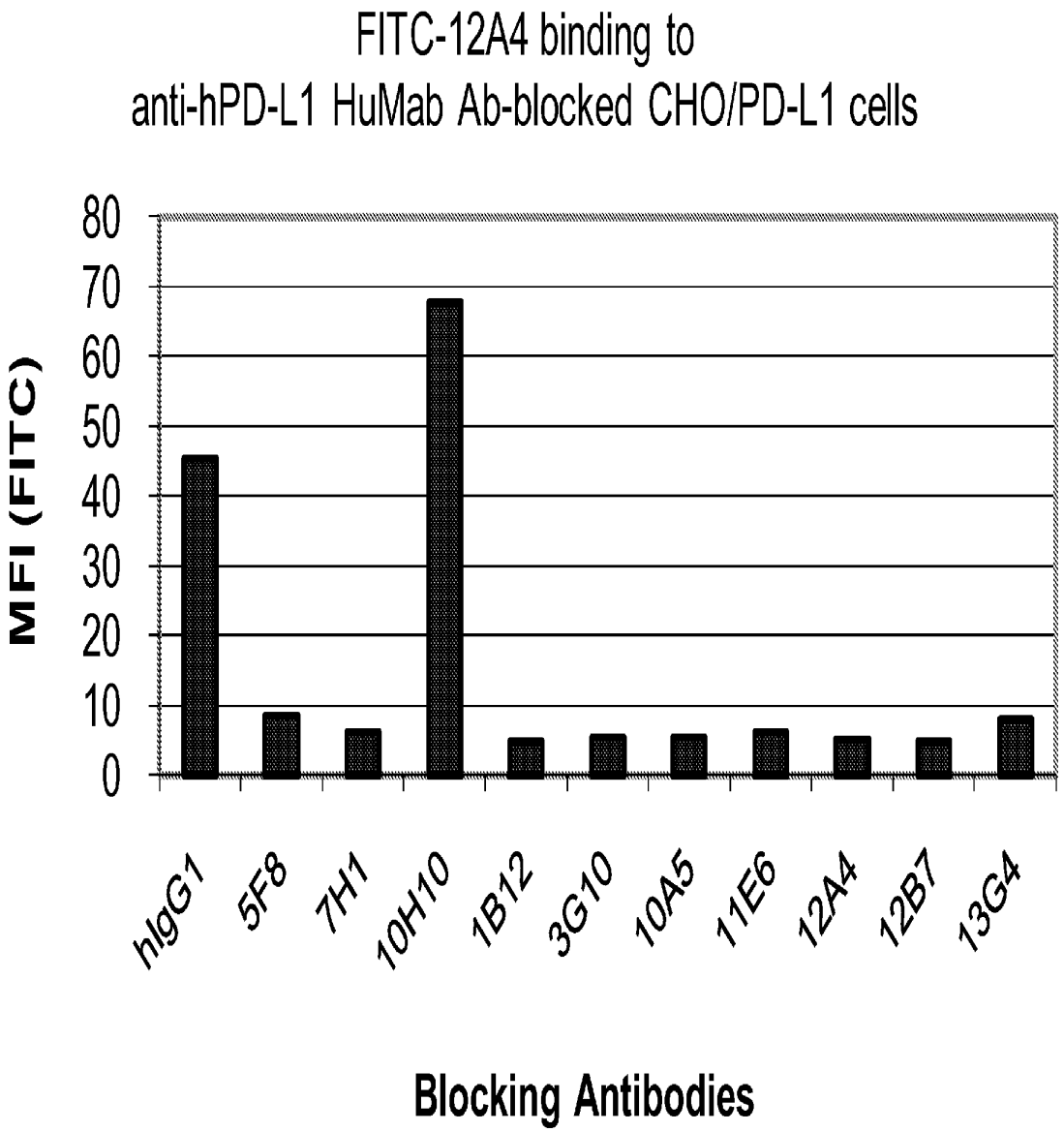

FITC-12A4 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
80
70
60
50
40
30
20
10
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2F

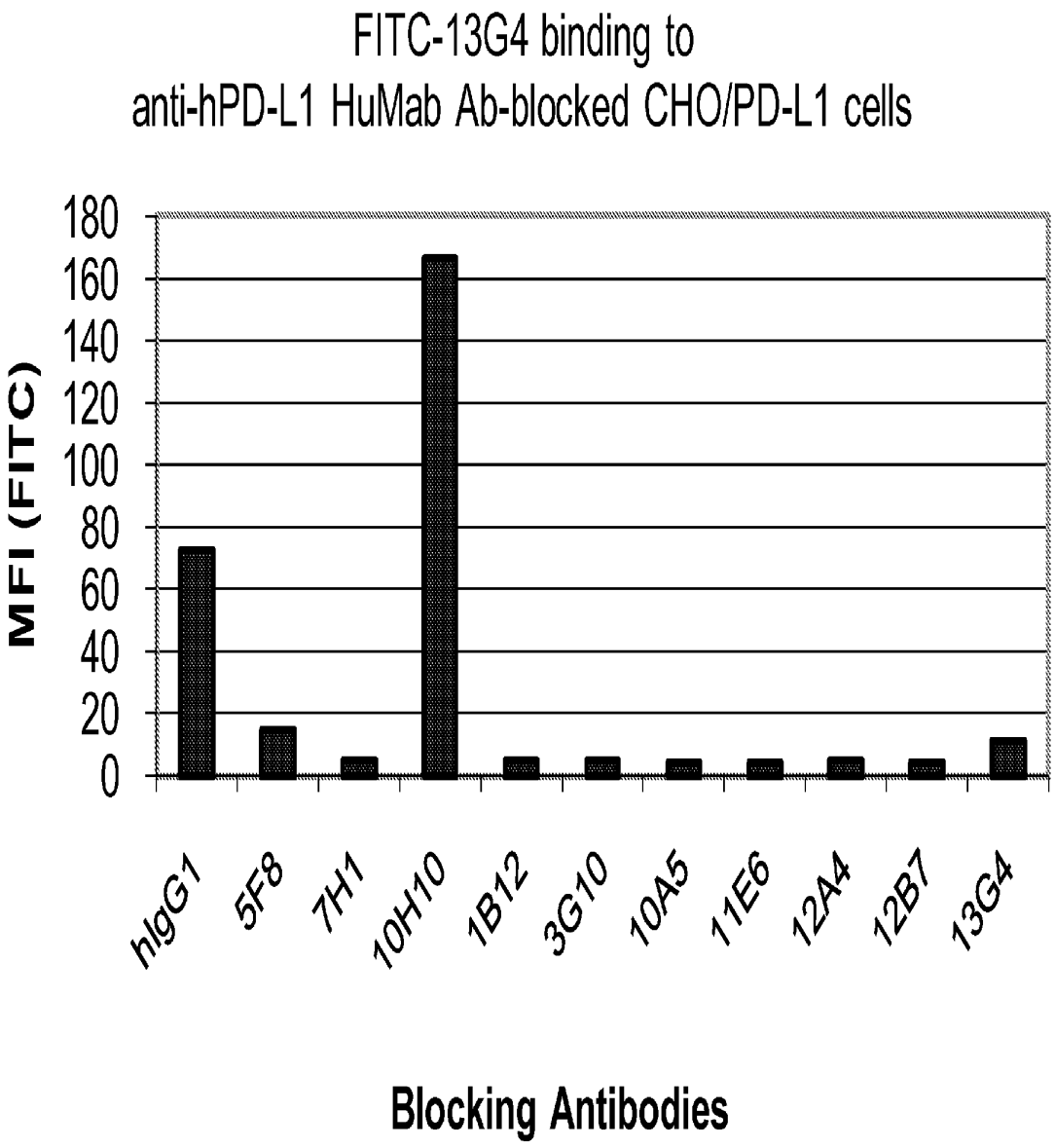

FITC-13G4 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
180
160
140
120
100
80
60
40
20
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 3

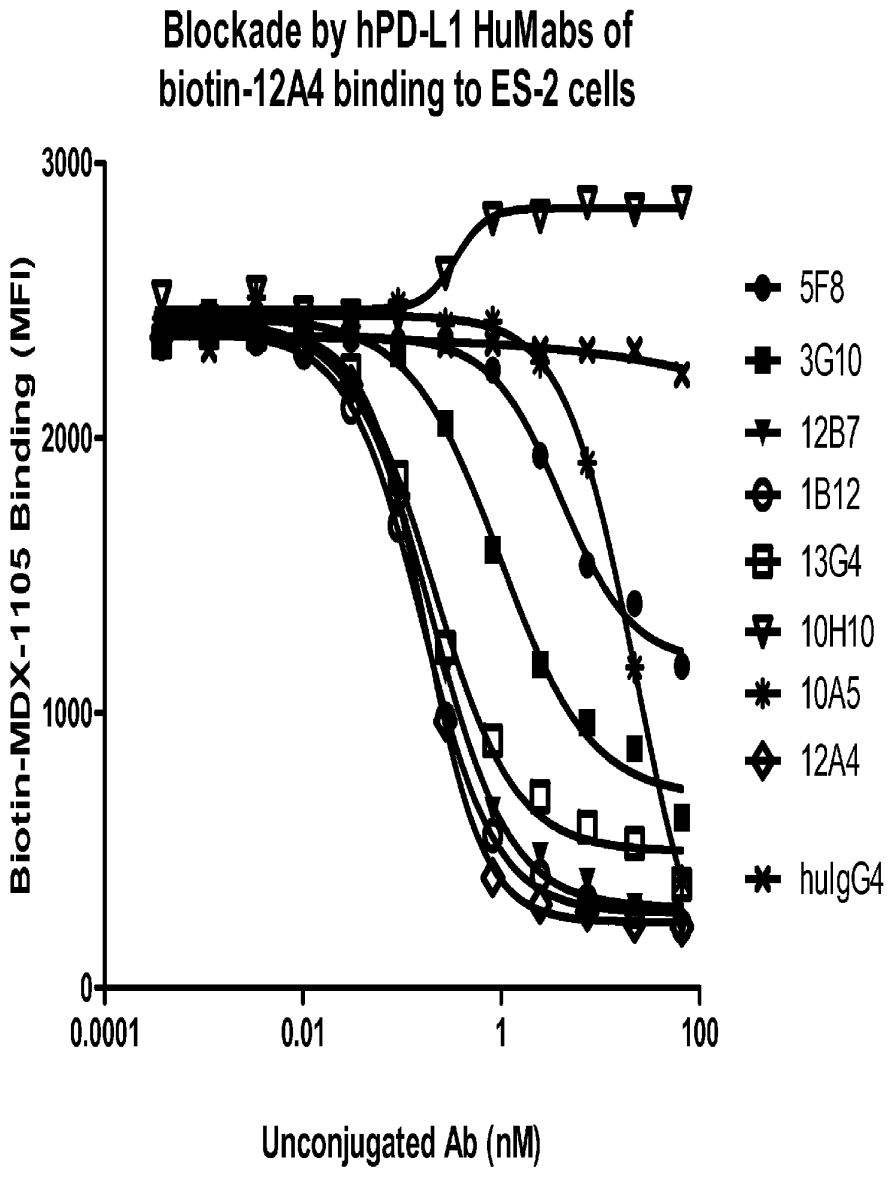

Blockade by hPD-L1 HuMabs of
biotin-12A4 binding to ES-2 cells
Biotin-MDX-1105 Binding (MFI)
3000
2000
1000
0
0.0001
0.01
1
100
Unconjugated Ab (nM)
5F8
3G10
12B7
1B12
13G4
10H10
10A5
12A4
hulgG4

FIG. 4

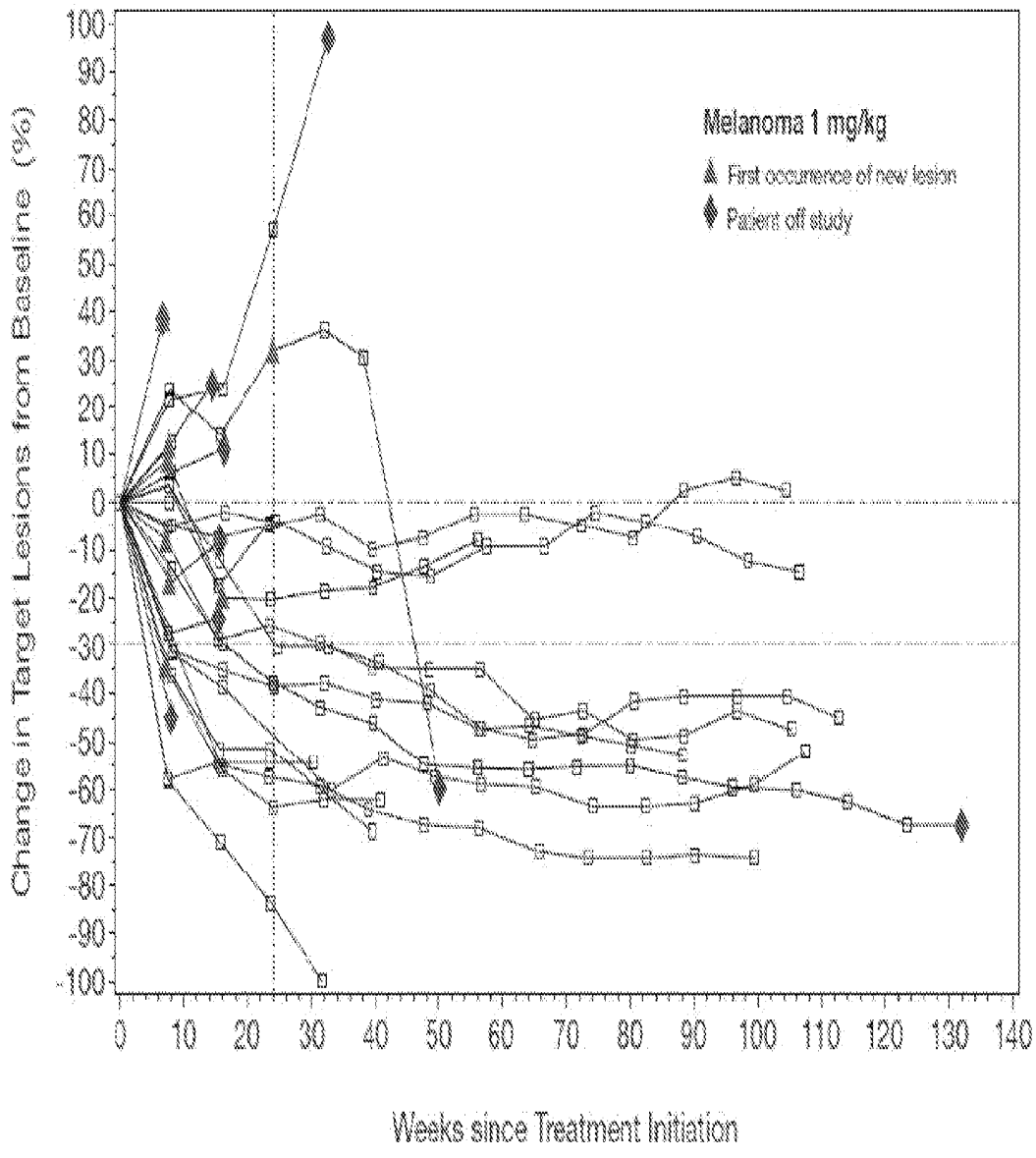

Melanoma 1 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
Weeks since Treatment Initiation
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140

FIG. 5

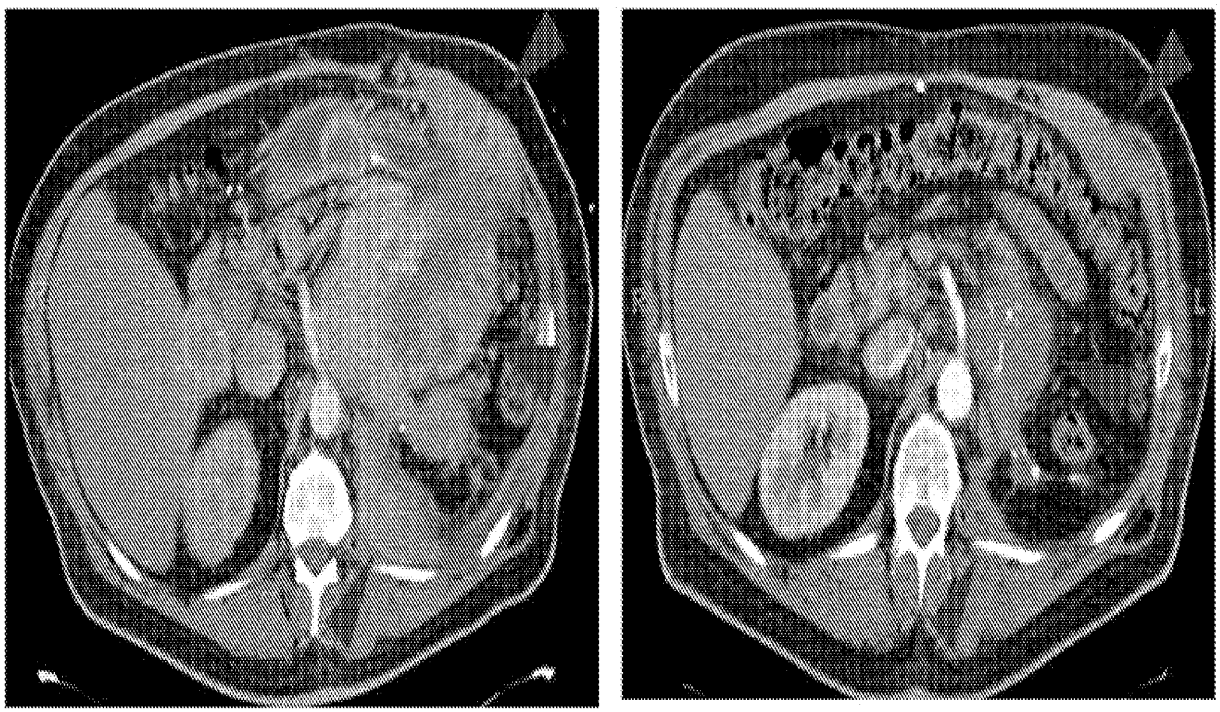


FIG. 6

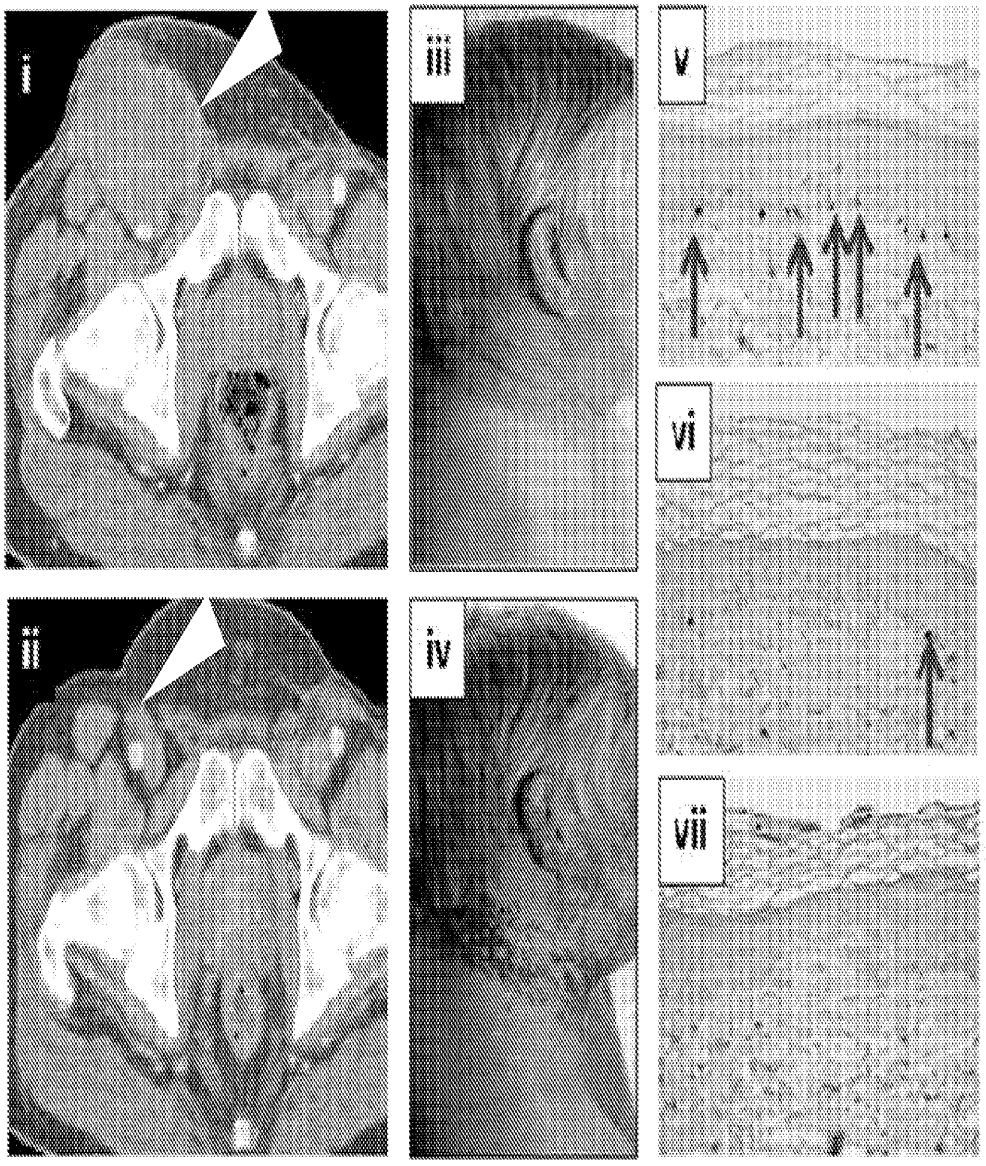


FIG. 7

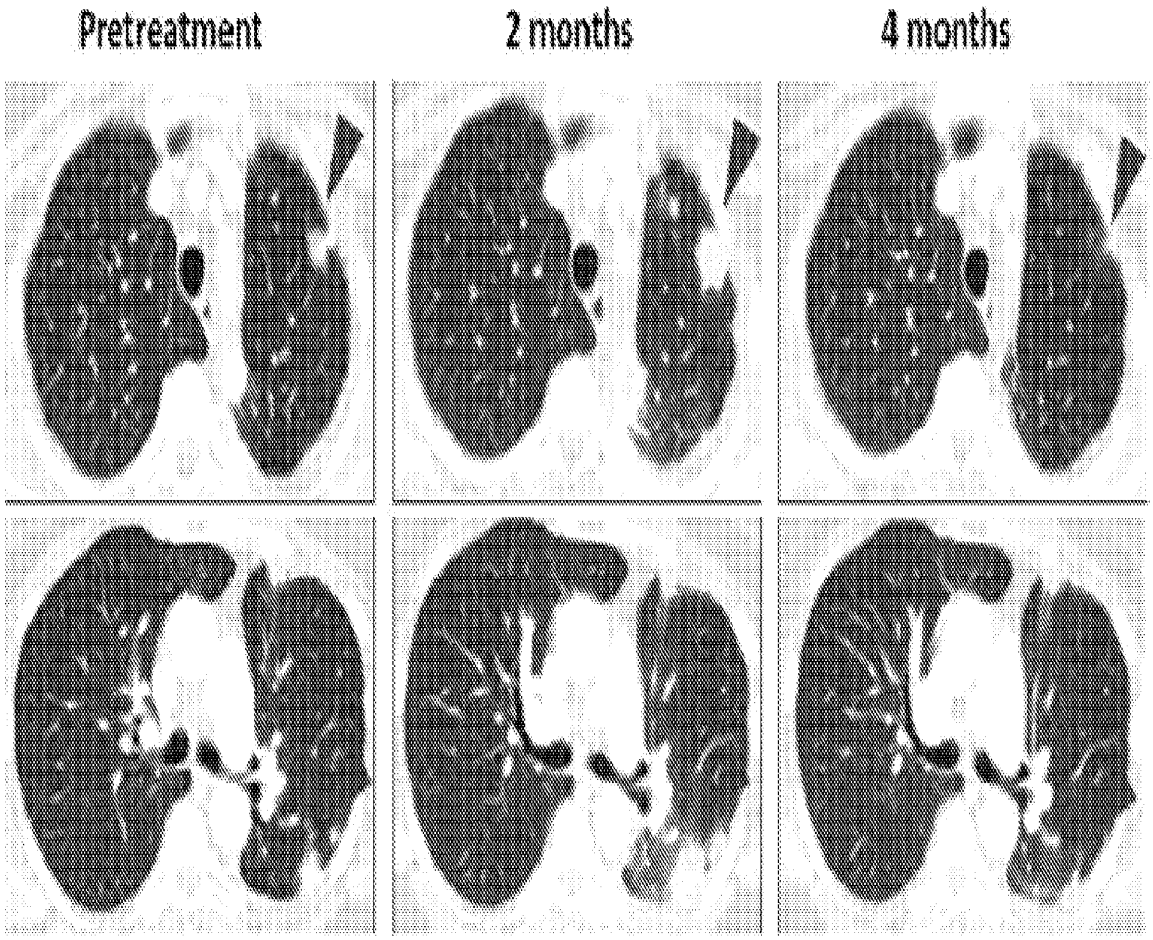
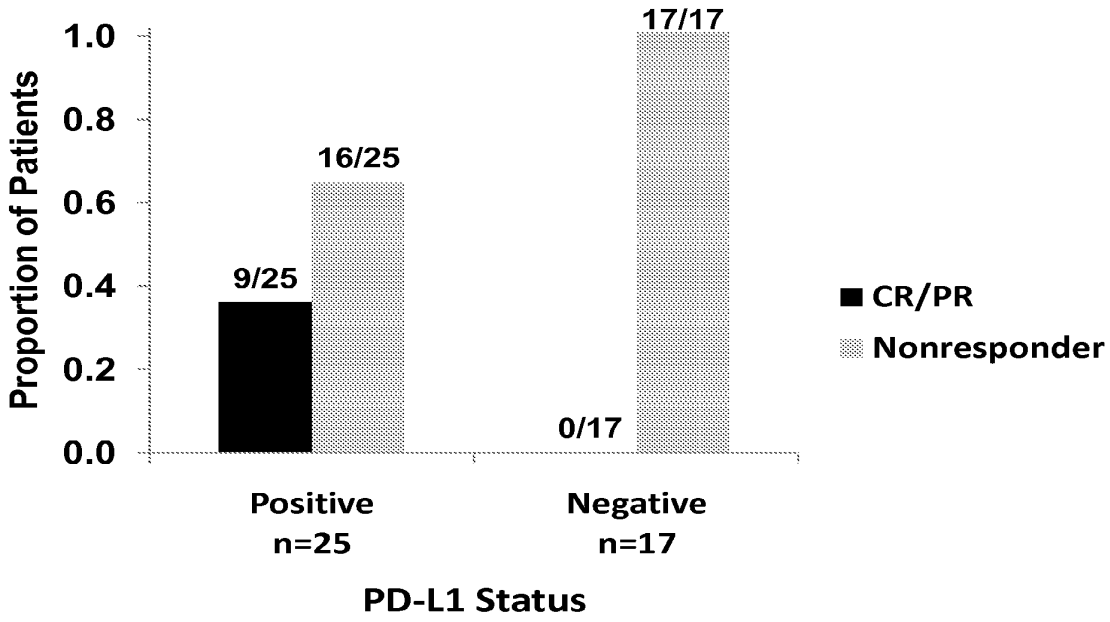


FIG. 8A



Association Between Pretreatment Tumor PD-L1 Expression and Clinical Response			
Response Status	**PD-L1-Positive no. (%)**	**PD-L1-Negative no. (%)**	**Total no. (%)**
CR/PR	**9 (36)**	**0**	**9 (21)**
Nonresponder	**16* (64)**	**17 (100)**	**33 (79)**
All Patients	**25**	**17**	**42**

Fisher's exact test for association P=0.006.

***Two patients categorized as nonresponders at the time of data analysis are still under evaluation.**

FIG. 8B

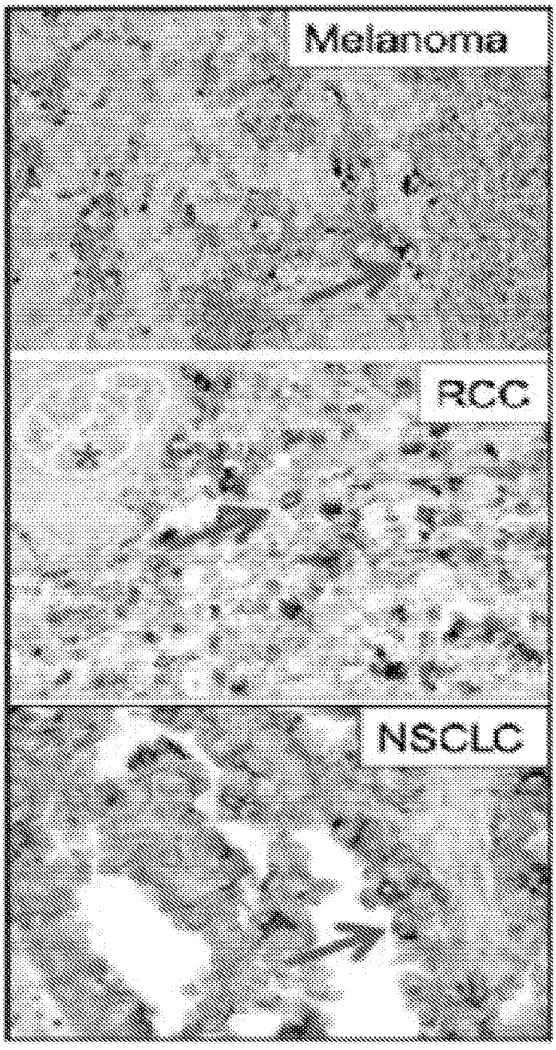


FIG. 9

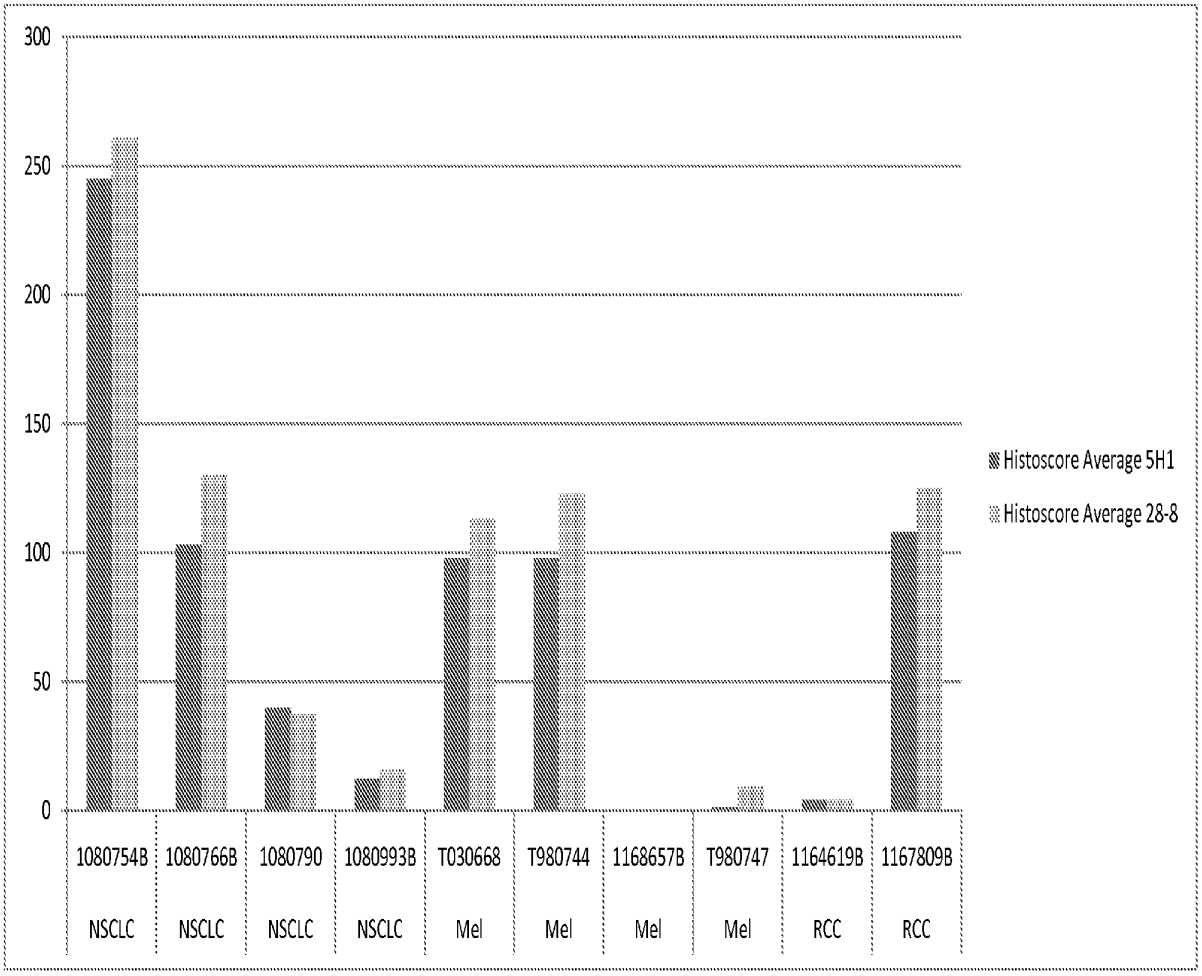

300
250
200
150
100
50
0
Histoscore Average 5H1
Histoscore Average 28-8
1080754B NSCLC
1080766B NSCLC
1080790 NSCLC
1080993B NSCLC
T030668 Mel
T980744 Mel
1168657B Mel
T980747 Mel
1164619B RCC
1167809B RCC

FIG. 10A

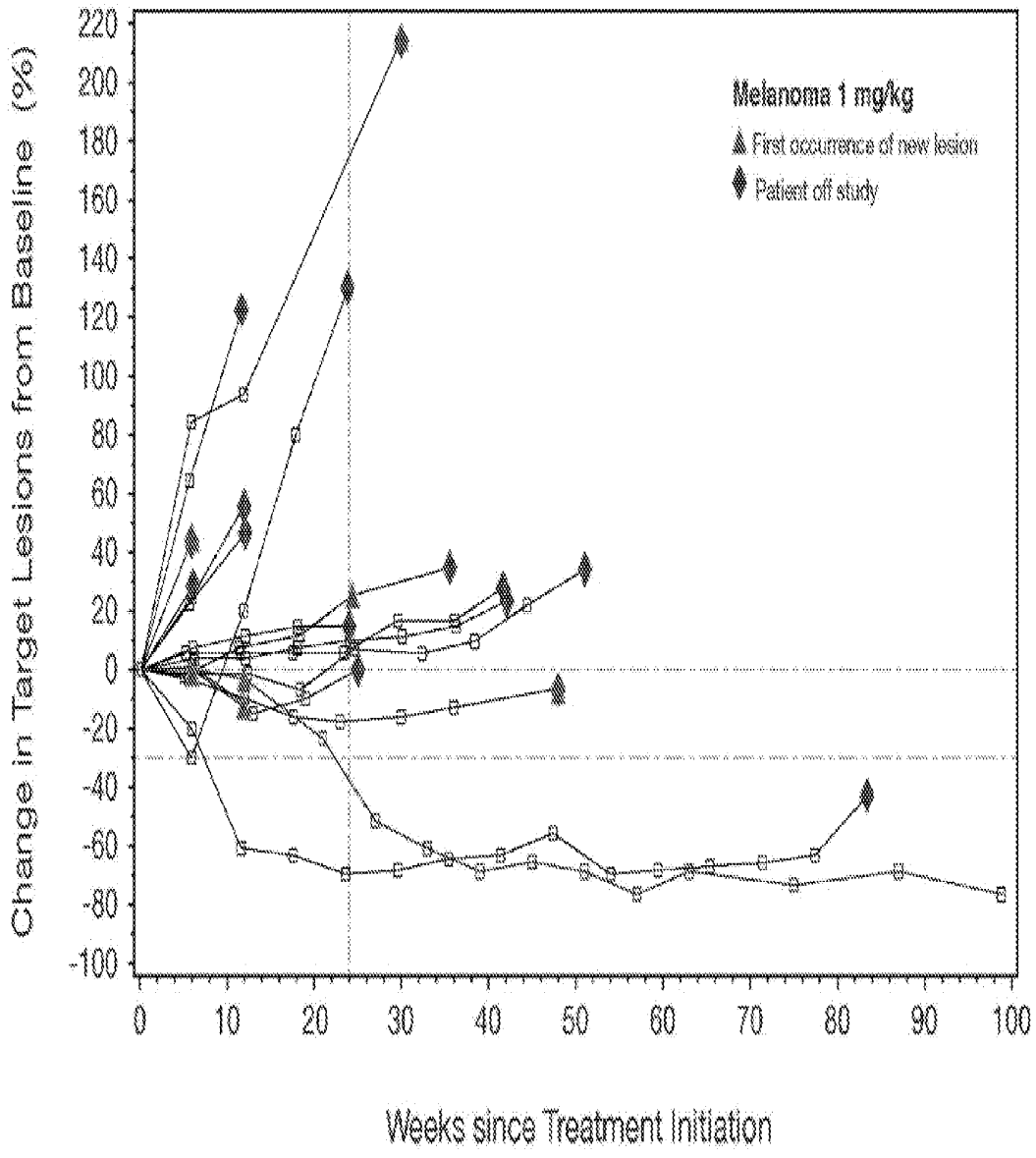

Melanoma 1 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
220
200
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
Weeks since Treatment Initiation

FIG. 10B

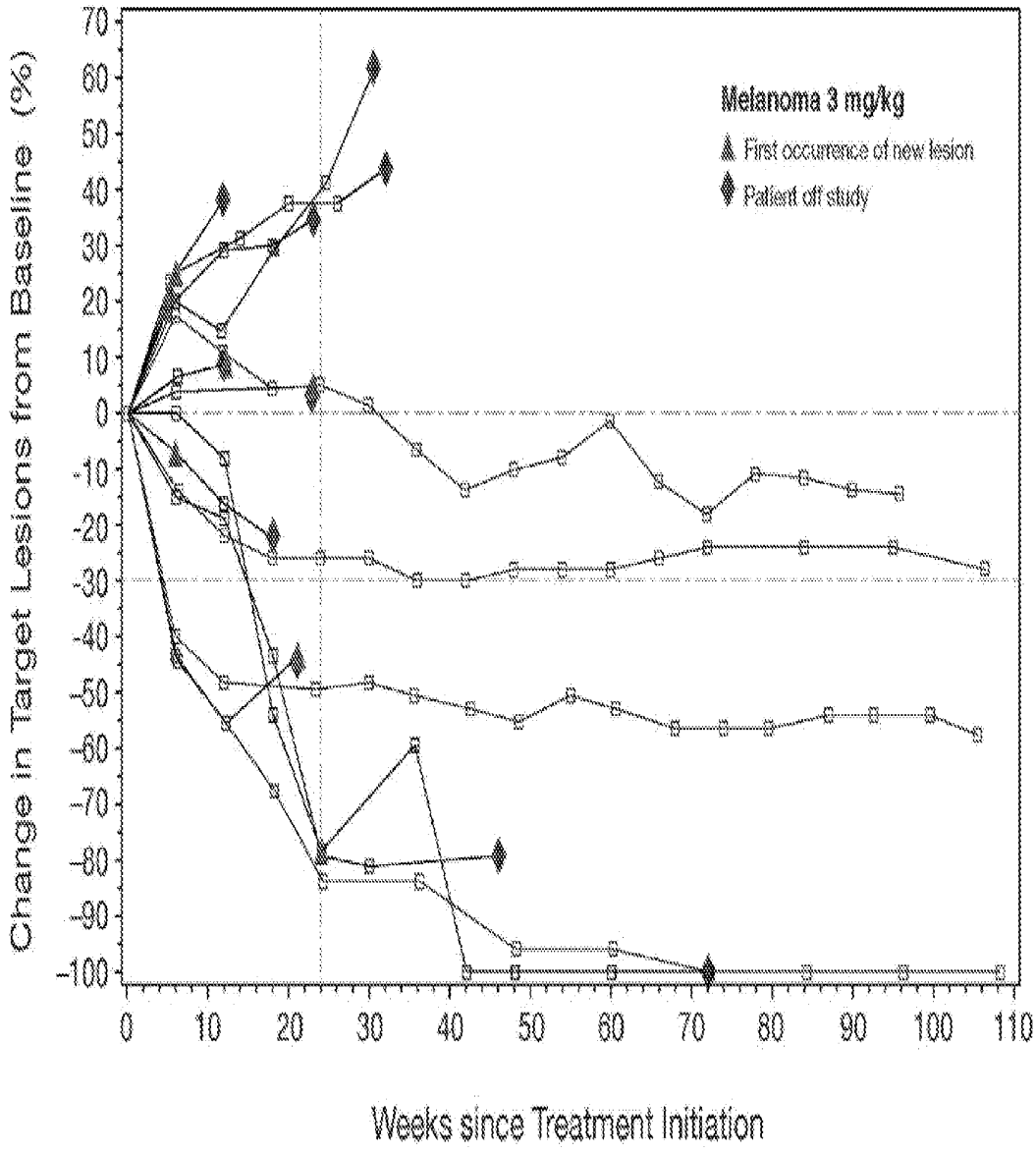

Melanoma 3 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
10
20
30
40
50
60
70
80
90
100
110
Weeks since Treatment Initiation

FIG. 10C

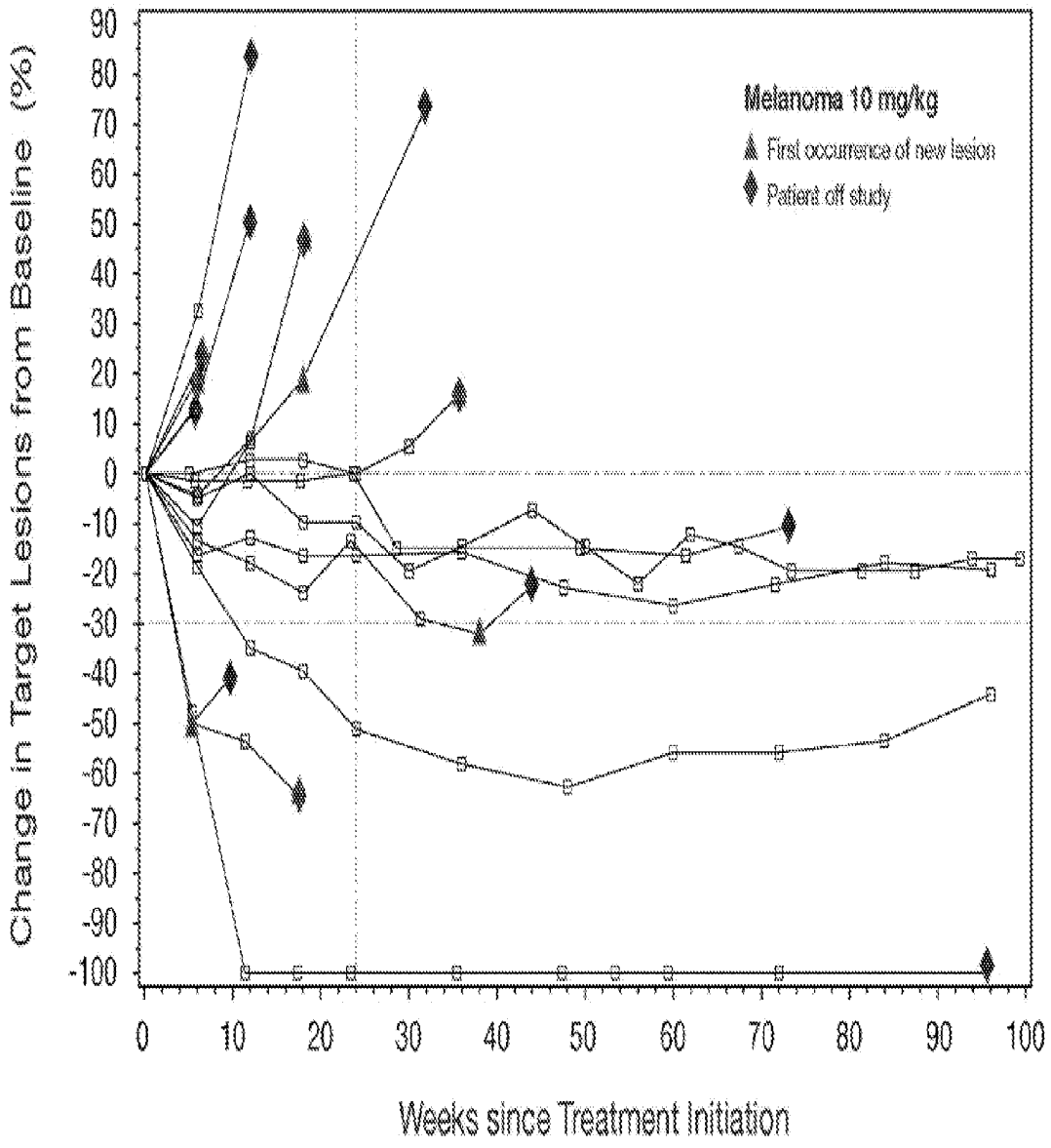

Melanoma 10 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
10
20
30
40
50
60
70
80
90
100
Weeks since Treatment Initiation

FIG. 10D

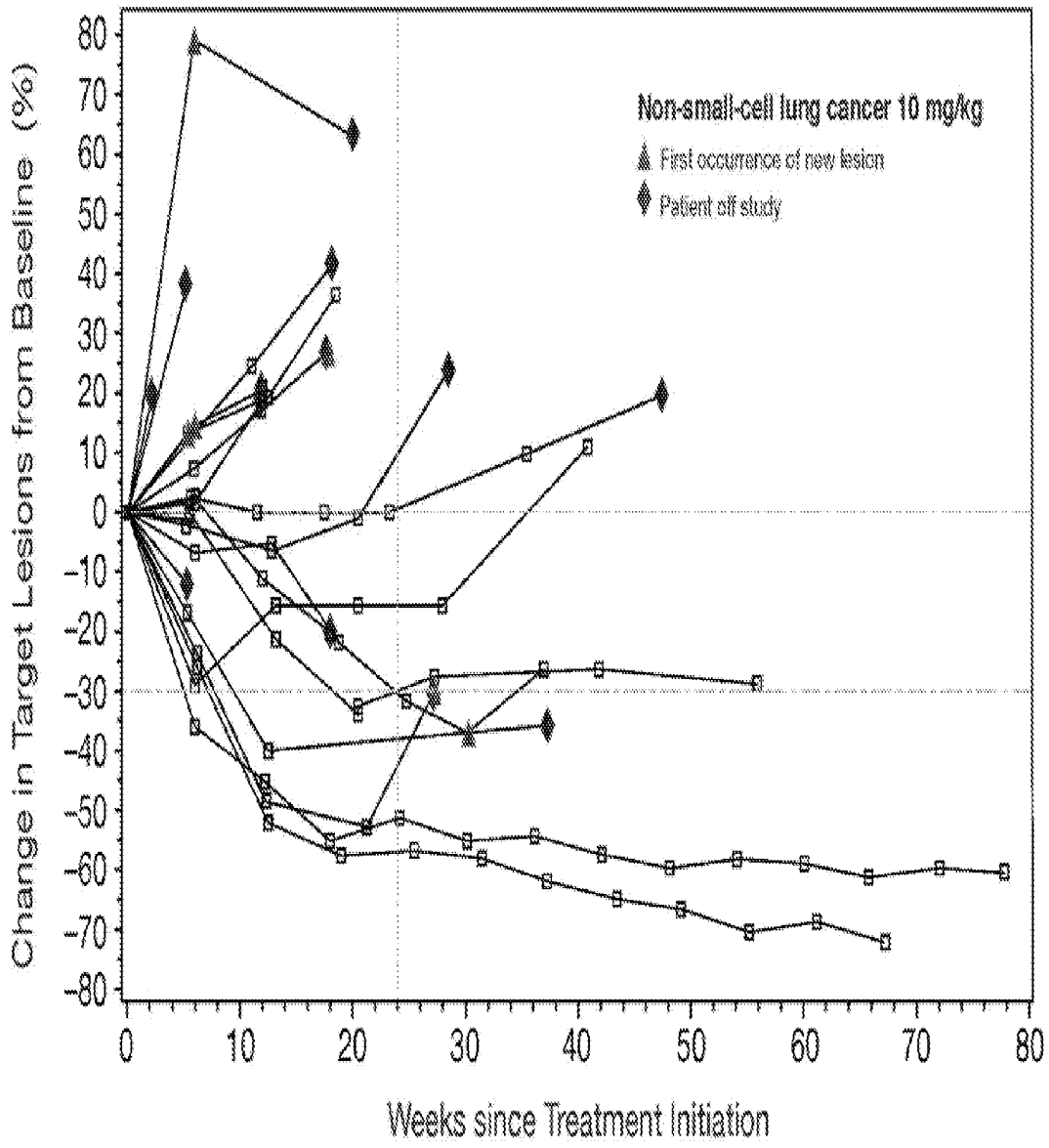
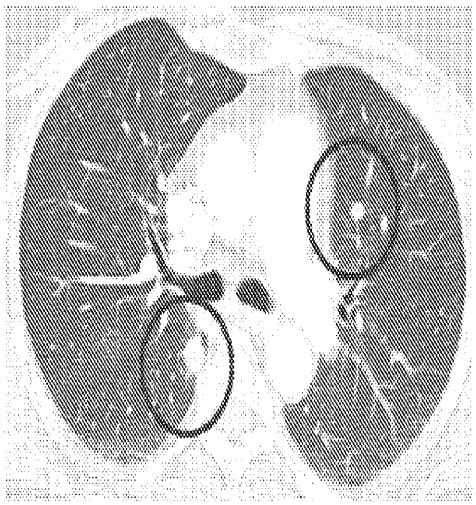
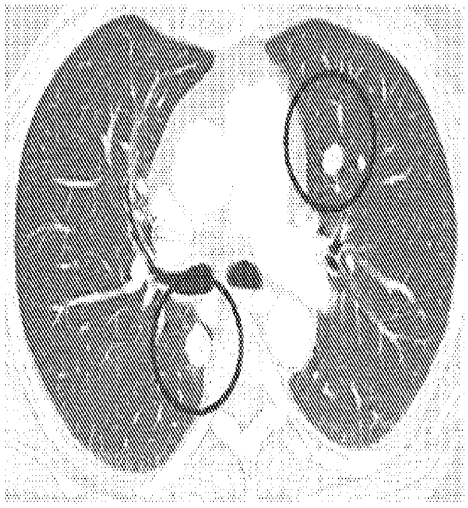

Non-small-cell lung cancer 10 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
0
10
20
30
40
50
60
70
80
Weeks since Treatment Initiation

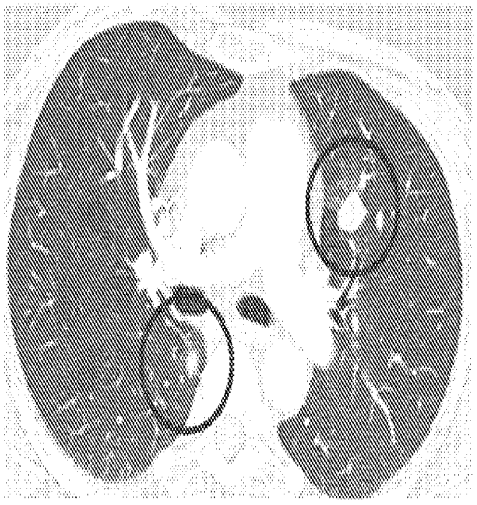
FIG. 11



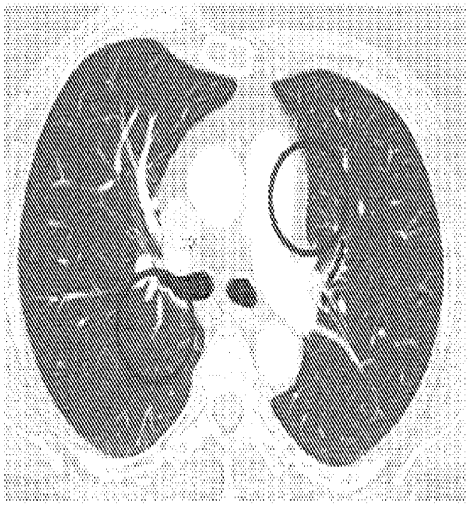
Pretreatment



1.5 months



3 months



10 months

FIG. 12

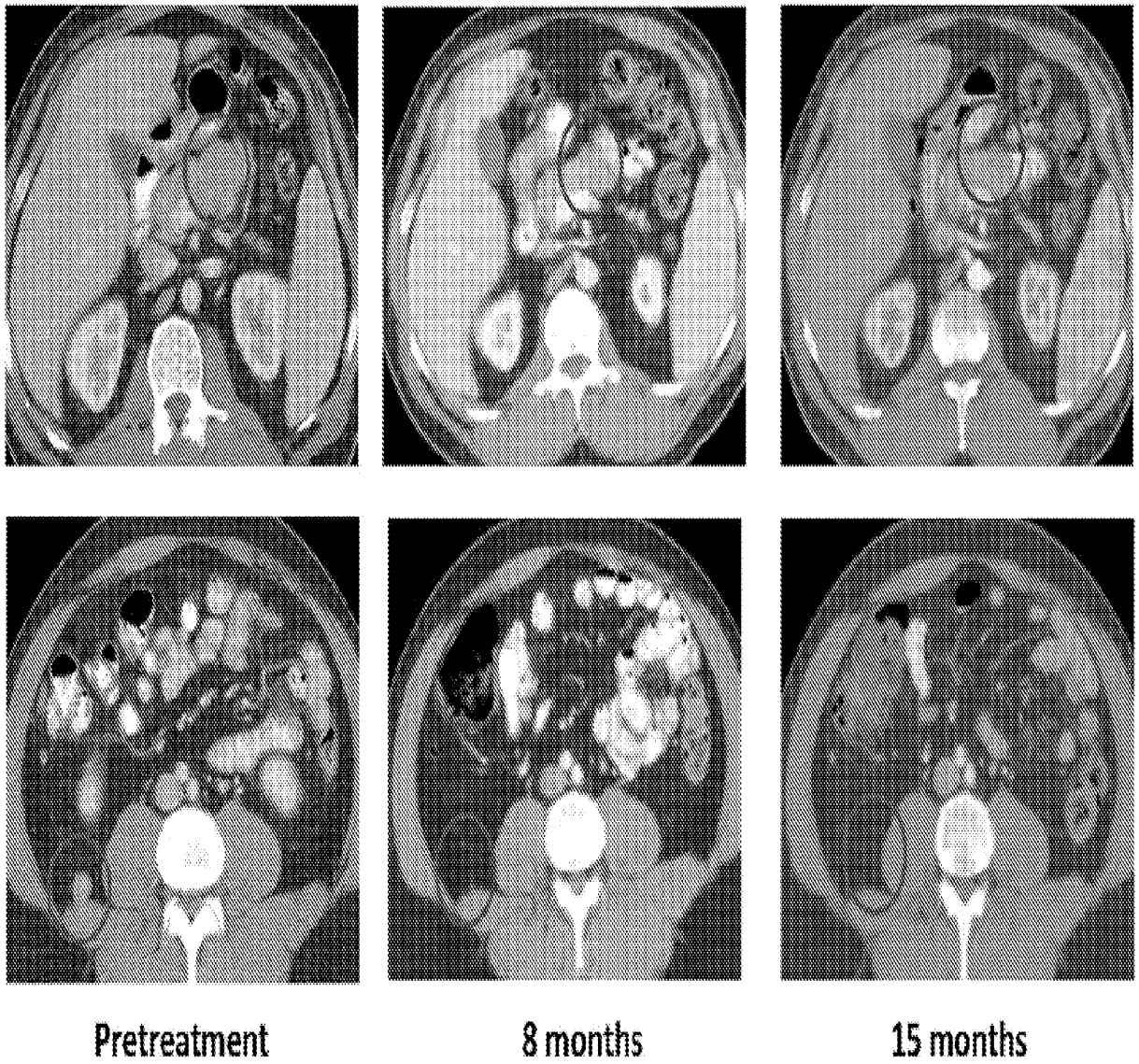
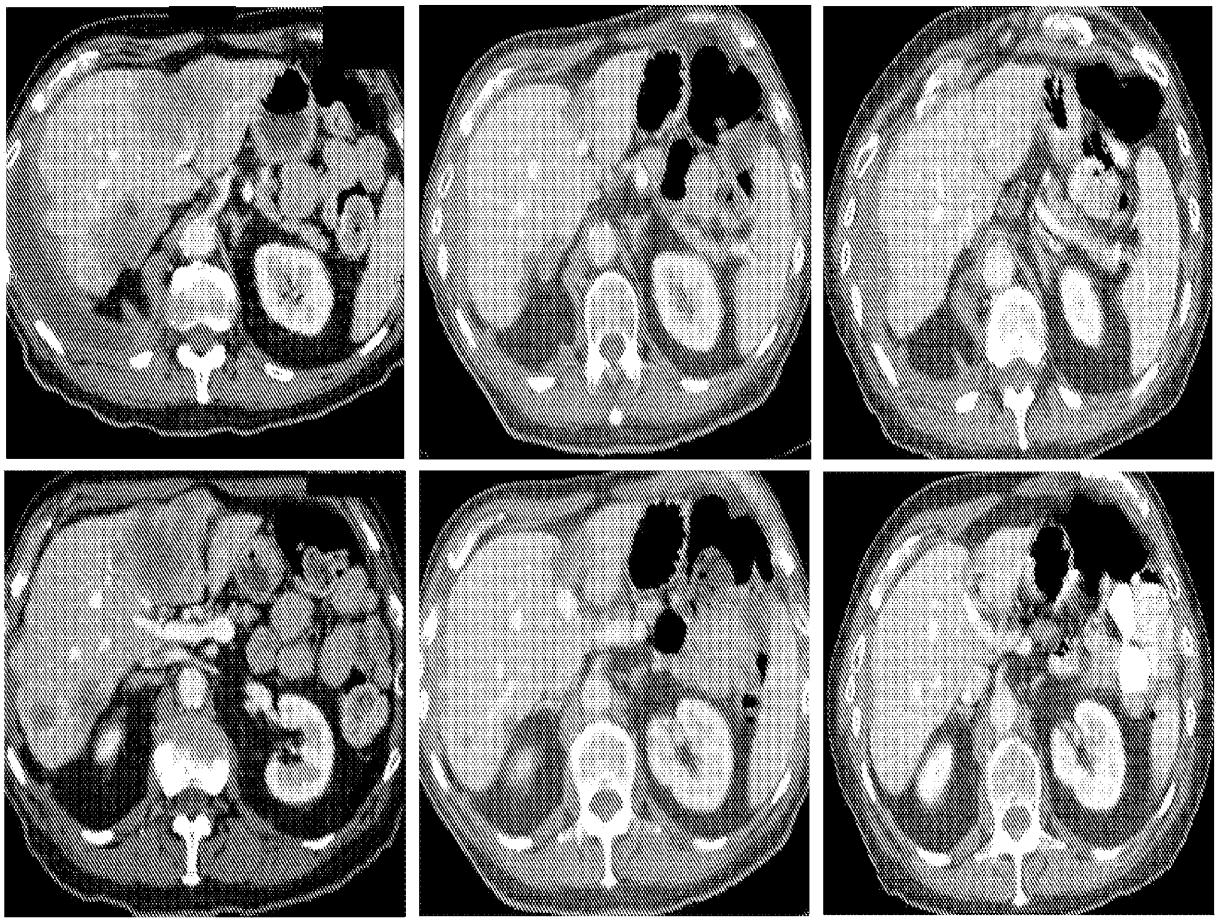


FIG. 13



Application Data Sheet 37 CFR 1.76

Application Information

Title of the Invention	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
Attorney Docket Number	11981-US-PSP
Large Entity Status Claimed	YES
Application Type	Provisional
Subject Matter	UTILITY
Total Number of Drawing Sheets (if any)	24
Sequence Submissions (if any)	YES

Inventor Information

Authority Type	Inventor
Primary Citizenship Country	US
Given Name	John
Middle Name/Initial	
Family Name	Cogswell
Residence City	Plainsboro
Residence State/Province	PA
Country of Residence	US
Mailing Address	1206 Evergreen Rd.
Mailing Address City	Yardley
Mailing Address Postal Code	19067
Mailing Address State/Province	PA
Mailing Address Country	US

Inventor Information

Authority Type	Inventor
Primary Citizenship Country	US
Given Name	Maria
Middle Name/Initial	
Family Name	Jure-Kunkel
Residence City	Plainsboro
Residence State/Province	NJ
Country of Residence	US
Mailing Address	31 Silvers Lane
Mailing Address City	Plainsboro
Mailing Address Postal Code	08536
Mailing Address State/Province	NJ
Mailing Address Country	US

Correspondence Information:

Customer Number	23914
Main E-Mail address	patents@bms.com

Representative Information:

Customer Number	23914

Application Data Sheet 37 CFR 1.76

Domestic Priority Information:

Application No	Continuity Type	Parent Application No.	Parent Filing Date
This Application			

Assignee Information:

Assignee	Bristol-Myers Squibb Company
Assignee is an Organization	Yes

Signature:

Signature	/ashton j. Delauney/
Date	5/15/12
Attorney/Agent Name	Ashton J. Delauney
Registration Number	64,286
Telephone Number	609-252-4560

Electronic Acknowledgement Receipt	
EFS ID:	12787430
Application Number:	61647442
International Application Number:	
Confirmation Number:	5263
Title of Invention:	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
First Named Inventor/Applicant Name:	John Cogswell
Customer Number:	23914
Filer:	Ashton J. Delauney/Hilda Franco
Filer Authorized By:	Ashton J. Delauney
Attorney Docket Number:	11981-US-PSP
Receipt Date:	15-MAY-2012
Filing Date:	
Time Stamp:	20:05:37
Application Type:	Provisional

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$250
RAM confirmation Number	7020
Deposit Account	193880
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		20120515_SPEC_11981USPSP.pdf	425624 28dcfde3be99c9c1de1c2e6154a4f2948e95a231	yes	91

Multipart Description/PDF files in .zip description

Document Description	Start	End
Specification	1	89
Claims	90	90
Abstract	91	91

Warnings:

Information:

2	Drawings-only black and white line drawings	20120515_DRW_11981USPSP.pdf	7606484 dccf2a05394c125a8ad5d67d7d67472fed077395	no	24

Warnings:

Information:

3	Application Data Sheet	20120515_ADS_11981USPSP.pdf	60830 0061d18d8d1ec36ca8da5f70ad580d27f703b2a	no	2

Warnings:

Information:

This is not an USPTO supplied ADS fillable form

4	Sequence Listing (Text File)	20120515_SEQ_11981USPS.txt	37594	no	0

Warnings:

Information:

5	Fee Worksheet (SB06)	fee-info.pdf	29792 be9e54a0fde8dd31cc90c895c9c4c506c722ada8	no	2

Warnings:

Information:

Total Files Size (in bytes):	8160324

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.

DocCode – SCORE

SCORE Placeholder Sheet for IFW Content

Application Number: 61647442 Document Date: 05/15/2012

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

- Design Drawing

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

To access the documents in the SCORE database, refer to instructions developed by SIRA.

At the time of document entry (noted above):

- Examiners may access SCORE content via the eDAN interface.
- Other USPTO employees can bookmark the current SCORE URL (http://es/ScoreAccessWeb/).
- External customers may access SCORE content via the Public and Private PAIR interfaces.

Form Revision Date: February 8, 2006

DocCode – SEQ.TXT

SCORE Placeholder Sheet for IFW Content

Application Number: 61647442 Document Date: 05/15/2012

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

- Sequence Listing

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

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At the time of document entry (noted above):

- Examiners may access SCORE content via the eDAN interface.
- Other USPTO employees can bookmark the current SCORE URL (http://es/ScoreAccessWeb/).
- External customers may access SCORE content via the Public and Private PAIR interfaces.

Form Revision Date: February 8, 2006

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DOCUMENT MADE AVAILABLE UNDER THE PATENT COOPERATION TREATY (PCT)

International application number:		**PCT/US2013/040764**
International filing date:		**13 May 2013 (13.05.2013)**
Document type:		**Certified copy of priority document**
Document details:	Country/Office:	**US**
	Number:	**61/647,442**
	Filing date:	**15 May 2012 (15.05.2012)**
Date of receipt at the International Bureau:		**03 June 2013 (03.06.2013)**

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a),(b) or (b-*bis*)

34, chemin des Colombettes
1211 Geneva 20, Switzerland
www.wipo.int

PCT REQUEST

Original (for **SUBMISSION**)

0	**For receiving Office use only**	
0-1	International Application No.	PCT/US13/40764
0-2	International Filing Date	13 MAY 2013 (13.05.13)
0-3	Name of receiving Office and "PCT International Application"	PCT INTERNATIONAL APPLICATION RO/US

0-4	**Form PCT/RO/101 PCT Request**	
0-4-1	Prepared Using	**PCT-SAFE [EASY/EFS-Web mode] Version 3.51.059.235 MT/FOP 20130401/0.20.5.21**
0-5	**Petition** The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	**Receiving Office (specified by the applicant)**	**United States Patent and Trademark Office (USPTO) (RO/US)**
0-7	**Applicant's or agent's file reference**	**11981-WO-PCT**
I	**Title of Invention**	**CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING**
II	**Applicant**	
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II-2	Applicant for	**All designated States**
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II-10	e-mail	**patents@bms.com**
II-10(a)	E-mail authorization The receiving Office, the International Searching Authority, the International Bureau and the International Preliminary Examining Authority are authorized to use this e-mail address, if the Office or Authority so wishes, to send notifications issued in respect of this international application:	**exclusively in electronic form (no paper notifications will be sent)**

PCT REQUEST

Original (for **SUBMISSION**)

III-1	**Applicant and/or inventor**	
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PCT REQUEST

Original (for **SUBMISSION**)

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IV-1-4	Facsimile No.	609-252-4526
IV-1-5	e-mail	patents@bms.com
IV-1-5(a)	E-mail authorization The receiving Office, the International Searching Authority, the International Bureau and the International Preliminary Examining Authority are authorized to use this e-mail address, if the Office or Authority so wishes, to send notifications issued in respect of this international application:	exclusively in electronic form (no paper notifications will be sent)
IV-1-6	Agent's registration No.	64,286
IV-2	**Additional agent(s)**	additional agent(s) with same address as first named agent
IV-2-1	Name(s)	EPPERSON, James(52,867); BERGEN, Briana C.(39,123); BOGIE, Terence J.(44,544); CHAO, Yuan(32,118); CLAUSS, Isabelle M.(47,326); D'AMICO, Stephen C.(46,652); DUNCAN, Laurelee A.(44,096); FRANK, Eve L.(46,785); GIBBONS, Maureen S.(44,121); GOLIAN, Paul D.(42,591); GREENBLATT, Gary D.(47,609); GRIEFF, Edward D.(44,638); GUO, Z. Angela(54,144); HADAD, Henry(35,888); HAI, Bing(57,170); JACOBSEN, Barry(43,689); KORSEN, Elliott(32,705); LEVIS, John F.(34,210); LIU, Hong(54,891); MILLER, Melissa(52,988); MINGO, Pamela A.(48,256); PARLET, Nickki L.(44,996); RODNEY, Burton(22,076); SPALDING, Todd(55,638); SUN, Jing G.(45,914);

PCT REQUEST

Original (for **SUBMISSION**)

		VANATTEN, Mary, K.(39,408); VOLLES, Warren(33,810); WALSH, Brian J.(45,543)
V	**DESIGNATIONS**	
V-1	**The filing of this request constitutes under Rule 4.9(a), the designation of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents.**	
VI-1	**Priority claim of earlier national application**	
VI-1-1	Filing date	15 May 2012 (15.05.2012)
VI-1-2	Number	61/647,442
VI-1-3	Country	US
VI-2	**Priority claim of earlier national application**	
VI-2-1	Filing date	15 March 2013 (15.03.2013)
VI-2-2	Number	61/790,747
VI-2-3	Country	US
VI-3	**Priority document request**	
	The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s):	VI-1 VI-2
VI-4	**Incorporation by reference :**	
	where an element of the international application referred to in Article 11(1)(iii)(d) or (e) or a part of the description, claims or drawings referred to in Rule 20.5(a) is not otherwise contained in this international application but is completely contained in an earlier application whose priority is claimed on the date on which one or more elements referred to in Article 11(1)(iii) were first received by the receiving Office, that element or part is, subject to confirmation under Rule 20.6, incorporated by reference in this international application for the purposes of Rule 20.6.	
VII-1	**International Searching Authority Chosen**	European Patent Office (EPO) (ISA/EP)

PCT REQUEST

Original (for **SUBMISSION**)

VIII	**Declarations**	Number of declarations	
VIII-1	Declaration as to the identity of the inventor	–	
VIII-2	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	–	
VIII-3	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	–	
VIII-4	Declaration of inventorship (only for the purposes of the designation of the United States of America)	–	
VIII-5	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	–	
IX	**Check list**	Number of sheets	Electronic file(s) attached
IX-1	Request (including declaration sheets)	6	✓
IX-2	Description	138	–
IX-3	Claims	5	–
IX-4	Abstract	1	✓
IX-5	Drawings	34	–
IX-6a	Sequence listing part of the description (also to be used for the purposes of international search)	–	✓
IX-7	TOTAL	184	
	Accompanying Items	Paper document(s) attached	Electronic file(s) attached
IX-8	Fee calculation sheet	✓	–
IX-20	**Figure of the drawings which should accompany the abstract**	7	
IX-21	**Language of filing of the international application**	English	
X-1	**Signature of applicant, agent or common representative**	/Ashton J. Delauney, Reg. No. 64,286/	
X-1-1	Name (LAST, First)	DELAUNEY, Ashton J.	
X-1-2	Name of signatory		
X-1-3	Capacity (if such capacity is not obvious from reading the request)		

FOR RECEIVING OFFICE USE ONLY

10-1	**Date of actual receipt of the purported international application**	13 MAY 2013 (13.05.13)
10-2	**Drawings:**	
10-2-1	Received	
10-2-2	Not received	
10-3	**Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application**	
10-4	**Date of timely receipt of the required corrections under PCT Article 11(2)**	
10-5	**International Searching Authority**	**ISA/EP**
10-6	**Transmittal of search copy delayed until search fee is paid**	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	**Date of receipt of the record copy by the International Bureau**	

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92*bis*.1 and Administrative Instructions, Section 422)

To:

DELAUNEY, Ashton J.
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Date of mailing *(day/month/year)*
12 November 2014 (12.11.2014)

Applicant's or agent's file reference
11981-WO-PCT

IMPORTANT NOTIFICATION

International application No.
PCT/US2013/040764

International filing date *(day/month/year)*
13 May 2013 (13.05.2013)

1. The following indications appeared on record concerning:

☐ the applicant ☒ the inventor ☐ the agent ☐ the common representative

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3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office
☐ the International Searching Authority
☐ the Authority(ies) specified for supplementary search
☐ the International Preliminary Examining Authority
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543644

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

June 02, 2013

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *61/790,747*
FILING DATE: *March 15, 2013*
RELATED PCT APPLICATION NUMBER: *PCT/US13/40764*

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS *US61/790,747*

Certified by

David J. Kappos

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

DocCode – SEQ.TXT

SCORE Placeholder Sheet for IFW Content

Application Number: 61790747 Document Date: 03/15/2013

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

- Sequence Listing

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

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Form Revision Date: February 8, 2006

CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING

Throughout this application, various publications are referenced in parentheses by author name and date, or by Patent No. or Patent Publication No. Full citations for these publications may be found at the end of the specification immediately preceding the claims. 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

This invention relates to methods for immunotherapy of a cancer patient comprising administering to the patient antibodies that disrupt the PD-1/PD-L1 signaling pathway. A biomarker may be used as part of this treatment for identifying suitable patients for immunotherapy and for predicting the efficacy of anti-PD-1 treatment.

BACKGROUND OF THE INVENTION

Human cancers harbor numerous genetic and epigenetic alterations, generating neoantigens potentially recognizable by the immune system (Sjoblom *et al*., 2006). Although an endogenous immune response to cancer is observed in preclinical models and patients, this response is ineffective, and established cancers are viewed as "self" and tolerated by the immune system. Contributing to this state of tolerance, tumors may exploit several distinct mechanisms to actively suppress the host immune response (Topalian *et al*., 2011; Mellman *et al*., 2011). Among these mechanisms, endogenous "immune checkpoints" that normally terminate immune responses to mitigate collateral tissue damage can be co-opted by tumors to evade immune destruction. Intensive efforts to develop specific immune checkpoint pathway inhibitors have begun to provide new immunotherapeutic approaches for treating cancer, including the development of the anti-CTLA-4 antibody (Ab), ipilimumab (Yervoy®), for the treatment of patients with advanced melanoma (Hodi *et al*., 2010).

Programmed Death-1 (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 downregulate T cell activation and cytokine secretion upon binding to PD-1 (Freeman *et al.*, 2000; Latchman *et al.*, 2001). Unlike CTLA-4, PD-1 primarily functions in peripheral tissues where activated T-cells may encounter the immunosuppressive PD-L1 (B7-H1) and PD-L2 (B7-DC) ligands expressed by tumor and/or stromal cells (Flies *et al.*, 2011; Topalian *et al.*, 2012a). 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), and the use of Ab inhibitors of the PD-1/PD-L1 interaction for treating cancer has entered clinical trials (Brahmer *et al.*, 2010; Flies *et al.*, 2011; Topalian *et al.*, 2012b; Brahmer *et al.*, 2012).

The promise of the emerging field of personalized medicine is that advances in pharmacogenomics will increasing 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 (Gleevac®), 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. Advances in validating PD-L1 expression as a biomarker for screening patients for anti-PD-1 immunotherapy are described herein.

SUMMARY OF THE INVENTION

The present invention provides a method for immunotherapy of a subject afflicted with cancer, which method comprises administering to the subject a composition comprising a therapeutically effective amount of an agent that reduces or suppresses signaling from an inhibitory immunoregulator. In preferred embodiments, the agent is an Ab. In other preferred embodiments, the inhibitory immunoregulator is a component of the PD-1/PD-L1 signaling pathway. In further preferred embodiments, the Ab disrupts the interaction between PD-1 and PD-L1. In certain embodiments, the Ab is an anti-PD-1 Ab

of the invention or an anti-PD-L1 Ab of the invention. In preferred embodiments, the anti-PD-1 Ab of the invention is nivolumab (BMS-936558) and the anti-PD-L1 Ab of the invention is BMS-936559. In certain embodiments, the subject has been pre-treated for the cancer. In other embodiments, the cancer is an advanced, metastatic and/or refractory cancer. In preferred embodiments, the administration of the antibody or antigen-binding portion to the subject thereof induces a durable clinical response in the subject.

This invention also provides a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is a suitable candidate for immunotherapy, the selecting comprising (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells, (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface, and (iii) selecting the subject as a suitable candidate based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and (b) administering a composition comprising a therapeutically effective amount of an anti-PD-1 Ab to the selected subject.

The invention further provides a method for treatment of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is not suitable for anti-PD-1 Ab immunotherapy, the selecting comprising (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; and (iii) selecting the subject as not suitable for anti-PD-1 Ab immunotherapy based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is less than a predetermined threshold level; and (b) administering a standard-of-care therapeutic other than an anti-PD-1 Ab to the selected subject.

In addition, the invention provides a method for selecting a cancer patient for immunotherapy with an anti-PD-1 Ab, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold proportion; and (d) selecting the patient for

immunotherapy based on an assessment that PD-L1 is expressed in cells of the test tissue sample.

This invention further provides a method for predicting the therapeutic effectiveness of an anti-PD-1 Ab for treating a cancer patient, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold value; and (d) predicting the therapeutic effectiveness of the anti-PD-1 Ab, wherein if the proportion of cells that express PD-L1 on the cell surface exceeds the threshold proportion the Ab is predicted to be effective in treating the patient, and wherein if the proportion of cells that express PD-L1 on the cell surface is below the threshold proportion the Ab is predicted to not be effective in treating the patient.

The present invention also provides a method for determining an immunotherapeutic regimen comprising an anti-PD-1 Ab for treating a cancer patient, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold proportion; and (d) determining an immunotherapeutic regimen comprising an anti-PD-1 Ab based on the determination that the proportion of cells that express PD-L1 on the cell surface exceeds the predetermined threshold proportion.

In certain embodiments of the methods described herein, the test tissue sample is a formalin-fixed and paraffin-embedded (FFPE) sample. In certain other embodiments, assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is achieved by immunohistochemical (IHC) staining of the FFPE sample. In preferred embodiments, the mAb 28-8 is used in an automated IHC assay to bind to PD-L1 on the surface of cells in the test tissue sample. In preferred embodiments of any of the methods disclosed herein, the cancer is melanoma (MEL), renal cell carcinoma (RCC), squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, colorectal cancer (CRC), castration-resistant prostate cancer (CRPC), hepatocellular carcinoma (HCC),

squamous cell carcinoma of the head and neck, carcinomas of the esophagus, ovary, gastrointestinal tract and breast, or a hematologic malignancy such as multiple myeloma, B-cell lymphoma, T-cell lymphoma, Hodgkin's lymphoma/primary mediastinal B-cell lymphoma, and chronic myelogenous leukemia.

This invention additionally provides a mAb or antigen-binding portion thereof that binds specifically to a cell surface-expressed human PD-L1 antigen in a FFPE tissue sample. In preferred embodiments, the mAb or antigen-binding portion thereof does not bind to a cytoplasmic PD-L1 antigen in the FFPE tissue sample. In other preferred embodiments, the monoclonal Ab (mAb) is the rabbit mAb designated 28-8, 28-1, 28-12, 29-8 or 20-12.

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 cited references, including scientific articles, newspaper reports, GenBank entries, patents and patent applications cited throughout this application are expressly incorporated herein by reference.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1C. Cross-competition between 5C4 and other HuMab anti-PD-1 mAbs for binding to hPD-1 expressed on CHO cells. A, the 5C4 Fab fragment substantially blocked the binding of mAbs 5C4 itself, as well as the binding of 2D3 and 7D3; B, the 5C4 Fab fragment substantially blocked the binding of mAb 4H1; C, the 5C4 mAb substantially blocked the binding of mAb 17D8.

Figures 2A-2F. Cross-competition of FITC-conjugated human anti-huPD-L1 mAbs for binding to hPD-L1 expressed on CHO cells. A, Binding of labeled 10H10 was partially blocked by 10A5, 11E6 and 13G4 and was significantly blocked by itself; B, Binding of labeled 3G10 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10; C, Binding of labeled 10A5 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10; D, Binding of labeled 11E6 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10; and E, Binding of labeled 12A4 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10; and F, Binding of labeled 13G4 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10.

Figure 3. Cross-competitive inhibition of binding of biotinylated mAb 12A4 to ES-2 cells by human anti-huPD-L1 mAbs. Fluorescence of bound biotin-12A4 is plotted

against the concentration of unlabeled hPD-L1 HuMabs.

Figure 4. Spider plot showing activity of anti-PD-1 mAb in patients with treatment-refractory melanoma (MEL). A representative plot of changes in tumor burden over time demonstrates the time course of change in the sum of the longest diameters of target lesions, compared with baseline, in 27 MEL patients treated with 5C4 at a dose of 1.0 mg/kg. In the majority of patients who achieved an objective response (OR), responses were durable and evident by the end of cycle 3 (6 months) of treatment (vertical dashed line). Tumor regressions followed conventional as well as "immune-related" patterns of response, such as prolonged reduction in tumor burden in the presence of new lesions.

Figure 5. Activity of anti-PD-1 mAb in patient with metastatic RCC. Partial regression of metastatic RCC in a 57-year-old patient treated with 5C4 at 1 mg/kg is illustrated. This patient had previously undergone radical surgery and had developed progressive disease after receiving sunitinib, temsirolimus, sorafenib, and pazopanib. Arrows show regression of recurrent tumor in the operative field.

Figures 6. Activity of anti-PD-1 mAb in patient with metastatic MEL. A complete response of metastatic MEL is illustrated in a 62-year-old patient treated with 5C4 at 3 mg/kg, associated with vitiligo. (i) Pretreatment CT scan, inguinal lymph node metastasis (arrow); (ii) after 13 months of treatment. Numerous metastases in the subcutaneous tissue and retroperitoneum also regressed completely (not shown). Vitiligo developed after 6 months of treatment; photos taken at 9 months under visible light (iii) and ultraviolet light (iv). Skin biopsies with immunohistochemistry for microphthalmia-associated transcription factor (MITF) show melanocytes (arrows) at the epidermal-dermal junction in normal skin (v), and scarce (vi) or absent (vii) melanocytes in skin partially or fully affected by vitiligo.

Figure 7. Activity of anti-PD-1 mAb in patient with metastatic NSCLC. A partial response is illustrated in a patient with metastatic NSCLC (nonsquamous histology) treated with 5C4 at 10 mg/kg. Arrows show initial progression in pulmonary lesions followed by regression ("immune-related" pattern of response).

Figures 8A-8B. Correlation between tumor PD-L1 expression and anti-PD-1 clinical response. Pretreatment tumor cell surface expression of PD-L1, as determined by IHC on formalin-fixed paraffin-embedded specimens, correlates with OR to PD-1 blockade. Forty-two subjects with advanced cancers including melanoma, non-small-cell

lung cancer, colorectal cancer, renal-cell cancer, and castration-resistant prostate cancer (n=18, 10, 7, 5, and 2, respectively) were studied. A, There was a significant correlation of tumor cell surface PD-L1 expression with objective clinical response. No patients with PD-L1 negative tumors experienced an OR. B, Examples of IHC analysis with the anti-PD-L1 mAb 5H1 are shown in a melanoma lymph node metastasis (top), a renal-cell cancer nephrectomy specimen (middle), and a lung adenocarcinoma brain metastasis (bottom). All 400X original magnification. Arrows indicate one of many tumor cells in each specimen with surface membrane staining for PD-L1. Asterisk indicates a normal glomerulus in the nephrectomy specimen, which is negative for PD-L1 staining.

Figure 9. Graphical comparison of the binding of mAbs 28-8 and 5H1 to PD-L1 antigen in tumor tissues by histoscore analysis.The rabbit mAb 28-8 showed higher histoscores in 7 out of 10 samples tested.

Figures 10A-9D. Spider plot showing activity of anti-PD-L1 mAb in patients with treatment-refractory MEL and NSCLC. Representative plots demonstrate the time course of target lesion tumor burden over time in patients with MEL treated with BMS-936559 at doses of 1 (A), 3 (B), 10 mg/kg (C) and in patients with NSCLC treated at 10 mg/kg (D). In the majority of patients who achieved ORs, responses were durable and were evident by the end of cycle 2 (3 months) of treatment, irrespective of dose or tumor type. Tumor regressions followed conventional as well as "immune-related" patterns of response.

Figure 11. Complete response in a patient with melanoma treated with BMS-936559 at 3 mg/kg. Circles indicate an initial increase in the size of pulmonary nodules at 6 weeks and 3 months followed by complete regression at 10 months ("immune-related" pattern of response).

Figure 12. Complete response in a patient with melanoma treated with BMS-936559 at 1 mg/kg. This patient developed an isolated brain metastasis 3 months after initiation of treatment that was successfully treated with stereotactic radiosurgery. A partial response in abdominal disease (circled) was noted at 8 months, with no evidence of disease at 15 months.

Figure 13. Partial response in a patient with NSCLC (non-squamous histology) treated with BMS-936559 at 10 mg/kg. Note the response in disease in right lung pleura and liver.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods for immunotherapy of a subject afflicted with diseases such as cancer or an infectious disease, which methods comprise administering to the subject a composition comprising a therapeutically effective amount of a compound or agent that potentiates an endogenous immune response, either stimulating the activation of the endogenous response or inhibiting the suppression of the endogenous response. More specifically, the invention provides methods for potentiating an endogenous immune response in a subject afflicted with cancer so as to thereby treat the patient, which method comprises administering to the subject a therapeutically effective amount of an Ab or an antigen-binding portion thereof that inhibits signaling from an inhibitory immunoregulator. In certain embodiments, the inhibitory immunoregulator is a component of the PD-1/PD-L1 signaling pathway. Accordingly, certain embodiments of the invention provide methods for immunotherapy of a subject afflicted with cancer, which methods comprise administering to the subject a therapeutically effective amount of an Ab or an antigen-binding portion thereof that disrupts the interaction between the PD-1 receptor and its ligand, PD-L1. In certain preferred embodiments, the Ab or antigen-binding portion thereof binds specifically to PD-1. In other preferred embodiments, the Ab or antigen-binding portion thereof binds specifically to PD-L1. In certain other embodiments, the subject is selected as suitable for immunotherapy in a method comprising measuring the surface expression of PD-L1 in a test tissue sample obtained from a patient with cancer of the tissue, for example, determining the proportion of cells in the test tissue sample that express PD-L1 on the cell surface, and selecting the patient for immunotherapy based on an assessment that PD-L1 is expressed on the surface of cells in the test tissue sample.

Terms

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.

"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. Preferred routes of administration for Abs of the invention 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. Alternatively, an Ab 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. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

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.

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 consant 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 Abs 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.

Antibodies typically bind specifically to their cognate antigen with high affinity, reflected by a dissociation constant (K_D) of 10^{-5} to 10^{-11} M^{-1} or less. Any K_D greater than about 10^{-4} M^{-1} is generally considered to indicate nonspecific binding. As used herein, an Ab that "binds specifically" to an antigen refers to an Ab that binds to the antigen and substantially identical antigens with high affinity, which means having a K_D of 10^{-7} M or less, preferably 10^{-8} M or less, even more preferably 5 x 10^{-9} M or less, and most preferably between 10^{-8} M and 10^{-10} M or less, but does not bind with high affinity to unrelated antigens. An antigen is "substantially identical" to a given antigen if it exhibits a high degree of sequence identity to the given antigen, for example, if it exhibits at least 80%, at least 90%, preferably at least 95%, more preferably at least 97%, or even more preferably at least 99% sequence identity to the sequence of the given antigen. By way of example, an Ab that binds specifically to human PD-1 may also have cross-reactivity with PD-1 antigens from certain primate species but may not cross-react with PD-1 antigens from certain rodent species or with an antigen other than PD-1, *e.g.*, a human PD-L1 antigen.

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 Ab class or subclass (*e.g.*, IgM or IgGl) 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 Abs; monoclonal and polyclonal Abs; chimeric and humanized Abs; human or nonhuman Abs; wholly synthetic Abs; and single chain Abs. A nonhuman Ab 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 Ab.

An "isolated antibody" refers to an Ab that is substantially free of other Abs having different antigenic specificities (*e.g.*, an isolated Ab that binds specifically to PD-1 is substantially free of Abs that bind specifically to antigens other than PD-1). An isolated

Ab that binds specifically to PD-1 may, however, have cross-reactivity to other antigens, such as PD-1 molecules from different species. Moreover, an isolated Ab may be substantially free of other cellular material and/or chemicals. By comparison, an "isolated" nucleic acid refers to a nucleic acid composition of matter that is markedly different, *i.e.*, has a distinctive chemical identity, nature and utility, from nucleic acids as they exist in nature. For example, an isolated DNA, unlike native DNA, is a free-standing portion of a native DNA and not an integral part of a larger structural complex, the chromosome, found in nature. Further, an isolated DNA, unlike native genomic DNA, can typically be used in applications or methods for which native genomic DNA is unsuited, *e.*g., as a PCR primer or a hybridization probe for, among other things, measuring gene expression and detecting biomarker genes or mutations for diagnosing disease or assessing the efficacy of a therapeutic. An isolated nucleic acid may be purified so as to be substantially free of other cellular components or other contaminants, *e.g.*, other cellular nucleic acids or proteins, using standard techniques well known in the art. Examples of isolated nucleic acids include fragments of genomic DNA, PCR-amplified DNA, cDNA and RNA.

The term "monoclonal antibody" ("mAb") refers to a preparation of Ab molecules of single molecular composition, *i.e.*, Ab 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 Ab. MAbs may be produced by hybridoma, recombinant, transgenic or other techniques known to those skilled in the art.

A "human" antibody (HuMAb) refers to an Ab having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the Ab contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The human Abs 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 Abs 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" Abs and "fully human" Abs and are used synonymously.

A "humanized" antibody refers to an Ab in which some, most or all of the amino

acids outside the CDR domains of a non-human Ab are replaced with corresponding amino acids derived from human immunoglobulins. In one embodiment of a humanized form of an Ab, 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 Ab to bind to a particular antigen. A "humanized" Ab retains an antigenic specificity similar to that of the original Ab.

A "chimeric antibody" refers to an Ab in which the variable regions are derived from one species and the constant regions are derived from another species, such as an Ab in which the variable regions are derived from a mouse Ab and the constant regions are derived from a human Ab.

An "antigen-binding portion" of an Ab (also called an "antigen-binding fragment") refers to one or more fragments of an Ab that retain the ability to bind specifically to the antigen bound by the whole Ab.

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 divide and grow 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.

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 Abs, 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.

An "immunoregulator" refers to a substance, an agent, a signaling pathway or a component thereof that regulates an immune response. "Regulating," "modifying" or "modulating" an immune response refers to any alteration in a cell of the immune system or in the activity of such cell. Such regulation includes stimulation or suppression of the immune system which may be manifested by an increase or decrease in the number of

various cell types, an increase or decrease in the activity of these cells, or any other changes which can occur within the immune system. Both inhibitory and stimulatory immunoregulators have been identified, some of which may have enhanced function in the cancer microenvironment.

The term "immunotherapy" refers to the treatment of a subject afflicted with, or at risk of contracting or suffering a recurrence of, a disease by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response. "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, condition or biochemical indicia associated with a disease.

"Potentiating an endogenous immune response" means increasing the effectiveness or potency of an existing immune response in a subject. This increase in effectiveness and potency may be achieved, for example, by overcoming mechanisms that suppress the endogenous host immune response or by stimulating mechanisms that enhance the endogenous host immune response.

A "predetermined threshold value," 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 being positive for cell surface PD-L1 expression. For cell surface expression assayed by IHC with the mAb 28-8, the predetermined threshold value for cells expressing PD-L1 on the cell surface ranges from at least about 0.01% to at least about 20% of the total number of cells. In preferred embodiments, the predetermined threshold value for cells expressing PD-L1 on the cell surface ranges from at least about 0.1% to at least about 10% of the total number of cells. More preferably, the predetermined threshold value is at least about 5%. Even more preferably, the predetermined threshold value is at least about 1%.

The "Programmed Death-1 (PD-1)" receptor 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.

"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 analogs having at least one common epitope with hPD-L1. The complete hPD-L1 sequence can be found under GenBank Accession No. Q9NZQ7.

A "signal transduction pathway" or "signaling 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 the cell. A "cell surface receptor" includes, for example, molecules and complexes of molecules that are located on the surface of a cell and are capable of receiving a signal and transmitting 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, which is located on the surface of activated B cells, activated T cells and myeloid cells, and transmits a signal that results in a decrease in tumor-infiltrating lymphocytes and a decrease in T cell proliferation. An "inhibitor" of signaling refers to a compound or agent that antagonizes or reduces the initiation, reception or transmission of a signal, be that signal stimulatory or inhibitory, by any component of a signaling pathway such as a receptor or its ligand.

A "subject" includes any human or nonhuman animal. The term "nonhuman animal" includes, but is not limited to, vertebrates such as nonhuman primates, sheep, dogs, cats, rabbits, ferrets, rodents such as mice, rats and guinea pigs, avian species such as chickens, amphibians, and reptiles. In preferred embodiments, the subject is a mammal such as a nonhuman primate, sheep, dog, cat, rabbit, ferret or rodent. In more preferred embodiments, the subject is a human. The terms, "subject," "patient" and "individual" are used interchangeably herein.

A "therapeutically effective amount" or "therapeutically effective dosage" of a drug or therapeutic agent, such as an Ab of the invention, 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.

By way of example, an anti-cancer agent promotes cancer regression in a subject. In preferred embodiments, a therapeutically effective amount of the drug promotes cancer regression to the point of eliminating the cancer. "Promoting cancer regression" means that administering an effective amount of the drug, alone or in combination with an anti-neoplastic agent, results in a reduction in tumor growth or size, necrosis of the tumor, a decrease in severity of at least one disease symptom, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. In addition, the terms "effective" and "effectiveness" with regard to a treatment includes both pharmacological effectiveness and physiological safety. Pharmacological effectiveness refers to the ability of the drug to promote cancer regression in the patient. Physiological safety refers to the level of toxicity, or other adverse physiological effects at the cellular, organ and/or organism level (adverse effects) resulting from administration of the drug.

By way of example for the treatment of tumors, a therapeutically effective amount of the drug 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. In other preferred embodiments of the invention, tumor regression may be observed and continue for a period of at least about 20 days, more preferably at least about 40 days, or even more preferably at least about 60 days. Notwithstanding these ultimate measurements of therapeutic effectiveness, evaluation of immunotherapeutic drugs must also make allowance for "immune-related" response patterns.

An "immune-related" response pattern refers to a clinical response pattern often observed in cancer patients treated with immunotherapeutic agents that produce antitumor effects by inducing cancer-specific immune responses or by modifying native immune processes. This response pattern is characterized by a beneficial therapeutic effect that follows an initial increase in tumor burden or the appearance of new lesions, which in the evaluation of traditional chemotherapeutic agents would be classified as disease progression and would be synonymous with drug failure. Accordingly, proper evaluation of immunotherapeutic agents may require long-term monitoring of the effects of these agents on the target disease.

A therapeutically effective amount of a drug includes a "prophylactically effective amount," which is any amount of the drug that, when administered alone or in combination with an anti-neoplastic agent to a subject at risk of developing a cancer (*e.g.*, a subject having a pre-malignant condition) or of suffering a recurrence of cancer, inhibits the development or recurrence of the cancer. In preferred embodiments, the prophylactically effective amount prevents the development or recurrence of the cancer entirely. "Inhibiting" the development or recurrence of a cancer means either lessening the likelihood of the cancer's development or recurrence, or preventing the development or recurrence of the cancer entirely.

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.

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.

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 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.

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.

Various aspects of the invention are described in further detail in the following subsections.

Antibodies of the Invention

Abs of the present invention include a variety of Abs having structural and functional properties described herein, including high-affinity binding to PD-1 or PD-L1, respectively. These Abs may be used, for example, as therapeutic Abs to treat subjects afflicted with disease or as reagents in diagnostic assays to detect their cognate antigens. Human mAbs (HuMAbs) that 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) with high affinity have been disclosed in U.S. Patent No. 8,008,449, and HuMAbs that bind specifically to PD-L1 with high affinity have been disclosed in U.S. Patent No. 7,943,743. The Abs of the invention include, but are not limited to, all of the anti-PD-1 and anti-PD-L1 Abs disclosed in U.S. Patent Nos. 8,008,449 and 7,943,743, respectively. Other anti-PD-1 mAbs have been described in, for example, U.S. Patent Nos. 7,488,802 and 8,168,757, and anti-PD-L1 mAbs have been described in, for example, U.S. Patent Nos. 7,635,757 and 8,217,149, and U.S. Publication No. 2009/0317368. To the extent these anti-PD-1 and anti-PD-L1 mAbs exhibit the structural and functional properties of antibodies of the invention, they too are included as antibodies of the invention.

Anti-PD-1 Antibodies of the Invention

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 Ab responses; and (j) inhibits tumor cell growth *in vivo*. Anti-PD-1 Abs of the present invention include mAbs that bind specifically to human PD-1 and exhibit at least one, preferably at least five, of the preceding characteristics.

U.S. Patent No. 8,008,449 exemplifies seven anti-PD-1 HuMAbs: 17D8, 2D3, 4H1, 5C4 (also referred to herein as nivolumab or BMS-936558), 4A11, 7D3 and 5F4. Isolated DNA molecules encoding the heavy and light chain variable regions of these Abs

have been sequenced, from which the amino acid sequences of the variable regions were deduced. The V_H amino acid sequences of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are provided herein as 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 provided herein as SEQ ID NOs. 8, 9, 10, 11, 12, 13 and 14, respectively.

Preferred anti-PD-1 Abs of the present invention include the anti-PD-1 HuMAbs 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4. These preferred Abs bind specifically to human PD-1 and comprise: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 1 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 8; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 2 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 9; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 3 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 10; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 5 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 12; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 6 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 13; or (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 7 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 14.

Given that each of these Abs can bind to PD-1, the V_H and V_L sequences can be "mixed and matched" to create other anti-PD-1 Abs of the invention. PD-1 binding of such "mixed and matched" Abs can be tested using binding assays, *e.g.*, enzyme-linked immunosorbent assays (ELISAs), western blots, radioimmunoassays and Biacore analysis

that are well known in the art (*see, e.g.*, U.S. Patent No. 8,008,449). 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, anti-PD-1 Abs of the invention include an isolated mAb 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 Ab specifically binds PD-1, preferably human PD-1.

The CDR domains of the above Abs have been delineated using the Kabat system, and these Abs may also be defined by combinations of their 3 heavy chain and 3 light chain CDRs (*see* U.S. Patent No. 8,008,449). Since each of these Abs can bind to PD-1 and antigen-binding specificity is provided primarily by the CDR1, CDR2, and CDR3 regions, the V_H CDR1, CDR2, and CDR3 sequences and V_κ CDR1, CDR2, and CDR3 sequences can be "mixed and matched" (*i.e.*, CDRs from different Abs can be mixed and match, although each Ab must contain a V_H CDR1, CDR2, and CDR3 and a V_κ CDR1, CDR2, and CDR3) to create other anti-PD-1 Abs that also constitute Abs of the invention. PD-1 binding of such "mixed and matched" Abs can be tested using the binding assays described above (*e.g.*, ELISAs, western blots, radioimmunoassays and Biacore analysis).

Abs of the invention also include isolated Abs that bind specifically to PD-1 and comprise a heavy chain variable region derived from a particular germline heavy chain immunoglobulin and/or a light chain variable region derived from a particular germline light chain immunoglobulin. Specifically, in certain embodiments, Abs of the invention include isolated Abs comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-33 or 4-39 germline sequence, and/or a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6, or L15 germline sequence. The amino acid sequences of the V_H and V_κ regions encoded by the V_H 3-33, V_H 4-39, V_κ L6 and V_κ L15 germline genes are provided in U.S. Patent No. 8,008,449.

As used herein, an Ab can be identified as comprising a heavy or a light chain variable region that is "derived from" a particular human germline immunoglobulin by comparing the amino acid sequence of the human Ab to the amino acid sequences

encoded by human germline immunoglobulin genes, and selecting the human germline immunoglobulin sequence that is closest in sequence (*i.e.*, greatest percentage of sequence identity) to the sequence of the human Ab. A human Ab that is "derived from" a particular human germline immunoglobulin 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 Ab 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 Ab 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 Ab 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, the sequence of a human Ab 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 other embodiments, the human Ab may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid differences from the amino acid sequence encoded by the germline immunoglobulin gene.

Preferred Abs of the invention also include isolated Abs or antigen-binding portions thereof comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-33 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6 germline sequence; or (b) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 4-39 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L15 germline sequence. Examples of Abs having a V_H and a V_κ derived from V_H 3-33 and V_κ L6 germline sequences, respectively, include 17D8, 2D3, 4H1, 5C4, and 7D3. Examples of Abs having V_H and V_κ regions derived from V_H 4-39 and V_κ L15 germline sequences, respectively, include 4A11 and 5F4.

In yet other embodiments, anti-PD-1 Abs of the invention comprise heavy and light chain variable regions having amino acid sequences that are highly similar or

homologous to the amino acid sequences of the preferred anti-PD-1 Abs described herein, wherein the Ab retains the functional properties of the preferred anti-PD-1 Abs of the invention. For example, Abs of the invention include mAbs comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises consecutively linked amino acids having a sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs. 1, 2, 3, 4, 5, 6 and 7, and the light chain variable region comprises consecutively linked amino acids having a sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs. 8, 9, 10, 11, 12, 13 and 14. In other embodiments, the V_H and/or V_L amino acid sequences may exhibit at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the sequences set forth above.

As used herein, the percent sequence identity (also referred to as the percent sequence homology) between two sequences (amino acid or nucleotide sequences) is a function of the number of identical positions shared by the sequences relative to the length of the sequences compared (*i.e.*, % identity = number of identical positions/total number of positions being compared x 100), taking into account the number of any gaps, and the length of each such gap, introduced to maximize the degree of sequence identity between the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using mathematical algorithms that are well know to those of ordinary skill in the art (*see, e.g.*, U.S. Patent No. 8,008,449).

Antibodies having very similar amino acid sequences are likely to have essentially the same functional properties where the sequence differences are conservative modifications. As used herein, "conservative sequence modifications" refer to amino acid modifications that do not significantly affect the binding characteristics of the Ab containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Thus, for example, one or more amino acid residues within the CDR regions of an Ab of the invention can be replaced with other amino acid residues from the same side chain family and the altered Ab can be tested for retained function using functional assays that are well known in the art. Accordingly, certain embodiments of the anti-PD-1 Abs of the invention comprise heavy and light chain variable regions each comprising CDR1, CDR2 and CDR3 domains, wherein one or more of these CDR domains comprise

consecutively linked amino acids having sequences that are the same as the CDR sequences of the preferred anti-PD-1 Abs described herein (*e.g.*, 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4), or conservative modifications thereof, and wherein the Abs retain the desired functional properties of the preferred anti-PD-1 Abs of the invention.

Further, it is well known in the art that the heavy chain CDR3 is the primary determinant of binding specificity and affinity of an Ab, and that multiple Abs can predictably be generated having the same binding characteristics based on a common CDR3 sequence (*see, e.g.*, Klimka *et al.*, 2000; Beiboer *et al.*, 2000; Rader *et al.*, 1998; Barbas *et al.*, 1994; Barbas *et al.*, 1995; Ditzel *et al.*, 1996; Berezov *et al.*, 2001; Igarashi *et al.*, 1995; Bourgeois *et al.*, 1998; Levi *et al.*, 1993; Polymenis and Stoller, 1994; and Xu and Davis, 2000). The foregoing publications demonstrate that, in general, once the heavy chain CDR3 sequence of a given Ab is defined, variability in the other five CDR sequences will not greatly affect the binding specificity of that Ab. Thus, Abs of the invention comprising 6 CDRs can be defined by specifying the sequence of the heavy chain CDR3 domain.

Anti-PD-1 Abs of the invention also include isolated Abs that bind specifically to human PD-1 and cross-compete for binding to human PD-1 with any of HuMAbs 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4. Thus, anti-PD-1 Abs of the invention include isolated Abs or antigen-binding portions thereof that cross-compete for binding to PD-1 with a reference Ab or a reference antigen-binding portion thereof comprising: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 1 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 8; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 2 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 9; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 3 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 10; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11; (e) a human heavy chain variable region

comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 5 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 12; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 6 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 13; or (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 7 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 14.

The ability of Abs to cross-compete for binding to an antigen indicates that these Abs bind to the same epitope region (*i.e.*, the same or an overlapping epitope) of the antigen and sterically hinder the binding of other cross-competing Abs to that particular epitope region. Thus, the ability of a test Ab to competitively inhibit the binding of, for example, 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4, to human PD-1 demonstrates that the test Ab binds to the same epitope region of human PD-1 as 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4, respectively. All isolated Abs that bind to the same epitope region of human PD-1 as does HuMAb 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4 are included among the Abs of the invention. These cross-competing Abs are expected to have very similar functional properties by virtue of their binding to the same epitope region of PD-1. For example, cross-competing anti-PD-1 mAbs 5C4, 2D3, 7D3, 4H1 and 17D8 have been shown to have similar functional properties (*see* U.S. Patent No. 8,008,449 at Examples 3-7). The higher the degree of cross-competition, the more similar will the functional properties be. Further, cross-competing Abs can be readily 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 Abs of the invention (*see, e.g.*, Examples 1 and 2). In preferred embodiments, the Abs that cross-compete for binding to human PD-1 with, or bind to the same epitope region of human PD-1 as, 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4 are mAbs, preferably chimeric Abs, or more preferably humanized or human Abs. Such human mAbs can be prepared and isolated as described in U.S. Patent No. 8,008,449. Data provided in Example 1 show that 5C4 or a Fab fragment thereof cross-competes with each of 2D3, 7D3, 4H1 or 17D8 for binding to hPD-1 expressed on the surface of a cell, indicating that all five anti-PD-1 mAbs bind to the same epitope

region of hPD-1 (Figures 1A-1C).

An anti-PD-1 Ab of the invention further can be prepared using an Ab having one or more of the V_H and/or V_L sequences disclosed herein as starting material to engineer a modified Ab, which modified Ab may have altered properties from the starting Ab. An Ab 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 Ab can be engineered by modifying residues within the constant region(s), for example, to alter the effector function(s) of the Ab. Specific modifications to Abs include CDR grafting, site-specific mutation of amino acid residues within the V_H and/or V_κ CDR1, CDR2 and/or CDR3 regions to thereby improve one or more binding properties (*e.g.*, affinity) of the Ab, site-specific mutation of amino acid residues within the V_H and/or V_κ framework regions to decrease the immunogenicity of the Ab, modifications within the Fc region, typically to alter one or more functional properties of the Ab, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity, and chemical modification such as pegylation or alteration in glycosylation patterns to increase or decrease the biological (*e.g.*, serum) half life of the Ab. Specific examples of such modifications and methods of engineering Abs are described in detail in U.S. Patent No. 8,008,449. Anti-PD-1 Abs of the invention include all such engineered Abs that bind specifically to human PD-1 and are obtained by modification of any of the above-described anti-PD-1 Abs.

Anti-PD-1 Abs of the invention also include antigen-binding portions of the above Abs. It has been amply demonstrated that the antigen-binding function of an Ab can be performed by fragments of a full-length Ab. Examples of binding fragments encompassed within the term "antigen-binding portion" of an Ab 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 Ab.

These fragments, obtained initially through proteolysis with enzymes such as papain and pepsin, have been subsequently engineered into monovalent and multivalent antigen-binding fragments. For example, 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 peptide 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 variable fragments (scFv). Divalent or bivalent scFvs (di-scFvs or bi-scFvs) can be engineered by linking two scFvs in within a single peptide chain known as a tandem scFv which contains two V_H and two V_L regions. ScFv dimers and higher multimers can also be created using linker peptides of fewer than 10 amino acids that are too short for the two variable regions to fold together, which forces the scFvs to dimerize and produce diabodies or form other multimers. Diabodies have been shown to bind to their cognate antigen with much higher affinity than the corresponding scFvs, having dissociation constants up to 40-fold lower than the K_D values for the scFvs. Very short linkers ($\leq$ 3 amino acids) lead to the formation of trivalent triabodies or tetravalent tetrabodies that exhibit even higher affinities for to their antigens than diabodies. Other variants include minibodies, which are scFv-C_{H3} dimers, and larger scFv-Fc fragments (scFv-C_{H2}-C_{H3} dimers), and even an isolated CDR may exhibit antigen-binding function. These Ab fragments are engineered using conventional recombinant techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are intact Abs. All of the above proteolytic and engineered fragments of Abs and related variants (*see* Hollinger and Hudson, 2005; Olafsen and Wu, 2010, for further details) are intended to be encompassed within the term "antigen-binding portion" of an Ab.

Anti-PD-L1 Antibodies of the Invention

Each of the anti-PD-L1 HuMAbs disclosed in U.S. Patent No. 7,943,743 has been demonstrated to exhibit one or more of the following characteristics (a) binds to human PD-L1 with a K_D of 1 x 10^{-7} M or less; (b) increases T-cell proliferation in a Mixed Lymphocyte Reaction (MLR) assay; (c) increase interferon-γ production in an MLR assay; (d) increase IL-2 secretion in an MLR assay; (e) stimulates Ab responses; (f) inhibits the binding of PD-L1 to PD-1; and (g) reverses the suppressive effect of T regulatory cells on T cell effector cells and/or dendritic cells. Anti-PD-L1 Abs of the present invention include mAbs that bind specifically to human PD-L1 and exhibit at least one, preferably at least four, of the preceding characteristics.

U.S. Patent No. 7,943,743 exemplifies ten anti-PD-1 HuMAbs: 3G10, 12A4 (also referred to herein as BMS-936559), 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4. Isolated DNA molecules encoding the heavy and light chain variable regions of these Abs have been sequenced, from which the amino acid sequences of the variable

regions were deduced. The V_H amino acid sequences of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4 are shown in SEQ ID NOs. 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24, respectively, whereas their V_L amino acid sequences are shown in SEQ ID NOs. 25, 26, 27, 28, 29, 30, 31, 32, 33 and 34, respectively.

Preferred anti-PD-L1 Abs of the present invention include the anti-PD-L1 HuMAbs 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4. These preferred Abs bind specifically to human PD-L1 and comprise: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 15 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 25; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 17 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 27; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 18 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 28; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 19 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 29; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 20 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 30; (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 21 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 31; (h) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 22 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 32; (i) a human heavy chain variable region comprising consecutively linked amino acids having

the sequence set forth in SEQ ID NO: 23 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 33; or (j) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 24 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 34.

Given that each of these Abs can bind to PD-L1, the V_H and V_L sequences can be "mixed and matched" to create other anti-PD-L1 Abs of the invention. PD-L1 binding of such "mixed and matched" Abs can be tested using binding assays *e.g.*, ELISAs, western blots, radioimmunoassays and Biacore analysis that are well known in the art (*see, e.g.*, U.S. Patent No. 7,943,743). 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, Abs of the invention also include a mAb, or antigen binding portion thereof, comprising a heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in any of SEQ ID NOs. 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24, and a light chain variable region comprising consecutively linked amino acids having the sequence set forth in any of SEQ ID NOs. 25, 26, 27, 28, 29, 30, 31, 32, 33 or 34, wherein the Ab binds specifically to PD-L1, preferably human PD-L1.

The CDR domains of the above anti-PD-L1 HuMAbs have been delineated using the Kabat system, and these Abs may also be defined by combinations of their 3 heavy chain and 3 light chain CDRs (*see* U.S. Patent No. 7,943,743). Since each of these Abs can bind to PD-L1 and antigen-binding specificity is provided primarily by the CDR1, CDR2, and CDR3 regions, the V_H CDR1, CDR2, and CDR3 sequences and V_κ CDR1, CDR2, and CDR3 sequences can be "mixed and matched" (*i.e.*, CDRs from different Abs can be mixed and match, although each Ab must contain a V_H CDR1, CDR2, and CDR3 and a V_κ CDR1, CDR2, and CDR3) to create other anti-PD-1 Abs that also constitute Abs of the invention. PD-L1 binding of such "mixed and matched" Abs can be tested using, for example, ELISAs, western blots, radioimmunoassays and Biacore analysis.

Antibodies of the invention also include Abs that bind specifically to PD-L1 and comprise a heavy chain variable region derived from a particular germline heavy chain immunoglobulin and/or a light chain variable region derived from a particular germline

light chain immunoglobulin. Specifically, in certain embodiments, Abs of the invention include Abs comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-18, 1-69, 1-3 or 3-9 germline sequence, and/or a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6, L15, A27 or L18 germline sequence. The amino acid sequences of the V_H and V_κ regions encoded by the V_H 1-18, V_H 1-3, V_H 1-69, V_H 3-9, V_κ L6, V_κ L15 and V_κ A27 germline genes are provided in U.S. Patent No. 7,943,743.

Preferred Abs of the invention include isolated Abs or antigen-binding portions thereof comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-18 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6 germline sequence; (b) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-69 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6 germline sequence; (c) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-3 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L15 germline sequence; (d) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-69 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ A27 germline sequence; (e) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-9 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L15germline sequence; or (f) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-9 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L18germline sequence.

An example of an Ab having a V_H and a V_κ derived from V_H 1-18 and V_κ L6 germline sequences, respectively, is 3G10. Examples of Abs having V_H and V_κ regions

derived from V_H 1-69 and V_κ L6 germline sequences, respectively, include 12A4, 1B12, 7H1 and 12B7. An example of an Ab having a V_H and a V_κ derived from V_H 1-3 and V_κ L15 germline sequences, respectively, is 10A5. Examples of Abs having V_H and V_κ regions derived from V_H 1-69 and V_κ A27 germline sequences, respectively, include 5F8, 11E6 and 11E6a. An example of an Ab having a V_H and a V_K derived from V_H 3-9 and V_κ L15 germline sequences, respectively, is 10H10. An example of an Ab having a V_H and a V_κ derived from V_H 1-3 and V_κ L15 germline sequences, respectively, is 10A5. An example of an Ab having a V_H and a V_κ derived from V_H 3-9 and V_κ L18 germline sequences, respectively, is 13G4.

In certain embodiments, anti-PD-L1 Abs of the invention comprise heavy and light chain variable regions having amino acid sequences that are highly similar or homologous to the amino acid sequences of the preferred anti-PD-L1 Abs described herein, wherein the Ab retains the functional properties of the aforementioned anti-PD-L1 Abs of the invention. For example, Abs of the invention include mAbs comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises consecutively linked amino acids having a sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs. 15, 16, 17, 18, 19, 20, 21, 22, 23, and 24, and the light chain variable region comprises consecutively linked amino acids having a sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs. 25, 26, 27, 28, 29, 30, 31, 32, 33, and 34. In other embodiments, the V_H and/or V_L amino acid sequences may exhibit at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the sequences set forth above.

Certain embodiments of the anti-PD-L1 Abs of the invention comprise heavy and light chain variable regions each comprising CDR1, CDR2 and CDR3 domains, wherein one or more of these CDR domains comprise consecutively linked amino acids having sequences that are the same as the CDR sequences of the preferred anti-PD-L1 Abs described herein (*e.g.*, 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 and 13G4), or conservative modifications thereof, and wherein the Abs retain the desired functional properties of the preferred anti-PD-L1 Abs of the invention.

On the basis of the evidence that the heavy chain CDR3 is the primary determinant of binding specificity and affinity of an Ab, it is generally true that once the heavy chain CDR3 sequence of a given Ab is defined, variability in the other five CDR

sequences will not greatly affect the binding specificity of that Ab. Accordingly, anti-PD-L1 Abs of the invention include isolated Abs comprising 6 CDRs, wherein the Abs are defined by specifying the sequence of the heavy chain CDR3 domain.

Anti-PD-L1 Abs of the invention also include isolated Abs that bind specifically to human PD-L1 and cross-compete for binding to human PD-L1 with any of HuMAbs 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 and 13G4. Thus, anti-PD-L1 Abs of the invention include isolated Abs or antigen-binding portions thereof that cross-compete for binding to PD-L1 with a reference Ab or a reference antigen-binding portion thereof comprising: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 15 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 25; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 17 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 27; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 18 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 28; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 19 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 29; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 20 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 30; (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 21 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 31; (h) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 22 and a human light chain variable region comprising consecutively linked amino acids having the sequence set

forth in SEQ ID NO: 32; (i) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 23 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 33; or (j) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 24 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 34.

The ability of an Ab to cross-compete with any of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 and 13G4 for binding to human PD-L1 demonstrates that such Ab binds to the same epitope region of each of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 and 13G4, respectively. All isolated Abs that bind to the same epitope region of human PD-L1 as does HuMAb 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 or 13G4 are included among the Abs of the invention. These cross-competing Abs are expected to have very similar functional properties by virtue of their binding to the same epitope region of PD-L1. For example, cross-competing anti-PD-L1 mAbs 3G10, 1B12, 13G4, 12A4 (BMS-936559), 10A5, 12B7, 11E6 and 5F8 have been shown to have similar functional properties (*see* U.S. Patent No. 7,943,743 at Examples 3-11), whereas mAb 10H10, which binds to a different epitope region, behaves differently (U.S. Patent No. 7,943,743 at Example 11). The higher the degree of cross-competition, the more similar will the functional properties be. Further, cross-competing Abs can be identified in standard PD-L1 binding assays, *e.g.*, Biacore analysis, ELISA assays or flow cytometry, that are well known to persons skilled in the art. In preferred embodiments, the Abs that cross-compete for binding to human PD-1 with, or bind to the same epitope region of human PD-L1 as, 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 or 13G4 are mAbs, preferably chimeric Abs, or more preferably humanized or human Abs.. Such human mAbs can be prepared and isolated as described in U.S. Patent No. 7,943,743.

Data provided in Example 2 show that each of the anti-PD-L1 HuMAbs 5F8, 7H1, 1B12, 3G10, 10A5, 11E6, 12A4, 12B7 and 13G4, *i.e.*, all of the HuMAbs tested except 10H10, substantially blocked binding of mAbs 3G10, 10A5, 11E6, 12A4 and 13G4 to Chinese Hamster Ovary (CHO) cells expressing PD-L1 cells. HuMAb 10H10 substantially blocked the binding only of itself to CHO/PD-L1 cells. These data show that 3G10, 10A5, 11E6, 12A4 and 13G4 cross-compete with all of the HuMAbs tested, except

for 10H10, for binding to the same epitope region of human PD-L1 (Figures 2A-F).

Data provided in Example 3 show that the binding of HuMAb 12A4 to ES-2 ovarian carcinoma cells expressing PD-L1 cells was substantially blocked by 12A4 itself and by 1B12 and 12B7, and was moderately to significantly blocked by mAbs 5F8, 10A5, 13G4 and 3G10, but was not blocked by mAb 10H10. These data, largely consistent with the data in Example 2, show that 12A4 itself, and 2 other HuMabs, 12B7 and 1B12, substantially cross-compete with 12A4 for binding to the same epitope region, possibly the same epitope, of human PD-L1; 5F8, 10A5, 13G4 and 3G10, exhibit a significant but lower level of cross-competition with 12A4, suggesting that these mAbs may bind to epitopes that overlap the 12A4 epitope; whereas 10H10 does not cross-compete at all with 12A4 (Figure 3), suggesting that this mAb binds to a different epitope region from 12A4.

Anti-PD-L1 Abs of the invention also include Abs engineered starting from Abs having one or more of the V_H and/or V_L sequences disclosed herein, which engineered Abs may have altered properties from the starting Abs. An anti-PD-L1 Ab can be engineered by a variety of modifications as described above for the engineering of modified anti-PD-1 Abs of the invention.

Anti-PD-L1 Abs of the invention also include isolated Abs 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. Yet, studies on measuring PD-L1 expression have often been conducted on frozen specimens because of the difficulty in isolating anti-human PD-L1 Abs that can be used to stain PD-L1 in FFPE specimens by IHC in general (Hamanishi *et al.*, 2007) and, in particular, Abs that bind specifically to membranous PD-L1 in these tissues. The use of different Abs to stain PD-L1 in frozen versus FFPE tissues, and the ability of certain Abs 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.*, 2007; Gadiot et al., 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.

Rabbit and mouse anti-hPD-L1 mAbs were produced as described in the Examples. Out of a total of one hundred and eighty-five rabbit Ab multiclones and

purified mouse subclones screened, only ten rabbit multiclone Abs specifically detected the membranous form of PD-L1, and the top five multiclones (designated Nos. 13, 20, 28, 29 and 49) were subsequently subcloned. The lead 15 rabbit subclones were purified for confirmatory screening. The clone that produced the most robust detection specifically of membranous PD-L1, rabbit clone 28-8, was selected for the IHC assays. The sequences of the variable regions of mAb 28-8 are set forth in SEQ ID NOs. 35 and 36, respectively. Rabbit clones 28-1, 28-12, 29-8 and 20-12 were the next best mAbs in terms of robust detection of membranous PD-L1 in FFPE tissues.

Anti-PD-L1 Abs of the invention also include antigen-binding portions of the above Abs, including Fab, F(ab')$_2$ Fd, Fv, and scFv, di-scFv or bi-scFv, and scFv-Fc fragments, diabodies, triabodies, tetrabodies, and isolated CDRs (*see* Hollinger and Hudson, 2005; Olafsen and Wu, 2010, for further details).

Nucleic Acid Molecules Encoding Antibodies of the Invention

Another aspect of the present disclosure pertains to isolated nucleic acid molecules that encode any of the Abs of the invention. These nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. 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.

Nucleic acids of the invention can be obtained using standard molecular biology techniques. For Abs 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 Ab made by the hybridoma can be obtained by standard PCR amplification or cDNA cloning techniques. Nucleic acids encoding Abs obtained from an immunoglobulin gene library (*e.g.*, using phage display techniques) can be recovered from the library.

Preferred nucleic acid molecules of the invention are those encoding the V_H and V_κ sequences of the anti-PD-1 HuMAbs, 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 (disclosed in U.S. Patent No. 8,008,449), and those encoding the V_H and V_κ sequences of the anti-PD-L1 HuMAbs, 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4 (disclosed in U.S. Patent No. 7,943,743). An isolated DNA encoding the V_H region can be converted to a full-length heavy chain gene by operatively linking the V_H-encoding DNA to another DNA molecule encoding heavy chain constant regions (C_{H1}, C_{H2} and C_{H3}), the sequences of which are known in the art and 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. Similarly, an isolated DNA encoding the V_L region can be converted to a full-length light chain gene by operatively linking the V_L-encoding DNA to another DNA molecule encoding the light chain constant region (C_L), the sequence of which is known in the art and 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.

Pharmaceutical Compositions

Antibodies of the present invention may be constituted in a composition, *e.g.*, a pharmaceutical composition, containing one Ab or a combination of Abs, or an antigen-binding portion(s) thereof, and a pharmaceutically acceptable carrier. As used herein, a "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). A pharmaceutical composition of the invention may include one or more pharmaceutically acceptable salts, anti-oxidant, aqueous and nonaqueous carriers, and/or adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents.

Dosage regimens are adjusted to provide the optimum desired response, *e.g.*, a therapeutic response or minimal adverse effects. For administration of an anti-PD-1 or anti-PD-L1 Ab, the dosage ranges from about 0.0001 to about 100 mg/kg, usually from about 0.001 to about 20 mg/kg, and more usually from about 0.01 to about 10 mg/kg, of the subject's body weight. Preferably, the dosage is within the range of 0.1-10 mg/kg body weight. For example, dosages can be 0.1, 0.3, 1, 3, 5 or 10 mg/kg body weight, and more preferably, 0.3, 1, 3, or 10 mg/kg body weight. The dosing schedule is typically designed to achieve exposures that result in sustained receptor occupancy (RO) based on typical pharmacokinetic properties of an Ab. 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. The dosage and scheduling may change during a course of treatment. For example, dosing schedule may comprise administering the Ab: (i) every two weeks in 6-week cycles; (ii)

every four weeks for six dosages, then every three months; (iii) every three weeks; (iv) 3-10 mg/kg body weight once followed by 1 mg/kg body weight every 2-3 weeks. Considering that an IgG4 Ab typically has a half-life of 2-3 weeks, a preferred dosage regimen for an anti-PD-1 or anti-PD-L1 Ab of the invention comprises 0.3-10 mg/kg body weight, preferably 3-10 mg/kg body weight, more preferably 3 mg/kg body weight via intravenous administration, with the Ab being given every 14 days in up to 6-week or 12-week cycles until complete response or confirmed progressive disease.

In some methods, two or more mAbs with different binding specificities are administered simultaneously, in which case the dosage of each Ab administered falls within the ranges indicated. Antibody is usually administered on multiple occasions. Intervals between single dosages can be, for example, weekly, every 2 weeks, every 3 weeks, monthly, every three months or yearly. Intervals can also be irregular as indicated by measuring blood levels of Ab to the target antigen in the patient. In some methods, dosage is adjusted to achieve a plasma Ab concentration of about 1-1000 μg/ml and in some methods about 25-300 μg/ml.

Alternatively, the Ab 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 Ab in the patient. In general, human Abs show the longest half-life, followed by humanized Abs, chimeric Abs, and nonhuman Abs. 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 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.

Uses and Methods of the Invention

The Abs, Ab compositions, nucleic acids and methods of the present invention have numerous *in vitro* and *in vivo* utilities including, for example, methods to determine and quantify the expression of PD-1 or PD-L1 comprising binding of the Abs to the target polypeptides or measuring the amount of nucleic acid encoding these polypeptides, and a method for immunotherapy of a subject afflicted with a disease comprising administering to the subject a composition comprising a therapeutically effective amount of a therapeutic agent that inhibits signaling from an inhibitory immunoregulator. In preferred embodiments of the latter method, the inhibitory immunoregulator is a component of the PD-1/PD-L1 signaling pathway, and the therapeutic agent disrupts signaling of this pathway. More preferably, the therapeutic agent is an Ab that interferes with the interaction between PD-1 and PD-L1. In certain preferred embodiments of this method, the Ab binds specifically to PD-1 and blocks the interaction of PD-1 with PD-L1 and/or PD-L2. In other preferred embodiments, the therapeutic agent is an Ab that binds specifically to PD-L1 and blocks the interaction of PD-L1 with PD-1 and/or B7-1 (CD80). Thus, the invention provides methods for potentiating an immune response in a subject comprising administering an anti- PD-1 and/or an anti-PD-L1 Ab in order to disrupt the interaction between PD-1 and PD-L1, and methods of treating diseases mediated by such a potentiation of the immune response. When Abs to PD-1 and PD-L1 are administered together, the two can be administered sequentially in either order or simultaneously. In certain aspects, this disclosure provides methods of modifying an immune response in a subject comprising administering to the subject an anti-PD-1 and/or an anti-PD-L1 Ab of the invention, or antigen-binding portion thereof, such that the immune response in the subject is modified. Preferably, the immune response is potentiated, enhanced, stimulated or up-regulated. In preferred embodiments, the Abs of

the present invention are human Abs.

Preferred subjects include human patients in need of enhancement of an immune response. The immunotherapeutic methods disclosed herein are particularly suitable for treating human patients having a disorder that can be treated by potentiating a T-cell mediated immune response. In certain embodiments, the methods are employed for treatment of subjects afflicted with a disease caused by an infectious agent. In preferred embodiments, the methods are employed for treatment of subjects afflicted with, or at risk of being afflicted with, a cancer.

Cancer Immunotherapy

Blockade of PD-1/PD-L1 interaction has been shown to potentiate immune responses *in vitro* (U.S. Patent Nos. 8,008,449 and 7,943,743; Fife *et al.*, 2009) and mediate preclinical antitumor activity (Dong *et al.*, 2002; Iwai *et al.*, 2002). However, the molecular interactions potentially blocked by these two Abs are not identical: anti-PD-1 Abs of the invention disrupt PD-1/PD-L1 and potentially PD-1/PD-L2 interactions; in contrast, whereas anti-PD-L1 Abs of the invention also disrupt PD-1/PD-L1 interactions, they do not block PD-1/PD-L2 interactions but instead may disrupt the PD-1-independent PD-L1/CD80 interaction, which has also been shown to down-modulate T-cell responses *in vitro* and *in vivo* (Park *et al.*, 2010; Paterson *et al.*, 2011; Yang *et al.*, 2011; Butte *et al.*, 2007; Butte *et al.*, 2008). Thus, it is possible that among these varied ligand-receptor pairings, different interactions may dominate in different cancer types, contributing to dissimilar activity profiles for the two Abs.

Disruption of the PD-1/PD-L1 interaction by antagonistic Abs can enhance the immune response to cancerous cells in a patient. PD-L1 is not expressed in normal human cells, but is abundant in a variety of human cancers (Dong *et al.*, 2002). The interaction between PD-1 and PD-L1 impairs T cell responses as manifested by a decrease in tumor-infiltrating lymphocytes (TILs) and a decrease in T-cell receptor mediated proliferation, resulting in T cell anergy, exhaustion or apoptosis, and immune evasion by the cancerous cells (Zou and Chen, 2008; Blank *et al.*, 2005; Konishi *et al.*, 2004; Dong *et al.*, 2003; Iwai *et al.*, 2002). Immune suppression can be reversed by inhibiting the local interaction between PD-L1 and PD-1 using an anti-PD-1 and/or an anti-PD-L1 Ab. These Abs may be used alone or in combination to inhibit the growth of cancerous tumors. In addition, either or both of these Abs may be used in conjunction with other immunogenic agents including cytokines, standard cancer chemotherapies,

vaccines, radiation, surgery, or other Abs.

Immunotherapy of Cancer Patients Using an Anti-PD-1 Antibody

This disclosure provides a method for immunotherapy of a subject afflicted with cancer, which method comprises administering to the subject a composition comprising a therapeutically effective amount of an Ab or an antigen-binding portion thereof that disrupts the interaction of PD-1 with PD-L1 and/or PD-L2. The invention also provides a method of inhibiting growth of tumor cells in a subject, comprising administering to the subject an Ab or an antigen-binding portion thereof that disrupts the interaction of PD-1 with PD-L1 and/or PD-L2 in an amount effective to inhibit growth of the tumor cells. In preferred embodiments, the subject is a human. In other preferred embodiments, the Ab or antigen-binding portion thereof is an anti-PD-1 Ab of the invention or an antigen-binding portion thereof. In certain embodiments, the Ab or antigen-binding portion thereof is of an IgG1 or IgG4 isotype. In certain embodiments, the Ab or antigen-binding portion thereof is a mAb or an antigen-binding portion thereof. In certain other embodiments, the Ab or antigen-binding portion thereof is a chimeric, humanized or human Ab or an antigen-binding portion thereof. In preferred embodiments for treating human subjects, the Ab or antigen-binding portion thereof is a human Ab or an antigen-binding portion thereof.

The clinical trials described in the Examples employed the anti-PD-1 HuMAb, nivolumab (designated 5C4 in U.S. Patent No. 8,008,449), to treat cancer. While 5C4 was selected as the lead Ab for entering the clinic, it is notable that several anti-PD-1 Abs of the invention share with 5C4 functional properties that are important to the therapeutic activity of 5C4, including high affinity binding specifically to human PD-1, increasing T-cell proliferation, IL-2 secretion and interferon-γ production in an MLR assay, inhibiting the binding of PD-L1 and/or PD-L2 to PD-1, and inhibiting tumor cell growth *in vivo*. Moreover, certain of the anti-PD-1 Abs of the invention, 17D8, 2D3, 4H1 and 7D3 are structurally related to 5C4 in comprising V_H and a V_κ regions that have sequences derived from V_H 3-33 and V_κ L6 germline sequences, respectively. In addition, 5C4, 2D3, 7D3, 4H1 and 17D8 all cross-compete for binding to the same epitope region of hPD-1 (Example 1). Thus, the preclinical characterization of nivolumab and other anti-PD-1 HuMabs indicate that the methods of treating cancer provided herein may be performed using different Abs selected from the broad genus of anti-PD-1 Abs of the invention.

Accordingly, certain embodiments of the immunotherapy methods disclosed

herein comprise administering to a patient an anti-PD-1 Ab or antigen-binding portion thereof comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-33 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6 germline sequence, or (b) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 4-39 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L15 germline sequence.

In certain other embodiments, the Ab or antigen-binding portion thereof that is administered to the patient cross-competes for binding to PD-1 with a reference Ab or a reference antigen-binding portion thereof comprising: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 1 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 8; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 2 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 9; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 3 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 10; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 5 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 12; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 6 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 13; or (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 7 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 14. In preferred

embodiments, the Ab or antigen-binding portion thereof cross-competes for binding to PD-1 with nivolumab.

In certain preferred embodiments of the immunotherapy methods disclosed herein, the anti-PD-1 Ab or antigen-binding portion thereof administered to the patient comprises: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 1 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 8; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 2 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 9; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 3 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 10; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 5 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 12; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 6 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 13; or (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 7 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 14. In more preferred embodiments, the anti-PD-1 Ab or antigen-binding portion comprises a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11. In yet more preferred embodiments, the anti-PD-1 Ab is nivolumab.

In the clinical trials of anti-PD-1 immunotherapy described in the Examples below, intriguing ORs with durable clinical responses, even in heavily pretreated patients,

were observed across multiple tumor types including a substantial proportion of NSCLC, MEL, and RCC patients and in various sites of metastasis including liver, lung, lymph nodes, and bone. *See, also*, Topalian *et al.* (2012b). MEL and RCC are considered to be immunogenic neoplasms, having previously been demonstrated to be responsive to cancer immunotherapy, *e.g.*, IL-2 (McDermott and Atkins, 2006) and/or anti-CTLA-4 Ab (Hodi *et al.*, 2010). In contrast, NSCLC has been considered "non-immunogenic" and poorly responsive to immune-based therapies (Holt *et al.*, 2011). Thus, the results with NSCLC are particularly striking, unexpected and surprising. In NSCLC patients, based on data analyzed up to February 2012, 14 ORs were observed at BMS-936558 doses of 1, 3, or 10 mg/kg with response rates of 6%, 32%, and 18%, respectively. ORs were observed across NSCLC histologies: 6 responders of 18 squamous (33%), 7 of 56 nonsquamous (13%), and 1 of 2 unknown. This level of activity seen with anti-PD-1 in NSCLC patients with significant prior therapy (47% with 3 lines of previous therapy) and across histologies is unique, particularly in the squamous histology patients (*cf.* Gridelli *et al.*, 2008; Miller, 2006), and provides a very favorable benefit/risk dynamic regarding efficacy and safety compared to existing standard-of-care.

Durability of clinical responses in heavily-pretreated cancer patients

The durability of ORs across multiple cancer types in patients treated with the anti-PD-1 Ab is particularly notable. The objective response rate (ORR) in heavily pretreated NSCLC patients receiving anti-PD-1 Ab, including patients with squamous histology, is particularly surprising and unexpected, as standard salvage therapies historically show modest benefit in these patients (Scagliotti *et al.*, 2011). As measured by standard RECIST in this study, ORs were long-lasting, with response durations ≥ 1 year in 20 of 31 responders in the data analyzed up to February 2012. In addition, patterns of tumor regression consistent with immune-related patterns of response were observed.

These findings have established the PD-1 pathway as a new therapeutic focus in oncology (Pardoll, 2012; Topalian *et al.*, 2012c; McDermott and Atkins, 2013). In the current study, in which 47% of patients had progressive disease following 3 or more prior systemic regimens, preliminary analysis has been conducted up to March 2013. This updated analysis has supported and reinforced the data obtained and conclusions reached from the earlier February 2012 analyses. Thus, conventional ORs were documented in patients with NSCLC (16%), MEL (31%) and RCC (29%), and prolonged disease stabilization in others (9%, 6%, 27%, respectively) across all doses tested (*see* Example 7,

Table 2). Additionally, 13 patients (4%) manifested unconventional, "immune-related" response patterns as previously described with anti-CTLA-4 therapy, several of which were sustained (Sharma *et al.*, 2011). The updated analyses again underscored the durability of survival in nivolumab-treated patients, which has not been observed with chemotherapy or small molecule inhibitors to date, but has been observed in patients with advanced melanoma receiving ipilimumab, another immune checkpoint blocking agent (Hodi *et al.*, 2010).

Of particular importance, the objective tumor regression and disease stabilization induced by nivolumab in heavily-pretreated patients with advanced NSCLC, MEL, and RCC translate to survival outcomes that compare very favorably with historical data for these patient populations treated with conventional chemotherapy and/or tyrosine-kinase inhibitor (TKI) treatments. In NSCLC, nivolumab induced median overall survivals of 9.6 and 9.2 months in patients with squamous and non-squamous histologies, respectively. Landmark survival rates of 43% (1-year), 32% (2-year) and 24% (3-year) were achieved (*see* Example 7, Table 2). This high level of efficacy is especially impressive since 55% of these patients had received 3 or more prior therapies. Historically, 2L chemotherapeutics for lung cancer (*i.e.*, docetaxel and pemetrexed) have achieved a median overall survival of 7.5-8.3 months (Shepherd *et al.*, 2000; Hanna *et al.*, 2004). In a 2/3-L population, erlotinib-treated patients had a median survival of 6.7 months, versus 4.7 months in placebo-treated patients (Shepherd *et al.*, 2005). No therapy is currently approved for use in lung cancer beyond the 3L setting, and minimal data exist to benchmark survival in this patient population.

In nivolumab-treated MEL patients, overall survival (OS) of 16.8 months was achieved, with landmark survival rates of 61% (1-year), 44% (2-year) and 40% (3-year) (*see* Example 7, Table 2). Survival outcomes in pretreated melanoma patients supported the recent PDA approvals of ipilimumab and vemurafenib. In patients with at least one prior treatment for metastatic disease, ipilimumab increased median OS from 6.4 to 10.1 months, compared to a gp100 peptide vaccine (Hodi *et al.*, 2010). In phase 2 trials of ipilimumab in previously treated patients, landmark 2-year survival rates ranged from 24.2-32.8% (Lebbe *et al.*, 2012). Median OS in previously treated MEL patients enrolled on a large phase 2 of vemurafenib was 15.9 months (Sosman *et al.*, 2012).

In nivolumab-treated patients with RCC, among whom 44% received 3 or more prior therapies and 74% received prior anti-angiogenic therapy, the median OS has not

been achieved and exceeds 22 months. Landmark survival rates of 70% (1-year), 52% (2-year) and 52% (3-year) were achieved (*see* Example 7, Table 2). In a recent Phase 3 trial enrolling kidney cancer patients whose disease progressed following anti-angiogenic therapy, everolimus was compared with placebo: median OS was 14.8 versus 14.4 months, respectively (Motzer *et al*., 2008; Motzer *et al*., 2008). A recent Phase 3 trial comparing sorafenib to temsirolimus in a sunitinib-refractory kidney cancer population yielded median OS of 16.6 and12.3 months, respectively (Hutson *et al*., 2012). Thus, treatment of a heavily-pretreated patient population with nivolumab has yielded a considerably longer median OS (> 22 months) than treatment of a less refractory population with standard-of-care chemotherapies. Controlled Phase 3 trials with prospective survival endpoints are underway in NSCLC, MEL and RCC (NCT01673867, NCT01721772, NCT01642004, NCT01668784, and NCT01721746 (*see* Clinical Trials Website). The results from these trials are expected to further demonstrate the high efficacy of, and durability of responses to, nivolumab in these cancers compared standard-of-care therapies.

The data disclosed herein demonstrating the high efficacy, durability and broad applicability of anti-PD-1 immunotherapy for treating cancer has led to nivolumab being tested for additional types of cancer. For example, on the basis that increased PD-L1 expression has been reported with various hematologic malignancies and may prevent the host immune response from exerting a beneficial impact on the malignant cells, a trial to confirm the ability of nivolumab to mediate antitumor activity in patients with hematologic malignancies (multiple myeloma, B-cell lymphoma, T-cell lymphoma, Hodgkin's lymphoma/primary mediastinal B-cell lymphoma, and chronic myelogenous leukemia) has been initiated (NCT01592370). Nivolumab is also being tested as a monotherapy in advanced hepatocellular carcinoma (NCT01658878).

In summary, the results of anti-PD-1 immunotherapy disclosed herein are remarkable in at least the following three respects. First, anti-PD-1 has been shown to be highly efficacious compared to historical data for patients on standard-of-care treatments for cancer. Notably, this efficacy has been demonstrated in patient in heavily pretreated populations in which about half of the patients had progressive disease following 3 or more prior systemic regimens. Such patients, afflicted with advanced, metastatic and/or refractory cancers, are notoriously difficult to treat. Accordingly, this disclosure provides methods for immunotherapy of a patient afflicted with an advanced, metastatic and/or

refractory cancer, which method comprises administering to the patient a therapeutically effective amount of an Ab or an antigen-binding portion thereof that disrupts the interaction of PD-1 with PD-L1 and/or PD-L2. In certain embodiments of any of the therapeutic methods disclosed herein, the subject has been pre-treated for the cancer; for example, the subject had undergone at least one, two, or three prior lines of therapy for cancer.

Second, the present therapeutic methods have been shown to be applicable to a broad genus of different cancers. Based on the surprising discovery that even a "non-immunogenic" cancer such as NSCLC (Holt *et al.*, 2011) and a hard-to-treat cancers such as ovarian and gastric cancers (as well as other cancers tested, including MEL, RCC, and CRC) are amendable to treatment with anti-PD-1 and/or anti-PD-L1 (*see* Examples 7 and 14), this disclosure provides methods for immunotherapy of a patient afflicted with any cancer.

Third, treatment with an anti-PD-1 or anti-PD-L1 Ab has been shown to produce strikingly durable clinical activity in cancer patients. Accordingly, this disclosure provides immunotherapeutic methods of inducing a durable clinical response in a cancer patient comprising administering to the patient a therapeutically effective amount of an Ab or an antigen-binding portion thereof that disrupts the interaction of PD-1 with PD-L1 and/or PD-L2.. In preferred embodiments of any of the therapeutic methods described herein, the clinical response is a durable response.

As used herein, a "durable" response is a therapeutic or clinical response that exceeds the anticipated median OS rate in a patient population. The anticipated median OS rate varies with different cancer and different patient populations. In certain embodiments, a durable response exceeds the anticipated median OS rate in the relevant patient population by at least 10%, preferably by at least 20%, more preferably by at least 30%, and even more preferably by at least 50%. A major benefit of immunotherapeutic approaches based on PD-1 pathway blockade may be the functional restoration of exhausted T cells with long-term generation of memory T cells that may maintain antitumor immune surveillance and inhibit tumor growth for prolonged periods extending to many years, even in the absence of continued therapy (Kim and Ahmed, 2010). Indeed, long-term follow-up studies on patients following cessation of nivolumab therapy have confirmed that a patient with CRC experienced a complete response which was ongoing after 3 years; a patient with RCC experienced a partial response lasting 3 years off

therapy, which converted to a complete response that was ongoing at 12 months; and a patient with melanoma achieved a partial response that was stable for 16 months off therapy, and recurrent disease was successfully treated with reinduction anti-PD-1 therapy (Lipson *et al.*, 2013).

Combination therapy with anti-PD-1 Abs

While monotherapy with anti-PD-1 and anti-PD-L1 Abs has been shown herein to significantly increase the survival of patients with lung cancer, melanoma, kidney cancer, and potentially other malignancies, preclinical evidence indicates that synergistic treatment combinations based on PD-1 pathway blockade could have even more potent effects. Clinical evaluation of nivolumab combined with ipilimumab (anti-CTLA-4), whose mechanism of action is similar yet distinct from nivolumab's (Mellman *et al.*, 2011; Topalian *et al.*, 2012c), is ongoing (Wolchok *et al.*, 2013), as are studies of nivolumab in combination with melanoma vaccines (NCT01176461, NCT01176474; Weber *et al.*, 2013), and BMS-986015, an anti-KIR Ab (NCT01714739).

Anti-PD-1 Abs can be combined with an immunogenic agent, for example a preparation of cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), antigen-presenting cells such as dendritic cells bearing tumor-associated antigens, and/or cells transfected with genes encoding immune stimulating cytokines (He *et al.*, 2004). 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. PD-1 blockade may also be effectively combined with standard cancer treatments, including chemotherapeutic regimes, radiation, surgery, hormone deprivation and angiogenesis inhibitors, as well as another immunotherapeutic Ab (*e.g.*, an anti-PD-L1, anti-CTLA-4 and/or anti-LAG-3 Ab).

Immune-related clinical responses

It has become evident that conventional response criteria may not adequately assess the activity of immunotherapeutic agents because progressive disease (by initial radiographic evaluation) does not necessarily reflect therapeutic failure. For example, treatment with the anti-CTLA-4 Ab, ipilimumab, has been shown to produce four distinct response patterns, all of which were associated with favorable survival: (a) shrinkage in baseline lesions, without new lesions; (b) durable stable disease (in some patients followed by a slow, steady decline in total tumor burden); (c) response after an increase in

total tumor burden; and (d) response in the presence of new lesions. Accordingly, to properly evaluate immunotherapeutic agents, long-term effects on the target disease must also be captured. In this regard, systematic immune-related response criteria (irRC) that make allowances for an early increase in tumor burden and/or the appearance of new lesions, and which seek to enhance the characterization of immune-related response patterns, have been proposed (Wolchok *et al.*, 2009). While the full impact of these unconventional response patterns remains to be defined in randomized trials of nivolumab with survival endpoints, the present observations are reminiscent of findings with ipilimumab in which a significant extension of OS was observed in treated patients (Hodi *et al.*, 2010; Robert *et al.*, 2011).

The overall risk/benefit profile of anti-PD-1 immunotherapy is also favorable, with a low incidence of more severe drug-related adverse events (AEs; > grade 3), the specific events observed to date being consistent with other immunotherapeutic agents. This suggests that anti-PD-1 immunotherapy can be delivered in an outpatient setting with minimal supportive care.

Broad spectrum of cancers treatable by immunotherapy

The clinical data presented herein demonstrate that immunotherapy based on PD-1 blockade is not limited to only "immunogenic" tumor types, such as MEL and RCC, but extends to tumor types not generally considered to be immune-responsive, including NSCLC. The unexpected successes with treatment-refractory metastatic NSCLC underscore the possibility that any neoplasm can be "immunogenic" in the context of proper immune modulation, and suggest that PD-1 blockade as an immunotherapeutic approach is broadly applicable across a very diverse range of tumor types. Cancers that may be treated using the anti-PD-1 Abs of the invention also include cancers typically responsive to immunotherapy. Non-limiting examples of preferred cancers for treatment include NSCLC, MEL, RCC, CRC, CRPC, HCC, squamous cell carcinoma of the head and neck, carcinomas of the esophagus, ovary, gastrointestinal tract and breast, and a hematologic malignancy. Although NSCLC is not generally considered responsive to immunotherapy, data disclosed herein unexpectedly demonstrate that both squamous and non-squamous NSCLC are responsive to treatment with an anti-PD-1 Ab. Additionally, the disclosure provides for the treatment of refractory or recurrent malignancies whose growth may be inhibited using an anti-PD-1 Ab of the invention.

Examples of other cancers that may be treated using an anti-PD-1 Ab in the methods of the present invention, based on the indications of very broad applicability of anti-PD-1 immunotherapy provided herein, 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 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 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 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.

One aspect of this invention is the use of any anti-PD-1 Ab or antigen-binding portion thereof of the invention for the preparation of a medicament for inhibiting signaling from the PD-1/PD-L1 pathway to thereby potentiate an endogenous immune response in a subject afflicted with cancer. Another aspect is the use of any anti-PD-1 Ab or an antigen-binding portion thereof of the invention for the preparation of a medicament for immunotherapy of a subject afflicted with cancer comprising disrupting the interaction between PD-1 and PD-L1. These uses for the preparation of medicaments are broadly applicable to the full range of cancers disclosed herein. In preferred embodiments of these uses, the cancers include squamous NSCLC, non-squamous NSCLC, MEL,

RCC, CRC, CRPC, HCC, squamous cell carcinoma of the head and neck, and carcinomas of the esophagus, ovary, gastrointestinal tract and breast, and a hematologic malignancy. This invention also provides medical uses of any anti-PD-1 Ab or antigen-binding portion thereof of the invention corresponding to all the embodiments of the methods of treatment employing an anti-PD-1 Ab described herein.

The invention also provides an anti-PD-1 Ab or an antigen-binding portion thereof of the invention for use in potentiating an endogenous immune response in a subject afflicted with cancer by inhibiting signaling from the PD-1/PD-L1 pathway. The invention further provides an anti-PD-1 Ab or an antigen-binding portion thereof of the invention for use in immunotherapy of a subject afflicted with cancer comprising disrupting the interaction between PD-1 and PD-L1. These Abs may be used in potentiating an endogenous immune response against, or in immunotherapy of, the full range of cancers disclosed herein. In preferred embodiments, the cancers include squamous NSCLC, non-squamous NSCLC, MEL, RCC, CRC, CRPC, HCC, squamous cell carcinoma of the head and neck, and carcinomas of the esophagus, ovary, gastrointestinal tract and breast, and a hematologic malignancy.

Immunotherapy of Cancer Patients Using an Anti-PD-L1 Antibody

PD-L1 is the primary PD-1 ligand up-regulated within solid tumors, where it can inhibit cytokine production and the cytolytic activity of PD-1-positive, tumor-infiltrating CD4-positive and CD8-positive T-cells, respectively (Dong *et al.*, 2002; Hino *et al.*, 2010; Taube *et al.*, 2012). These properties make PD-L1 a promising target for cancer immunotherapy. The clinical trials of anti-PD-L1 immunotherapy described in the Examples demonstrate for the first time that mAb blockade of the immune inhibitory ligand, PD-L1, produces both durable tumor regression and prolonged (≥ 24 weeks) disease stabilization in patients with metastatic NSCLC, MEL, RCC and OV, including those with extensive prior therapy. The human anti-PD-L1 HuMAb, BMS-936559, had a favorable safety profile overall at doses up to and including 10 mg/kg, as is evident from the low (9%) incidence of grade 3-4 drug-related AEs. These findings are consistent with the mild autoimmune phenotype seen in PD-L1$^{-/-}$ mice (Dong *et al.*, 2004) and the more severe hyperproliferation seen in CTLA-4$^{-/-}$ mice relative to PD-1$^{-/-}$ mice (Phan *et al.*, 2003; Tivol *et al.*, 1995; Nishimura *et al.*, 1999). Most of the toxicities associated with anti-PD-L1 administration in patients were immune-related, suggesting on-target effects. The spectrum and frequency of adverse events of special interest (AEOSIs) is somewhat

different between anti-PD-L1 and anti-CTLA-4, emphasizing the distinct biology of these pathways (Ribas *et al.*, 2005). Infusion reactions were observed with BMS-936559, although they were mild in most patients. Severe colitis, a drug-related AE observed in ipilimumab-treated patients (Beck *et al.*, 2006), was infrequently noted with anti-PD-L1.

As noted above for anti-PD-1 immunotherapy, another important feature of anti-PD-L1 therapy is the durability of responses across multiple tumor types. This is particularly notable considering the advanced disease and prior treatment of patients on the current study. Although not compared directly, this durability appears greater than that observed with most chemotherapies and kinase inhibitors used in these diseases.

Because peripheral blood T-cells express PD-L1, it is possible to assess *in vivo* RO by BMS-963559 as a pharmacodynamic measure. Median RO was 65.8%, 66.2%, and 72.4% for the doses tested. Whereas these studies provide a direct assessment and evidence of target engagement in patients treated with BMS-936559, relationships between RO in peripheral blood and the tumor microenvironment remain poorly understood.

Based on the clinical data disclosed herein, this invention provides a method for immunotherapy of a subject afflicted with cancer, which method comprises administering to the subject a composition comprising a therapeutically effective amount of an anti-PD-L1 Ab of the invention or an antigen-binding portion thereof. The disclosure also provides a method of inhibiting growth of tumor cells in a subject, comprising administering to the subject an anti-PD-L1 Ab of the invention or an antigen-binding portion thereof. In preferred embodiments, the subject is a human. In certain embodiments, the Ab or antigen-binding portion thereof is of an IgG1 or IgG4 isotype. In certain embodiments, the Ab or antigen-binding portion thereof is a mAb or an antigen-binding portion thereof. In certain other embodiments, the Ab or antigen-binding portion thereof is a chimeric, humanized or human Ab or an antigen-binding portion thereof. In preferred embodiments for treating human subjects, the Ab or antigen-binding portion thereof is a human Ab or an antigen-binding portion thereof.

Clinical trials described in the Examples employed the anti-PD-L1 HuMAb BMS-936559 to treat cancer. While BMS-936559 (designated HuMAb 12A4 in U.S. Patent No. 7,943,743) was selected as the lead anti-PD-L1 Ab for entering the clinic, it is notable that several anti-PD-L1 Abs of the invention share with 12A4 functional properties that are important to the therapeutic activity of 12A4, including high affinity binding

specifically to human PD-L1, increasing T-cell proliferation, IL-2 secretion and interferon-γ production in an MLR assay, inhibiting the binding of PD-L1 to PD-1, and reversing the suppressive effect of T regulatory cells on T cell effector cells and/or dendritic cells. Moreover, certain of the anti-PD-L1 Abs of the invention, namely 1B12, 7H1 and 12B7 are structurally related to 12A4 in comprising V_H and a V_κ regions that have sequences derived from V_H 1-69 and V_κ L6 germline sequences, respectively. In addition, at least 12B7, 3G10, 1B12 and 13G4 cross-compete with 12A4 for binding to the same epitope region of hPD-L1, whereas 5F8 and 10A5 may bind to the same or an overlapping epitope region as 12A4 (Examples 2 and 3). Thus, the preclinical characterization of 12A4 and other anti-PD-L1 HuMabs indicate that the methods of treating cancer provided herein may be performed using any of the broad genus of anti-PD-L1 Abs of the invention.

Accordingly, this invention provides immunotherapy methods comprising administering to a patient an anti-PD-L1 Ab or antigen-binding portion thereof comprising (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-18 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6 germline sequence; (b) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-69 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6 germline sequence; (c) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-3 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L15 germline sequence; (d) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-69 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ A27 germline sequence; (e) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-9 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L15germline sequence; or (f) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-9

germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L18germline sequence.

In certain other embodiments, the anti-PD-L1 Ab or antigen-binding portion thereof administered to the patient cross-competes for binding to PD-L1 with a reference Ab or a reference antigen-binding portion thereof comprising: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 15 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 25; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 17 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 27; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 18 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 28; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 19 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 29; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 20 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 30; (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 21 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 31; (h) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 22 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 32; (i) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 23 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID

NO: 33; or (j) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 24 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 34. In preferred embodiments, the Ab or antigen-binding portion thereof cross-competes for binding to PD-1 with a reference Ab or reference antigen-binding portion thereof comprising a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26.

In certain preferred embodiments of the immunotherapy methods disclosed herein, the anti-PD-L1 Ab or antigen-binding portion thereof administered to the subject comprises: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 15 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 25; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 17 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 27; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 18 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 28; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 19 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 29; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 20 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 30; (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 21 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ

ID NO: 31; (h) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 22 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 32; (i) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 23 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 33; or (j) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 24 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 34. In more preferred embodiments, the anti-PD-L1 Ab or antigen-binding portion comprises a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26.

The clinical activity of anti-PD-L1 in patients with advanced NSCLC, similar to the activity of anti-PD-1 in these patients, was surprising and unexpected since NSCLC has been considered to be poorly responsive to immune-based therapies (Holt *et al.*, 2011). The present clinical data obtained with BMS-936559, an anti-PD-L1 Ab of the invention, substantiate and extend the evidence obtained using the anti-PD-1 Ab that immunotherapy based on PD-1 blockade is not applicable only to "immunogenic" tumor types, such as MEL and RCC, but is also effective with a broad range of cancers, including treatment-refractory metastatic NSCLC, that are generally not considered to be immune-responsive. Preferred cancers that may be treated using the anti-PD-L1 Abs of the invention include MEL (*e.g.*, metastatic malignant melanoma), RCC, squamous NSCLC, non-squamous NSCLC, CRC, ovarian cancer (OV), gastric cancer (GC), breast cancer (BC), pancreatic carcinoma (PC) and carcinoma of the esophagus. Additionally, the invention includes refractory or recurrent malignancies whose growth may be inhibited using the anti-PD-L1 Abs of the invention.

Examples of other cancers that may be treated using an anti-PD-L1 Ab in the methods of the invention, based on the indications of very broad applicability of anti-PD-L1 immunotherapy provided herein, include bone cancer, skin cancer, cancer of the head or neck, breast cancer, lung cancer, cutaneous or intraocular malignant melanoma, renal cancer, uterine cancer, castration-resistant prostate cancer, colon 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, carcinomas of the ovary, gastrointestinal tract and breast, 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, multiple myeloma, environmentally induced cancers including those induced by asbestos, metastatic cancers, and any combinations of said cancers. The present invention is also applicable to treatment of metastatic cancers.

Optionally, Abs to PD-L1 can be combined with an immunogenic agent, for example a preparation of cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), antigen-presenting cells such as dendritic cells bearing tumor-associated antigens, and cells transfected with genes encoding immune stimulating cytokines (He *et al.*, 2004). 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. PD-1 blockade may also be effectively combined with standard cancer treatments, including chemotherapeutic regimes, radiation, surgery, hormone deprivation and angiogenesis inhibitors, as well as another immunotherapeutic Ab (*e.g.*, an anti-PD-1, anti-CTLA-4 or anti-LAG-3 Ab).

This invention provides the use of any anti-PD-L1 Ab or antigen-binding portion thereof of the invention for the preparation of a medicament for inhibiting signaling from the PD-1/PD-L1 pathway to thereby potentiate an endogenous immune response in a subject afflicted with cancer. The invention also provides the use of any anti-PD-L1 Ab or antigen-binding portion thereof of the invention for the preparation of a medicament for immunotherapy of a subject afflicted with cancer comprising disrupting the interaction between PD-1 and PD-L1. The invention provides medical uses of any anti-

PD-L1 Ab or antigen-binding portion thereof of the invention corresponding to all the embodiments of the methods of treatment employing an anti-PD-L1 Ab described herein.

This invention also provides an anti-PD-L1 Ab or an antigen-binding portion thereof of the invention for use in potentiating an endogenous immune response in a subject afflicted with cancer by inhibiting signaling from the PD-1/PD-L1 pathway. The invention further provides an anti-PD-L1 Ab or an antigen-binding portion thereof of the invention for use in immunotherapy of a subject afflicted with cancer comprising disrupting the interaction between PD-1 and PD-L1. These Abs may be used in potentiating an endogenous immune response against, or in immunotherapy of, the full range of cancers disclosed herein. In preferred embodiments, the cancers include MEL (*e.g.*, metastatic malignant MEL), RCC, squamous NSCLC, non-squamous NSCLC, CRC, ovarian cancer (OV), gastric cancer (GC), breast cancer (BC), pancreatic carcinoma (PC) and carcinoma of the esophagus.

Validation of Cancer Immunotherapy by Immune Checkpoint Blockade

A major implication of the clinical activity of immune checkpoint blockade is that significant endogenous immune responses to tumor antigens are generated and these responses may be harnessed therapeutically to mediate clinical tumor regression upon checkpoint inhibition. In fact, there is evidence that inhibitory ligands such as PD-L1 are induced in response to immune attack, a mechanism termed adaptive resistance (Gajewski *et al.*, 2010; Taube *et al.*, 2012). This potential mechanism of immune resistance by tumors suggests that PD-1/PD-L1-directed therapy might synergize with other treatments that enhance endogenous antitumor immunity. Follow-up studies have verified that patients continue to demonstrate tumor control after cessation of PD-1/PD-L1 pathway blockade (Lipson *et al.*, 2013). Such tumor control may reflect a persistent antitumor immune response and the generation of effective immunologic memory to enable sustained control of tumor growth.

The data disclosed herein on the clinical testing of Abs that block the immunoregulatory receptor, PD-1, and also of Abs that block one of its cognate ligands, PD-L1, are unprecedented. These data constitute the largest clinical experience to date with PD-1 pathway-directed cancer immunotherapy, and the first report specifically describing the safety, tolerability, and initial clinical activity of an anti-PD-L1-directed agent. These findings show that both anti-PD-1 and anti-PD-L1 have favorable overall safety profiles and provide clear evidence of clinical activity across diverse cancers,

including NSCLC, a tumor not historically considered responsive to immunotherapy, as well as tumors known to respond to immunotherapy, including MEL, RCC and OV. Thus, these data strongly validate the PD-1/PD-L1 pathway as an important target for therapeutic intervention in cancer.

The remarkable similarities observed between the patterns of clinical activity obtained with the anti-PD-1 and anti-PD-L1 mAbs, and among the tumor types analyzed to date, validate the general importance of the PD-1/PD-L1 signaling pathway in tumor immune resistance and as a target for therapeutic intervention. Although the molecular interactions blocked by these two Abs are not identical, it has been clearly demonstrated herein that, irrespective of mechanistic details, both anti-PD-1 and anti-PD-L1 Abs of the invention are effective in treating patients afflicted with a wide variety of cancers, including "immunogenic" cancers such as MEL and RCC as well as treatment-refractory metastatic NSCLC, a tumor that is generally not considered to be immune-responsive. In certain embodiments of the invention, either or both of these Abs can be administered in combination with another therapeutic agent such as a cytokine.

Infectious Diseases

Other methods of the invention are used to treat patients that have been exposed to particular toxins or pathogens. For example, another aspect of the invention provides a method of treating an infectious disease in a subject comprising administering to the subject an anti-PD1 or an anti-PD-L1 Ab, or antigen-binding portion thereof, of the invention such that the subject is treated for the infectious disease. Preferably, the Ab is a human anti-human PD-1 or PD-L1 Ab (such as any of the human Abs described herein). Alternatively, the Ab is a chimeric or humanized Ab.

Similar to its application to tumors as discussed above, Ab-mediated PD-1 or PD-L1 blockade can be used alone, or as an adjuvant, in combination with vaccines, to potentiate an immune response to pathogens, toxins, and/or 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, and C), Influenza, Herpes, Giardia, Malaria, Leishmania, Staphylococcus aureus, Pseudomonas aeruginosa. PD-1 and/or PD-L1 blockade is particularly useful against established infections by agents such as HIV that present altered antigens over the course of an infection. Novel epitopes on these antigens are

recognized as foreign at the time of anti-human PD-1 or PD-L1 administration, thus provoking a strong T cell response that is not dampened by negative signals through the PD-1/PD-L1 pathway.

In the above methods, PD-1 or PD-L1 blockade can be combined with other forms of immunotherapy such as cytokine treatment (*e.g.*, administration of interferons, GM-CSF, G-CSF or IL-2).

Kits

Also within the scope of the present invention are kits, including pharmaceutical kits, comprising an anti-PD-1 and/or an anti-PD-L1 Ab of the invention for therapeutic uses, and diagnostic kits comprising an anti-PD-L1 Ab of the invention for assaying membranous PD-L1 expression as a biomarker for screening patients for immunotherapy or for predicting the efficacy of an immunotherapeutic agent. 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 and/or anti-PD-L1 Abs may be co-packaged with other therapeutic agents in unit dosage form. In certain embodiments of a diagnostic kit, the anti-PD-L1 Ab may be co-packaged with other reagents for performing an assay to detect and/or quantify PD-L1 expression.

In certain preferred embodiments, the pharmaceutical kit comprises the anti-human PD-1 HuMAb, nivolumab. In other preferred embodiments, the pharmaceutical kit comprises the anti-human PD-L1 HuMAb, BMS-936559. In certain preferred embodiments, the diagnostic kit comprises the rabbit anti-human PD-L1 mAb, 28-8, comprising the V_H and V_κ regions whose amino acid sequences are set forth in SEQ ID NOs. 35 and 36, respectively. In other preferred embodiments, the diagnostic kit comprises the murine anti-human PD-L1 mAb, 5H1 (Dong *et al.*, 2002).

PD-L1 Biomarker for Predicting anti-PD-1 Efficacy

A particular challenge in cancer immunotherapy has been the identification of mechanism-based predictive biomarkers to enable patient selection and guide on-treatment management. Data disclosed in the Examples below indicate that cell surface PD-L1 expression in tumors is a useful molecular marker for predicting the efficacy of, and selecting patients for, anti-PD-1 immunotherapy.

There are conflicting reports in the literature about the clinical implications of PD-L1 being expressed in tumors. Several studies have concluded that PD-L1 expression in tumors correlates with a poor prognosis for the patient. *See, e.g.*, Hino *et al.* (2010) (MEL); Hamanishi *et al.* (2007) (OV); Thompson *et al.* (2006) (RCC). These findings may be rationalized on the basis that the interaction of PD-L1 on tumor cells and PD-1 on T cells helps abrogate immune responses directed against the tumor, resulting in immune evasion from tumor-specific T cells. However, in contrast to the foregoing studies, Gadiot *et al.* (2011) and Taube *et al.* (2012) have recently reported that PD-L1 expression in melanoma tumors correlates with a trend toward better survival. These seemingly contradictory data may reflect the relatively small numbers of patients analyzed, different histologic subtypes studied, or different methodologies used, *e.g.*, the use of different Abs to stain PD-L1, the use of frozen versus paraffin-embedded material for IHC, and the detection of membranous and/or cytoplasmic staining of PD-L1. Taube *et al.* (2012) note that PD-L1 is a type I transmembrane molecule, and hypothesize that while the cytoplasmic presence of PD-L1 may represent intracellular stores of this polypeptide that may be deployed to the cell surface upon appropriate stimulation, it is cell surface PD-L1 expression of that is biologically relevant as a potential biomarker for predicting clinical response to PD-1 blockade. *See, also*, Brahmer *et al.* (2010), which describes preliminary evidence, obtained on a small sample size of only 9 patients, of a correlation between membranous PD-L1 expression and anti-PD-1 efficacy. The data described in the Examples below on the use of membranous PD-L1 expression as a biomarker for anti-PD-1 efficacy, which was obtained from analysis of a much larger sample, substantiate the hypothesis that PD-L1 expression may be used as a biomarker for predicting anti-PD-1 clinical response and for screening patients to identify suitable candidates for immunotherapy with an anti-PD-1 Ab or other inhibitors of inhibitory immunoregulators.

Specifically, membranous PD-L1 expression was assayed using an automated IHC protocol and a rabbit anti-hPD-L1 Ab. Strikingly, in the initial set of data analyzed (*see* Example 8), no patients with cell surface PD-L1-negative tumors (MEL, NSCLC, CRC, RCC and CRPC) experienced an OR following treatment with the anti-PD-1 Ab, nivolumab. In contrast, cell surface expression of PD-L1 on tumor cells in pretreatment biopsies may be associated with an increased rate of OR among patients treated with nivolumab. While tumor cell expression of PD-L1 may be driven by constitutive oncogenic pathways, it may also reflect "adaptive immune resistance" in response to an

endogenous antitumor immune response, part of a host inflammatory response, which may remain in check unless unleashed by blockade of the PD-1/PD-L1 pathway (Taube *et al.*, 2012). This emerging concept of adaptive immune resistance in cancer immunology suggests that inhibitory ligands such as PD-L1 are induced in response to immune attack (Gajewski *et al.*, 2010; Taube *et al.*, 2012). A major implication of the clinical activity of immune checkpoint blockade as described herein is that significant endogenous immune responses to tumor antigens are generated and these responses may be harnessed therapeutically to mediate clinical tumor regression upon checkpoint inhibition. This potential mechanism of immune resistance by tumors suggests that PD-1/PD-L1-directed therapy might synergize with other treatments that enhance endogenous antitumor immunity. It also suggests that cell surface expression of PD-L1 in tumors and/or inflammatory cells in the tumor microenvironment may be a marker not just for treatment of cancer patients with an anti-PD-1 Ab, but also for treatment with an anti-PD-L1 Ab as well as treatments targeting inhibitory immunoregulatory pathways other than the PD-1/PD-L1 pathway.

Assaying Cell-Surface PD-L1 Expression by Automated IHC

As described in the Examples, an automated IHC method was developed for assaying the expression of PD-L1 on the surface of cells in FFPE tissue specimens. The invention 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 Ab or portion thereof and human PD-L1. Preferably, 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.

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 post-primary blocking agent; incubating with NovoLink Polymer; adding a chromogen substrate and developing; and counterstaining with hematoxylin.

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%.

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.

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.

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)]

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).

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.*, 2012).

Cancer Immunotherapy with Anti-PD-1 Comprising a Patient Selection Step

This invention also provides a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is a suitable candidate for immunotherapy, the selecting comprising (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells, (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface, and (iii) selecting the subject as a suitable candidate based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and (b) administering to the selected subject a composition comprising a therapeutically effective amount of an agent that inhibits signaling from an inhibitory immunoregulator.

There is evidence that membranous PD-L1 expression is a surrogate for an endogenous antitumor immune response that is part of a host inflammatory response (Gajewski *et al.*, 2010; Taube *et al.*, 2012). Accordingly, cell surface expression of PD-L1 in tumors and/or tumor-infiltrating inflammatory cells is a marker not just for selecting cancer patients who would benefit from treatment with an anti-PD-1 Ab, but also for treatment with an anti-PD-L1 Ab as well as treatments targeting inhibitory immunoregulatory pathways other than the PD-1/PD-L1 pathway. For example, cell surface expression of PD-L1 in tumors and/or tumor-infiltrating inflammatory cells may be used as a marker for identifying or selecting suitable cancer patients who would benefit from immunotherapy with agents, including Abs, that target, and disrupt or inhibit signaling from, inhibitory immunoregulators such as PD-L1, Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4), B and T Lymphocyte Attenuator (BTLA), T cell Immunoglobulin

and Mucin domain-3 (TIM-3), Lymphocyte Activation Gene-3 (LAG-3), Killer cell Lectin-like Receptor G1 (KLRG-1), Natural Killer Cell Receptor 2B4 (CD244), and CD160 (Baitsch et al., 2012). In certain preferred embodiments, the inhibitory immunoregulator is a component of the PD-1/PD-L1 signaling pathway. In other preferred embodiments, the inhibitory immunoregulator is an anti-PD-1 Ab of the invention. In yet other preferred embodiments, the inhibitory immunoregulator is an anti-PD-L1 Ab of the invention.

Although many of the immunotherapy methods below comprising assaying PD-L1 expression, *i.e.*, employing a PD-L1 expression biomarker, are described as comprising the selection of a patient that is, or is not, suitable for anti-PD-1 immunotherapy or as comprising the administration of an anti-PD-1 Ab for immunotherapeutic purposes, it should be understood that these methods apply generally to the selection of a patient that is, or is not, suitable for immunotherapy with, or to the administration of an inhibitor of an inhibitory immunoregulator or a component or ligand thereof. Further, in any the methods comprising the measurement of PD-L1 expression in a test tissue sample, 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 preferred embodiments the "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 medical practitioner for use in selecting a suitable candidate for immunotherapy and/or administering an immunotherapeutic agent to the patient. In certain embodiments, the steps that provide the intermediate result may be performed by a medical practitioner or someone acting under the direction of a medical practitioner. In other embodiment, these steps are performed by an independent person or laboratory.

The invention further provides a method for treatment of a subject afflicted with

cancer, which method comprises: (a) selecting a subject that is not suitable for treatment with an agent that inhibits an inhibitory immunoregulator, *e.g.*, anti-PD-1 Ab immunotherapy, the selecting comprising (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the surface of the cells; and (iii) selecting the subject as not suitable for immunotherapy with an inhibitor of an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is less than a predetermined threshold level; and (b) administering a standard-of-care therapeutic other than an inhibitor of an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, to the selected subject.

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 preferred embodiments, PD-L1 expression is assayed by IHC. Flow cytometry may be particularly suitable for assaying PD-L1 expression in cells of hematologic tumors. In preferred embodiments of all of these methods, cell surface expression of PD-L1 is assayed using, *e.g.*, IHC or *in vivo* imaging.

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, 2010). Ab 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, 2010; Olafsen *et al.*, 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 certain preferred 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 further embodiments, the IHC assay is performed using an anti-PD-L1 mAb to bind to the PD-L1 polypeptide.

An Ab may bind to an antigen in fresh tissues but completely fail to recognize the antigen in an FFPE tissue sample. This phenomenon, well known in the art, is thought to be due primarily to intra- and inter-molecular cross-linking of polypeptides induced by formalin fixation, which alters the epitope recognized by the Ab (Sompuram *et al.*, 2006). In addition, several factors known to influence staining in FFPE tissue, including variable time to fixation, inadequate fixation period, differences in fixative used, tissue processing, Ab clone and dilution, antigen retrieval, detection system, and interpretation of results using different threshold points are important variables that can affect tissue antigenicity and IHC measurements (Bordeaux *et al.*, 2010). In particular, a lack of anti-human PD-L1 Abs that stain PD-L1 in FFPE specimens has been noted in the art (Hamanishi *et al.*, 2007). Thus, the contradictory results reported by different groups on the implications of PD-L1 expression for prognosis of a tumor may, in part, reflect the differential abilities of anti-PD-L1 Abs used to detect PD-L1 polypeptide in FFPE tissue samples. Indeed, our analysis of five commercially available anti-hPD-L1 Abs shows that these Abs failed to distinguish FFPE cells expressing PD-L1 from cells that did not express PD-L1 (see Example 9, Table 6). Accordingly, in order to detect hPD-L1 on the surface of cells using an IHC assay on FFPE tissues, there is a need for anti-hPD-L1 Abs that bind specifically to cell surface-expressed PD-L1 in FFPE tissue samples.

This disclosure provides a mAb or an antigen-binding portion thereof that binds specifically to a cell surface-expressed PD-L1 antigen in a FFPE tissue sample. In preferred embodiments, the mAb or antigen-binding portion thereof does not bind to a

cytoplasmic PD-L1 polypeptide in the FFPE tissue sample or exhibits a very low level of background binding. In certain other embodiments, the presence or absence of binding specifically to a cell surface-expressed or a cytoplasmic PD-L1 polypeptide is detected by immunohistochemical staining. In certain preferred aspects of the invention, the mAb or antigen-binding portion is a rabbit Ab or a portion thereof. In other preferred embodiments, the mAb is the rabbit mAb designated 28-8, 28-1, 28-12, 29-8 or 20-12. In more preferred embodiments, the mAb is the rabbit mAb designated 28-8 or an antigen-binding portion thereof. In further embodiments, the mAb is an Ab comprising a heavy chain variable region (V_H) comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 35 and a light chain variable region (V_κ) comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 36. In other embodiments, the mAb comprises the CDR1, CDR2 and CDR3 domains in a V_H having the sequence set forth in SEQ ID NO: 35, and the CDR1, CDR2 and CDR3 domains in a V_κ having the sequence set forth in SEQ ID NO: 36.

It is known in the art that rabbit Abs have certain advantages over murine Abs. For example, rabbit Abs generally exhibit more diverse epitope recognition, improved immune response to small-size epitopes, and higher specificity and affinity compared to murine Abs (*see, e.g.*, Fischer *et al.*, 2008; Cheang *et al.*, 2006; Rossi *et al.*, 2005). For example, the rabbit's lower immune dominance and larger B-cell repertoire results in greater epitope recognition compared to murine Abs. Further, the high specificity and novel epitope recognition of rabbit antibodies translates to success with recognition of post-translational modifications (Epitomics, 2013). In addition, many protein targets relevant to signal transduction and disease are highly conserved between mice, rats and humans, and can therefore be recognized as self-antigens by a mouse or rat host, making them less immunogenic. This problem is avoided by generating Abs in rabbits. In addition, in applications in which two antigen-specific Abs are required, it is more convenient to have the Abs come from two different species. Thus, for example, it is easier to multiplex a rabbit Ab such as 28-8 with other Abs (likely to be murine Abs since the best immune marking Abs are murine Abs) that can mark immune cells that also express PD-L1 (*e.g.*, macrophages and lymphocytes). Thus, rabbit anti-hPD-L1 mAbs, *e.g.*, 28-8, are particularly suited to IHC assays for detecting surface-expressed PD-L1 in FFPE tissue samples and have distinct advantages over murine Abs, such as 5H1.

As described in Example 9, a large number (185) of antibody multiclones from

both rabbit and mouse immunizations were screened, and only ten rabbit Ab, but no mouse Ab, multiclones specifically detected the membranous form of hPD-L1. After further extensive screening by multiple rounds of IHC, 15 purified rabbit subclones were selected based on their specificity and intensity of staining (see Table 4). Following further characterization of the antibodies to determine their binding affinity and cross-competition by surface plasmon resonance, as well as screening by IHC on FFPE tissues, mAb 28-8 was selected as the Ab with the best combination of binding to membranous PDF-L1 with high affinity and specificity, and low background staining.

In certain aspects of this invention, the mAb or antigen-binding portion cross-competes with mouse mAb 5H1 for binding to PD-L1, which indicates that these antibodies bind to the same epitope region of PD-L1. In certain other aspects, the mAb or antigen-binding portion thereof does not cross-compete with mouse mAb 5H1 for binding to PD-L1, indicating that they do not bind to the same epitope region of PD-L1.

The invention also provides nucleic acids encoding all of the rabbit anti-hPD-L1 Abs or portions thereof disclosed herein.

The availability of rabbit Abs that bind with high affinity specifically to membranous PD-L1 in FFPE tissue specimens facilitates methods comprising a step of detecting PD-L1 polypeptide on the surface of cells in FFPE tissue samples. Accordingly, this disclosure also provides a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is a suitable candidate for immunotherapy, the selecting comprising: (i) optionally providing a FFPE test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface by IHC using a rabbit anti-human PD-L1 Ab, *e.g.*, mAb 28-8, to bind to the PD-L1; and (iii) selecting the subject as a suitable candidate based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and (b) administering a composition comprising a therapeutically effective amount of an anti-PD-1 Ab to the selected subject.

In certain embodiments of methods employing IHC to assay PD-L1 expression in FFPE tissues, an automated IHC assay is used. The automated IHC process is performed on an autostainer and comprises: (a) de-paraffinizing the FFPE sample with xylene and rehydrating the sample; (b) retrieving the antigen using a decloaking chamber; (c)

blocking nonspecifc protein binding sites by incubation with a Protein Block; (d) incubating the sample with a primary anti-PD-L1 Ab; (e) adding a polymeric horseradish peroxidase (HRP)-conjugated secondary Ab; (f) detecting the bound secondary Ab comprising staining with a 3,3'-diaminobenzidine (DAB) chromogen; and/or (g) counterstaining with hematoxylin. This automated IHC process has been optimized by minimization of the number of steps, optimization of incubation times, and selection of primary Abs, blocking and detection reagents that produce strong specific staining with a low level of background staining. In preferred embodiments of this automated IHC assay, the primary anti-PD-L1 Ab is rabbit mAb 28-8 or murine mAb 5H1. In certain embodiments of this invention, this IHC assay, and any other IHC assay described herein to measure PD-L1 expression, may be used as part of a method of immunotherapy. In other embodiments, any of the IHC methods described herein is used independently of any therapeutic process requiring the administration of a therapeutic, *i.e.*, solely as a diagnostic method to assay PD-L1 expression.

In certain embodiments any of the immunotherapy methods described herein, the Ab administered to the selected subject is any anti-PD-1 or anti-PD-L1 Ab or antigen-binding portion thereof of the invention. In certain preferred embodiments, the subject is a human. In other preferred embodiments, the Ab is a human Ab or antigen-binding portion thereof. In more preferred embodiments, the anti-PD-1 Ab is nivolumab and the anti-PD-L1 Ab is BMS-936559. In certain other embodiments, the anti-PD-1 Ab is an Ab or antigen-binding portion thereof that cross-competes with nivolumab for binding to PD-1, and the anti-PD-L1 Ab is an Ab or antigen-binding portion thereof that cross-competes with BMS-936559 for binding to PD-L1. In certain preferred embodiments, the cancer to be treated is selected from the group consisting MEL, RCC, squamous NSCLC, non-squamous NSCLC, CRC, castration-resistant prostate cancer CRPC, HCC, squamous cell carcinoma of the head and neck, carcinomas of the esophagus, ovary, gastrointestinal tract and breast, and a hematological malignancy.

In certain embodiments of the disclosed methods, the predetermined threshold is based on a proportion of (a) tumor cells, (b) tumor-infiltrating inflammatory cells, (c) particular tumor-infiltrating inflammatory cells, *e.g.*, TILs or macrophages, or (d) a combination of tumor cells and tumor-infiltrating inflammatory cells, in a test tissue sample that expresses PD-L1 on the cell surface. In certain embodiments, the predetermined threshold is at least 0.001% of tumor cells expressing membranous PD-L1

as determined by IHC. In other embodiments, the predetermined threshold is at least 0.01%, preferably at least 0.1%, more preferably at least 1% of tumor cells expressing membranous PD-L1, as determined by IHC. In certain embodiments, the predetermined threshold is at least 5% of tumor cells expressing membranous PD-L1 as determined by IHC. In certain embodiments, the predetermined threshold is at least 0.01%, at least 0.1%, at least 1%, or at least 5% of tumor cells expressing membranous PD-L1 as determined by IHC, and/or a single tumor-infiltrating inflammatory cell expressing membranous PD-L1 as determined by IHC. In certain other embodiments, the predetermined threshold is at least 0.01%, at least 0.1%, at least 1%, or at least 5% of a tumor-infiltrating inflammatory cell expressing membranous PD-L1 as determined by IHC. In certain other embodiments, the predetermined threshold is at least 0.01%, at least 0.1%, at least 1%, or at least 5% of a tumor-infiltrating lymphocyte expressing membranous PD-L1 as determined by IHC. In certain other embodiments, the predetermined threshold is at least 0.01%, at least 0.1%, at least 1%, or at least 5% of a tumor-infiltrating macrophage expressing membranous PD-L1 as determined by IHC. In yet other embodiments, the predetermined threshold is at least a single tumor cell or a single tumor-infiltrating inflammatory cell expressing membranous PD-L1 as determined by IHC. Preferably, PD-L1 expression is assayed by automated IHC using mAb 28-8 or 5H1 as the primary Ab.

This disclosure also provides a method for treatment of a subject afflicted with cancer, which method comprises: (a) screening a plurality of subjects to identify a subject that is not a suitable candidate for immunotherapy comprising the administration of an anti-PD-1 Ab to the subject, the screening comprising: (i) optionally providing test tissue samples from the plurality of subjects, the test tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue samples that express PD-L1 on the surface of the cells; and (iii) selecting the subject as a candidate that is not suitable for anti-PD-1 Ab immunotherapy based on an assessment that the proportion of cells that express PD-L1 on the surface of cells in the subject's test tissue sample is below a predetermined threshold level; and (b) administering a standard-of-care therapeutic other than an anti-PD-1 Ab to the selected subject.

This disclosure also provides a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) screening a plurality of subjects to identify a subject that is a suitable candidate for immunotherapy, the screening comprising: (i)

optionally providing test tissue samples from the plurality of subjects, the test tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue samples that express PD-L1 on the surface of the cells; and (iii) selecting the subject as a candidate that is suitable for anti-PD-1 Ab immunotherapy based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and (b) administering a composition comprising a therapeutically effective amount of an anti-PD-1 Ab to the selected subject.

This disclosure further provides a method for treatment of a subject afflicted with cancer, which method comprises: (a) screening a plurality of subjects to identify a subject that is a suitable candidate for the treatment, the screening comprising: (i) optionally providing test tissue samples from the plurality of subjects, the test tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue samples that express PD-L1 on the surface of the cells, wherein the subject is identified as a suitable candidate for anti-PD-1 Ab immunotherapy if the proportion of cells in the tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level, and the subject is identified as a candidate that is not a suitable candidate for anti-PD-1 Ab immunotherapy if the proportion of cells in the tissue sample that express PD-L1 on the cell surface is below a predetermined threshold level; and (b) administering a composition comprising a therapeutically effective amount of an anti-PD-1 Ab to the subject identified as a suitable candidate for anti-PD-1 Ab immunotherapy, or (c) administering a standard-of-care therapeutic other than an anti-PD-1 Ab to the subject identified as not a suitable candidate for anti-PD-1 Ab immunotherapy.

One aspect of this invention is a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) determining that a proportion of cells in the test tissue sample express PD-L1 above a predetermined threshold level on the cell surface; and (c) based on that determination administering a composition comprising a therapeutically effective amount of an anti-PD-1 Ab to the subject. Another aspect of the invention is a method for treatment of a subject afflicted with cancer, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with

cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) determining that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is below a predetermined threshold level; and (c) based on that determination administering a standard-of-care therapeutic other than an anti-PD-1 Ab to the subject.

Yet another aspect of the invention is a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) determining that cells in the test tissue sample express PD-L1 on the cell surface; (c) selecting an anti-PD-1 Ab as a treatment for the subject based on the recognition that an anti-PD-1 Ab is effective in patients whose test tissue sample contains a proportion of cells above a predetermined threshold level that express PD-L1 on the cell surface; and (d) administering a composition comprising a therapeutically effective amount of an anti-PD-1 Ab to the subject. In a further aspect, the invention provides a method for treatment of a subject afflicted with cancer, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) determining that cells in the test tissue sample do not express PD-L1 on the cell surface; (c) selecting a standard-of-care therapeutic other than an anti-PD-1 Ab as a treatment for the subject based on the recognition that an anti-PD-1 Ab is ineffective in patients whose test tissue sample contains a proportion of cells that express PD-L1 on the cell surface is below a predetermined threshold level; and (d) administering the standard-of-care therapeutic to the subject.

This disclosure also provides a method of selecting an immunotherapy for a subject afflicted with cancer, which method comprises: (a) assaying cells of a test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells to assess the proportion of cells in the test tissue sample that express PD-L1; and (b) based on an assessment that the proportion of cells that express membranous PD-L1 is above a predetermined threshold level, selecting an immunotherapy comprising a therapeutically effective amount of an anti-PD-1 Ab for the subject. The invention further provides a method of selecting a treatment for a subject afflicted with cancer, which method comprises: (a) assaying cells of a test tissue sample comprising tumor cells and tumor-

infiltrating inflammatory cells to assess the proportion of cells in the test tissue sample that express PD-L1; and (b) based on an assessment that the proportion of cells that express membranous PD-L1 is below a predetermined threshold level, selecting a standard-of-care treatment other than an anti-PD-1 Ab for the subject.

In addition, the invention provides a method for treatment of a subject afflicted with cancer, which method comprises administering to the subject a composition comprising a therapeutically effective amount of an anti-PD-1 Ab, the subject having been selected on the basis that the proportion of cells in a test tissue sample from the subject that express PD-L1 is determined to exceed a predetermined threshold level, wherein the test tissue sample comprises tumor cells and tumor-infiltrating inflammatory cells. This invention also provides a method for treatment of a subject afflicted with cancer, which method comprises administering to the subject a standard-of-care treatment other than an anti-PD-1 Ab, the subject having been selected on the basis that the proportion of cells in a test tissue sample from the subject that express PD-L1 is determined to be below a predetermined threshold level, wherein the test tissue sample comprises tumor cells and tumor-infiltrating inflammatory cells.

This invention further provides a method for selecting a cancer patient for immunotherapy with an anti-PD-1 Ab, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold proportion; and (d) selecting the patient for immunotherapy based on an assessment that the proportion of cells in the test tissue sample that express surface PD-L1 is above the predetermined threshold level. In any of the methods described herein comprising a step for assessing PD-L1 expression, the test tissue sample may be a FFPE tissue sample.

In addition, in any method where an immunotherapy is selected or administered based on an assessment that the proportion of cells in a test tissue sample from the subject expresses PD-L1 at a level above a predetermined threshold level, it follows that a complementary method of treatment may be performed wherein a standard-of-care treatment other than the immunotherapy is selected or administered based on an assessment that the proportion of cells in a test tissue sample from the subject expresses

PD-L1 at a level below the predetermined threshold level.

This invention further provides a method for predicting the therapeutic effectiveness of an anti-PD-1 Ab for treating a cancer patient, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold value; and (d) predicting the therapeutic effectiveness of the anti-PD-1 Ab, wherein if the proportion of cells that express PD-L1 on the cell surface exceeds the threshold proportion the Ab is predicted to be effective in treating the patient, and wherein if the proportion of cells that express PD-L1 on the cell surface is below the threshold proportion the Ab is predicted to not be effective in treating the patient.

This invention also provides a method for determining an immunotherapeutic regimen comprising an anti-PD-1 Ab for treating a cancer patient, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold proportion; and (d) determining an immunotherapeutic regimen comprising an anti-PD-1 Ab based on the determination that the proportion of cells that express PD-L1 on the cell surface exceeds the predetermined threshold proportion.

Standard-of-Care Therapeutics

Several of the methods of treatment described herein comprise the administration of a standard-of-care therapeutic to a patient. As used herein, a "standard-of-care therapeutic" is a treatment process, including a drug or combination of drugs, radiation therapy, surgery or other medical intervention that is recognized by medical practitioners as appropriate, accepted, and/or widely used for a certain type of patient, disease or clinical circumstance. Standard-of-care therapeutics for treating different types of cancer are well known by persons of skill in the art. For example, the National Comprehensive Cancer Network (NCCN), an alliance of 21 major cancer centers in the USA, publishes the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) that provide

detailed up-to-date information on the standard-of-care treatments for a wide variety of cancers (*see* NCCN Guidelines, 2013).

By way of example, for *in situ* or early-stage MEL, surgical treatment is the primary treatment. Where surgical excision is not feasible for *in situ* melanoma due to comorbidity or cosmetically sensitive tumor location, topical imiquimod and radiotherapy are emerging as treatments, especially for lentigo maligna. Chemothepeutic agents for treating MEL include dacarbazine, temozolomide, imatinib for melanoma with 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.

There is no consensus on the best treatments for metastatic melanoma, though a variety of treatments including excision to clear margins, intralesional injections, laser ablation, radiation and biochemotherapy (combination of chemotherapy and biological agents such as interferon-alpha and IL-2) are being investigated. The therapeutic landscape for metastatic melanoma has recently seen dramatic improvements with the development of novel drugs such as vemurafenib and ipilimumab. Vemurafenib specifically inhibits signaling by a mutated intracellular kinase, BRAF, that is present in about 50% of patients with metastatic melanoma (Flaherty *et al*., 2010). Ipilimumab is a HuMAb that inhibits the immune checkpoint receptor, CTLA-4, and thereby stimulates a T cell immune response (Hodi *et al*., 2010). Besides these two agents, no other agent has demonstrated an OS benefit in a Phase 3 randomized study. Dacarbazine is approved by the FDA and the EMA for treatment of metastatic MEL with a reported objective response rate of 5% to 20% and a median OS of 6.4 months, but these responses are short-lived. Other drugs such as temozolomide and fotemustine have not resulted in significant improvement in survival when compared to dacarbazine. IL-2 has also been approved by the FDA for the treatment of metastatic MEL as it is associated with a 15 to 20% response rate including 4-6% complete responses which can be durable, but it is associated with significant toxicities including hypotension, cardiac arrhythmias, and pulmonary edema. Further details on standard-of-care treatments for melanoma are provided by Garbe *et al*. (2012). Despite the recent approval of ipilimumab and vemurafenib for advanced MEL, there is still a large unmet need for patients who have progressed on anti-CTLA-4 therapy and a BRAF inhibitor (depending on BRAF status) or patients with previously untreated, unresectable or metastatic BRAF wild-type MEL. The

5-year survival rate for late-stage MEL is currently only 15%.

For clinically localized RCC (Stage IA and IB), surgical resection, including radical nephrectomy and nephron-sparing surgery, is an effective therapy. Partial nephrectomy is generally not suitable for patients with locally advanced tumors (Stage II and III), in which case radical nephrectomy is preferred. Patients with Stage IV disease may also benefit from surgery, and cytoreductive nephrectomy before systemic therapy is recommended for patients with a potentially surgically resectable primary and multiple resectable metastases.

Until recently, the cytokines IL-2 and IFNα were the only active systemic treatments for advanced or metastatic RCC. However, due to each of these agent's limited clinical benefit and substantial toxicity profile, newer targeted agents have largely replaced cytokines in the treatment of advanced or metastatic renal cell carcinoma. The recognition of the importance of hypoxia inducible factor alpha (HIFα) signaling in the pathogenesis of clear-cell RCC has led to widespread study of two classes of targeted therapies, anti-angiogenic agents and mammalian target of rapamycin (mTOR) inhibitors (Mulders, 2009). Targeting of angiogenesis is rational because constitutive HIFα activation leads to the upregulation or activation of several proteins including vascular endothelial growth factor (VEGF), which can subsequently lead to tumor proliferation and neovasculature formation. Targeting of the mTOR pathway is important because activation of the upstream PI3K/Akt/mTOR signaling pathway is one method by which constitutive HIFα activation or upregulation occurs (Mulders, 2009). Agents that target angiogenesis include VEGF-receptor (VEGFr) TKIs (*e.g.*, sorafenib, sunitinib, pazopanib, axitinib, and tivozanib) and VEGF-binding mAbs (*e.g.*, bevacizumab), while agents that target the mTOR pathway include the mTOR inhibitors (*e.g.*, everolimus and temsirolimus). However, most patients develop resistance, and overall survival (OS) improvement has only been shown in one Phase 3 trial in poor-risk patients. Everolimus demonstrated a 3-month improvement in median progression-free survival (PFS) versus placebo, with no OS improvement (Motzer et al., 2008). Among the five approved anti-angiogenic agents (sorafenib, sunitinib, bevacizumab, pazopanib, and axitinib) and two approved mTOR inhibitors (temsirolimus, everolimus), only everolimus is approved specifically for use after the failure of treatment with anti-angiogenic therapy. In the US, everolimus is indicated for the treatment of advanced RCC after failure of treatment with sunitinib or sorafenib, whereas in the EU, everolimus is more broadly indicated for

patients with advanced RCC, whose disease has progressed on or after treatment with VEGF-targeted therapy.

NSCLC is the leading cause of cancer death worldwide, exceeding breast, colon and prostate cancer combined. The majority of subjects (approximately 78%) are diagnosed with advanced/recurrent or metastatic disease. NSCLC therapies have incrementally improved OS, but benefit has reached a plateau (median OS for late stage patients is just 1 year). Progression after 1L therapy occurred in nearly all of these subjects and the 5-year survival rate is only 3.6% in the refractory setting.

There is a particular unmet need among patients who have squamous cell NSCLC (representing up to 25% of all NSCLC) as there are few treatment options after 1L therapy. Surgery, radiation therapy (RT) and chemotherapy are the three modalities commonly used to treat NSCLC patients. As a class, NSCLCs are relatively insensitive to chemotherapy, compared to small cell carcinoma. In general, for patients with Stage I or II disease, surgery provides the best chance for cure, with chemotherapy increasingly being used both pre-operatively and post-operatively. RT can also be used as adjuvant therapy for patients with resectable NSCLC, the primary local treatment, or as palliative therapy for patients with incurable NSCLC.

Patients with Stage IV disease who have a good performance status (PS) benefit from chemotherapy. Many drugs, including platinum agents (*e.g.*, cisplatin, carboplatin), taxanes agents (*e.g.*, paclitaxel, albumin-bound paclitaxel, docetaxel) vinorelbine, vinblastine, etoposide, pemetrexed and gemcitabine are useful for Stage IV NSCLC. Combinations using many of these drugs produce 1-year survival rates of 30% to 40% and are superior to single agents. Specific targeted therapies have also been developed for the treatment of advanced lung cancer. For example, bevacizumab (Avastin®) is a mAb that blocks vascular endothelial growth factor A (VEGF-A). Erlotinib (Tarceva®) is a small-molecule TKI of epidermal growth factor receptor (EGFR). Crizotinib (Xalkori®) is a small-molecule TKI that targets ALK and MET, and is used to treat NSCLC in patients carrying the mutated *ALK* fusion gene. Cetuximab (Erbitux®) is a mAb that targets EGFR.

Squamous cell carcinoma (SCC) represents one quarter of NSCLC cases and has limited treatment options. Currently, second-line treatment for SCC remains an area of unmet need. Single-agent chemotherapy is standard of care following progression with platinum-based doublet chemotherapy (Pt-doublet), resulting in median OS of

approximately 7 months. Docetaxel remains the benchmark treatment in this line of therapy although erlotinib may also used with less frequency. Pemetrexed has also been shown to produce clinically equivalent efficacy outcomes but with significantly fewer side effects compared with docetaxel in the 2L treatment of patients with advanced NSCLC (Hanna *et al.*, 2004). No therapy is currently approved for use in lung cancer beyond the 3L setting. Pemetrexed and bevacizumab are not approved in SCC, and molecularly targeted therapies have limited application.

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.

EXAMPLE 1

Cross-Competition between Anti-PD-1 HuMAbs for Binding to CHO Cells Expressing Human PD-1

Chinese Hamster Ovary (CHO) cells transfected to express human PD-1 (CHO/PD-1 cells) were incubated with 10 μg/ml of Fab fragment of the anti-PD-1 HuMAb 5C4 or human IgG1 (hIgG1) isotype control Ab for 30 minutes at 4°C before addition of anti-PD-1 HuMAbs 2D3, 7D3 or 4H1 at a concentration of 0.2 μg/ml. Binding of 4H1, 2D3 or 7D3 to CHO/PD-1 cells were detected by fluorescein isothiocyanate (FITC)-conjugated goat anti-hIgG, Fc-gamma specific Ab. In the case of cross-competition assay with 5C4 and 17D8, CHO/PD-1 cells were incubated with the whole molecule of 5C4 before addition of FITC-labeled 17D8. Binding of 2D3, 7D3, 4H1 or 17D8 to the CHO/PD-1 cells was measured by flow cytometric analysis using a FACScalibur flow cytometer (Becton Dickinson, San Jose, CA).

The results are depicted in Figure 1. The data show that the 5C4 Fab fragment substantially blocked the binding of mAbs 5C4 itself, as well as the binding of 2D3, 7D3 (Figure 1A) and 4H1 (Figure 1B), while the 5C4 whole mAb substantially blocked the binding of 17D8 (Figure 1C) to CHO/PD-1 cells as measured by mean fluorescent intensity (MFI) of staining.

EXAMPLE 2

Cross-Competition between Anti-PD-L1 HuMAbs for Binding to CHO Cells Expressing Human PD-L1

CHO cells transfected to express hPD-L1 (CHO/PD-L1 cells) were incubated with 10 μg/ml of each of ten unconjugated human anti-PD-L1 mAbs (5F8, 7H1, 10H10, 1B12, 3G10, 10A5, 11E6, 12A4, 12B7, and 13G4) or human IgG1 (hIgG1) isotype control Ab for 20 min at 4°C. FITC-conjugated 10H10 (A), 3G10 (B), 10A5 (C), 11E6 (D), 12A4 (E) or 13G4 (F) was added to the cells to a final concentration of 0.09 μg/ml (B, D), 0.27 μg/ml (A, C), 0.91 μg/ml (F), or 2.73 μg/ml (E) for an additional 20 min at 4°C without prior washout of unbound, unconjugated Ab. Different quantities of the various FITC-conjugated HuMAbs were used due to differences in binding efficiency following labeling, and the optimal amounts of these FITC-conjugated HuMAbs were previously determined by dose-titration analysis of binding to CHO/PD-L1 cells. Binding of FITC-conjugated 10H10, 3G10, 10A5, 11E6, 12A4 or 13G4 to the CHO/PD-L1 cells was measured by flow cytometry.

The results are depicted in Figure 2. Binding of labeled 10H10 was partially blocked by 10A5, 11E6 and 13G4, but was substantially blocked only by itself (Figure 2A). Conversely, 10H10 substantially blocked the binding only of itself to CHO/PD-L1 cells. Each of anti-PD-L1 HuMAbs 5F8, 7H1, 1B12, 3G10, 10A5, 11E6, 12A4, 12B7 and 13G4 substantially blocked binding of labeled mAbs 3G10 (Figure 2B), 10A5 (Figure 2C), 11E6 (Figure 2D), 12A4 (Figure 2E) and 13G4 (Figure 2F) to CHO/PD-L1 cells as measured by MFI, though mAbs 5F8 and 13G4 generally blocked binding of the labeled mAbs to a slightly lesser extent.

EXAMPLE 3

Cross-Competition between Anti-PD-L1 mAbs for Binding to Ovarian Carcinoma Cells Expressing Human PD-L1

Anti-PD-L1 HuMAbs 5F8, 12B7, 3G10, 1B12, 13G4, 10H10, 10A5 and 12A4, and a human IgG1 (huIgG1) isotype control Ab were serially diluted from 10 μg/ml and incubated with hPD-L1-expressing ES-2 ovarian carcinoma cells for 20 minutes at 4°C. Without washing, biotinylated-12A4 Ab was added to a final concentration of 0.4 μg/ml for an additional 20 minutes at 4°C. After washing, bound biotin-12A4 was detected using fluorescent streptavidin-PE secondary reagent and measured by flow cytometry. Figure 3 shows the fluorescence of bound biotin-12A4 plotted against the concentration

of unlabeled hPD-L1 HuMAbs. Binding of biotin-12A4 to ES-2 cells was substantially blocked by 12A4 itself and by 1B12 and 12B7, and was moderately to significantly blocked by mAbs 5F8, 10A5, 13G4 and 3G10, but was not blocked by mAb 10H10.

EXAMPLE 4

Design of Phase I Clinical Study of Anti-PD-1 Antibody

A Phase I study was conducted to assess the safety, antitumor activity, and pharmacokinetics of an anti-PD-1 in patients with selected advanced solid tumors. The human anti-PD-1 mAb, BMS-936558 (also referred to herein as nivolumab, and in U.S. Patent No. 8,008,449 as 5C4), was administered as an intravenous infusion every 2 weeks of each 8-week treatment cycle. Patients continued treatment for up to 2 years (12 cycles), unless they experienced complete remission, unacceptable toxicity, disease progression, or withdrew consent. In patients who were otherwise clinically stable, study treatment was continued beyond apparent initial disease progression until further progression was noted as recommended by proposed immune response criteria (Wolchok *et al*., 2009). Patients with stable disease (SD) or an ongoing objective response (OR: complete [CR] or partial response [PR]) at the end of treatment were followed for 1 year and were offered retreatment for 1 additional year in the event of progression.

Dose escalation

Patients with advanced melanoma (MEL), non-small-cell lung (NSCLC), renal cell carcinoma (RCC), castration-resistant prostate (CRPC) and colorectal cancer (CRC) were eligible to enroll. Cohorts of 3-6 patients per dose level were enrolled sequentially at 1.0, 3.0, and 10.0 mg/kg. Dose escalation proceeded when a minimum of 3 patients had completed the safety evaluation period (56 days) at the given dose level, with dose-limiting toxicity in less than one-third of patients. Intra-patient dose escalation was not permitted.

Cohort expansion

A maximum tolerated dose (MTD) was not reached. Initially, 5 expansion cohorts of approximately 16 patients each were enrolled at 10 mg/kg for MEL, NSCLC, RCC, CRPC and CRC. Based on initial signals of activity, additional expansion cohorts of approximately 16 patients each were enrolled for MEL (at 1.0 and 3.0 mg/kg, followed by cohorts randomized to 0.1, 0.3, or 1.0 mg/kg), NSCLC (squamous or nonsquamous histology cohorts randomized to 1, 3, or 10 mg/kg), and RCC (at 1.0 mg/kg).

Patients

Eligible patients had documented advanced solid tumors; age >18 years; life expectancy >12 weeks; Eastern Cooperative Oncology Group performance status of ≤2; measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST), v1.0 with modification (*see* Topalian *et al.*, 2012b); adequate hematologic, hepatic, and renal function; and received 1-5 prior systemic treatment regimens. Patients with stable treated brain metastases were enrolled. Exclusion criteria included a history of chronic autoimmune disease, prior therapy with T-cell modulating Abs (e.g., anti-CTLA-4), conditions requiring immunosuppressive medications, and chronic infections (HIV, hepatitis B or C).

A total of 296 patients with advanced solid tumors including MEL (n=104), NSCLC (n=122), RCC (n=34), CRPC (n=17), and CRC (n=19) were treated with BMS-936558 for 40 months up to February 2012. By March 2013, 304 patients including patients with non-small-cell lung cancer (n=127), melanoma (n=107), RCC (n=34), CRPC (n=17), and CRC (n=19) had been treated with BMS-936558 from October 2008 through March 2012, all with a minimum of one year and up to about 4.4 years of observation. Two patients did not receive a full cycle of treatment and were not considered response-evaluable. The majority of patients were heavily pretreated, with 47% having received at least 3 prior regimens. Notable prior therapies included immunotherapy (64%) and B-RAF inhibitor (8%) in MEL patients; platinum-based chemotherapy (94%) and tyrosine kinase inhibitors (TKIs, 34%) in NSCLC patients; and nephrectomy (94%), immunotherapy (59%), and anti-angiogenic therapy (74%) in RCC patients. Baseline characteristics of the total treated population (N=296) were similar to those of the efficacy population (response evaluable patients, N=236). Details on the patient pre-treatments are provided in Topalian *et al.* (2012b).

Statistical analysis

All patients (N=304) treated as of the date of analysis (March 2013) were used for summaries of baseline characteristics and AEs. Pharmacokinetic and molecular-marker populations consisted of treated patients with available data as of the date of analysis. The efficacy population consisted of response-evaluable patients commencing treatment at least 8 months before the date of analysis. Tumor measurements were collected after each treatment cycle (4 doses) by investigators. Individual best objective responses based on the tumor measurements were assessed by the sponsor per modified RECIST v1.0.

Objective response was confirmed by at least one sequential tumor assessment. Objective response and stable disease rates were estimated with confidence intervals using the Clopper-Pearson method. Time-to-event endpoints including progression-free survival, overall survival, survival rates, and duration of response, were estimated using the Kaplan–Meier method. AEs were coded using Medical Dictionary for Regulatory Activities (MedDRA), version 14.1. AEs of special interest (AEOSIs), with potential immune-related etiologies, , defined as adverse events that require more frequent monitoring and/or unique intervention, were identified using a pre-defined list of MeDRA terms. Individual best ORs were derived from investigator-reported data per modified RECIST v1.0. OR was confirmed by at least one sequential tumor assessment and OR rate (ORR={[CR + PR] ÷ n} × 100) was calculated.

EXAMPLE 5

Safety Evaluations on Patients Treated with Anti-PD-1 Antibody

Safety evaluations, including clinical examination and laboratory assessments, were conducted in all treated patients at baseline and regular intervals up to 100 days following last administration of drug. The severity of AEs was graded based on the NCI Common Terminology Criteria for Adverse Events (NCI CTCAE), v3.0. Computed tomography (CT) or magnetic resonance imaging was performed for tumor assessment at baseline and following each treatment cycle.

A MTD was not defined across the doses of BMS-936558 tested on this study. A relative BMS-936558 dose intensity of 90% or higher was achieved in 87% of patients (*see* Topalian *et al.*, 2012b, for details). AEs were coded using Medical Dictionary for Regulatory Activities (MedDRA), version 14.1. AEOSIs were identified using a pre-defined list of MeDRA terms. Fifteen of 296 (5%) patients discontinued treatment due to BMS-936558-related AEs. As of the date of analysis, 62 (21%) patients had died, with disease progression being the most common cause of death (Topalian *et al.*, 2012b).

The most common adverse events, regardless of causality included fatigue, decreased appetite, diarrhea, nausea, cough, dyspnea, constipation, vomiting, rash, pyrexia and pruritus (Topalian *et al.*, 2012b). Common BMS-936558-related AEs included fatigue, rash, diarrhea, decreased appetite, and nausea. The majority of the events were low grade, with grade 3-4 drug-related AEs observed in 41 of 296 (14%) patients. Drug-related serious AEs occurred in 32 of 296 (11%) patients (Topalian *et al.*, 2012b). The spectrum, frequency, and severity of BMS-936558-related AEs were

generally similar across the dose levels tested. Drug-related AEOSIs, with potential immune-related etiologies, included pneumonitis, vitiligo, colitis, hepatitis, hypophysitis, and thyroiditis among others. Hepatic or gastrointestinal AEOSIs were managed with treatment interruption and, as necessary, with administration of corticosteroids. Among patients treated to-date, these AEs (in 13 patients with diarrhea; 11 patients with hepatic AEs) were reversible in all cases. Endocrine AEOSIs were managed with replacement therapy. At the discretion of the treating physician, patients successfully reinitiated treatment with BMS-936558. Drug-related pneumonitis occurred in 9 of 296 (3%) patients. Grade 3-4 pneumonitis developed in 3 patients (1%). No clear relationship between the occurrence of pneumonitis and tumor type, dose level, or the number of doses received was noted. Early grade pneumonitis was generally reversible with treatment discontinuation and corticosteroid administration. In 3 patients with pneumonitis, infliximab and/or mycophenolate were utilized for additional immunosuppression; however, given the small number of patients and variable outcomes, the effectiveness of this treatment is unclear. There were 3 (1%) drug-related deaths due to pneumonitis (2 NSCLC patients, 1 CRC).

EXAMPLE 6

Pharmacokinetics/Pharmacodynamics Analyses on Anti-PD-1 Antibody

For pharmacokinetic (PK) analyses, serial blood samples were collected and serum concentrations of BMS-936558 were quantified using by ELISA. For pharmacodynamic (PD) analysis, peripheral blood mononuclear cells were isolated from patients at baseline and following cycle 1 to estimate PD-1 receptor occupancy (RO) by BMS-936558 on circulating CD3+ T-cells via flow cytometry (Brahmer *et al.*, 2010).

The maximum concentration of BMS-936558 was observed at a median T_{max} of 1-4 hours after the start of infusion. The PK of BMS-936558 was linear with a dose proportional increase in C_{max} and $AUC_{(0\text{-}14\ d)}$ in the dose range of 0.1-10 mg/kg (n=35). BMS-936558 PD was assessed by PD-1 RO on circulating T-cells. PBMCs from 65 MEL patients treated with one cycle of BMS-936558 at 0.1-10 mg/kg biweekly demonstrated median occupancy of PD-1 molecules on circulating $CD3^+$ T-cells by BMS-936558 ranging from 64%-70% (*see* Topalian *et al.*, 2012b, for details).

EXAMPLE 7

Antitumor Efficacy Exhibited by Anti-PD-1 Antibody

Analysis of data obtained since February 2012 is ongoing; thus, unless otherwise noted, the data presented below were obtained as of February 2012. Clinical antitumor activity was observed at all BMS-936558 doses tested. ORs (confirmed CR or PR) were observed in a substantial portion of patients with NSCLC, MEL, and RCC (Tables 1-3; Figure 4), and in various sites of metastatic disease including liver, lung, lymph nodes, and bone (Figures 5-7 and not shown). Tumor regressions followed conventional as well as "immune-related" patterns of response, such as prolonged reduction in tumor burden in the presence of new lesions. Individual best overall responses were derived from investigator-reported data according to modified RECIST v1.0. OR was confirmed by at least one sequential tumor assessment. At the time of data analysis, 2 patients with NSCLC who were treated with 10 mg/kg had unconfirmed responses, and 8 additional patients (with MEL, NSCLC, or RCC) had a persistent reduction in baseline target lesions in the presence of new lesions, *(i.e.*, an "immune-related" response pattern). None of these patients was categorized as a responder for the purpose of calculating OR rates. Antitumor responses and/or prolonged disease stabilization were observed in patients irrespective of prior therapies received (*see* summary of progression free interval for patients with OR and SD in Supplementary Appendix 4 of Topalian *et al*., 2012b).

In NSCLC patients, 14 ORs were observed at BMS-936558 doses of 1, 3, or 10 mg/kg with response rates of 6%, 32%, and 18%, respectively. ORs were observed across NSCLC histologies: 6 responders of 18 squamous (33%), 7 responders of 56 nonsquamous (13%), and 1 of 2 unknown. All 14 patients with ORs started treatment ≥24 weeks before data analysis, and of these, 8 had response duration ≥ 24 weeks (Table 1). Stable disease (SD) lasting ≥ 24 weeks was observed in 5 (7%) NSCLC patients, all with nonsquamous histology. Among MEL patients, 26 ORs were observed at doses ranging from 0.1-10 mg/kg, with response rates ranging from 19%-41% per dose level. At the 3 mg/kg dose level, ORs were noted in 7 of 17 (41%) patients. Of 26 MEL patients who achieved an OR, 17 started treatment ≥ 1 year before data analysis, and of these, 13 patients had an OR duration ≥ 1 yr. The remaining 8 patients with OR were on study < 1 year and 6 had responses ranging from 1.9-5.6 months. SD lasting ≥ 24 weeks was observed in 6 (6%) patients. In RCC patients, ORs occurred in 4 of 17 (24%) patients treated with a BMS-936558 dose of 1 mg/kg and 5 of 16 (31%) patients treated with 10

mg/kg. Among 8 RCC patients with OR who started treatment ≥ 1 year prior to data analysis, 5 (63%) had OR duration ≥ 1 yr. SD lasting ≥ 24 weeks was observed in an additional 9 (27%) patients.

Table 1. Clinical Activity of BMS-936558 in the Efficacy Population* (N=236)†

Tumor Type	Dose (mg/kg)	n	ORR‡ No. Patients (%) [95% CI]†	SD ≥ 24 wk No. Patients (%) [95% CI]	PFSR§ at 24 wk (%) [95% CI]
MEL	0.1	14	4 (29) [8-58]	1 (7) [0.2-34]	40 [13-66]
	1.0	27	8 (30) [14-50]	3 (11) [2-29]	45 [26-65]
	3.0	17	7 (41) [18-67]	1 (6) [0.1-29]	55 [30-80]
	10.0	20	4 (20) [6-44]	0	30 [9-51]
All MEL		94	26 (28) [19-38]	6 (6) [2-13]	41 [30-51]
NSCLC**					
All	1	18	1 (6) [0.1-27]	1 (6) [0.1-27]	16 [0-34]
Squamous	1	5	0	0	0
Nonsquamous	1	12	0	1 (8) [0.2-39]	14 [0-37]
Unknown	1	1	1(100) [3-100]	0	1
All	3	19	6 (32) [13-57]	2 (11) [1-33]	41 [18-64]
Squamous	3	6	3 (50) [12-88]	0	50 [10-90]
Nonsquamous	3	13	3 (23) [5-54]	2 (15) [2-45]	37 [10-64]
All	10	39	7 (18) [8-34]	2 (5) [0.6-17]	24 [11-38]
Squamous	10	7	3 (43) [10-82]	0	43 [6-80]
Nonsquamous	10	31	4 (13) [4-30]	2 (7) [0.8-21]	21 [6-36]
Unknown	10	1	0	0	0
ALL NSCLC		**76**	**14 (18) [11-29]**	**5 (7) [2-15]**	**26 [16-36]**
All Squamous		**18**	**6 (33) [13-59]**	**0**	**33 [12-55]**
All Nonsquamous		**56**	**7 (13) [5-24]**	**5 (9) [3-20]**	**22 [11-34]**
RCC	1	17	4 (24) [7-50]	4 (24) [7-50]	47 [23-71]
	10	16	5 (31) [11-59]‡	5 (31) [11-59]¶	67 [43-91]
ALL RCC		**33**	**9 (27) [13-46]**	**9 (27) [13-46]**	**56 [39-73**

*The efficacy population consists of response-evaluable patients whose treatment was initiated at least 8 months before data analysis in February 2012, and had measurable disease at baseline and one of the following: at least 1 on-treatment scan or clinical evidence of disease progression or death.

†CR denotes complete response, MEL melanoma, NSCLC non-small-cell lung cancer, ORR objective response rate, PFSR progression-free survival rate, PR partial response, RCC renal-cell cancer, SD stable disease, n number of patients.

‡Objective response rates ({[CR + PR] ÷ n} × 100) have been calculated based on confirmed responses with confidence intervals calculated using the Clopper-Pearson method. Individual patient responses were adjudicated per RECIST v1.0 with modification (*see* Topalian *et al.*, 2012b).

[§]Progression-free survival rate was the proportion of patients who did not progress and were alive at 24 weeks calculated by the Kaplan-Meier methodology with confidence intervals using the Greenwood method.

[¶]One CR.

**One NSCLC patient who was treated at the 3 mg/kg dose level had an initial evaluation of progressive disease, subsequently had a PR, and was classified as a responder.

Table 2. Clinical Activity of BMS-936558 in the Efficacy Population*

Tumor Type	**ORR[a]** ***No. of patients/total no. of patients (%) [95% CI]***	**Stable Disease ≥24 wk** ***No. of patients/total no. of patients (%) [95% CI]***	**Median Overall Survival (95% CI)**	**Overall Survival Rate (95% CI); patients at risk, n**		
				1 y	**2 y**	**3 y**
NSCLC	20/122 (16.4) [10.3, 24.2]	11/122 (9.0) [4.6, 15.6]	NA	43 (33-53); 24	32 (18-47); 4	24 (7-41); 1
Squamous	9/48 (18.8) [8.9 , 32.6]	NA	NA	NA	NA	NA
Non-sq.	11/73 (15.1) [7.8 , 25.4]	NA	NA	NA	NA	NA
MEL	33/106 (31.1) [22.5, 40.9]	6/106 (5.7) [2.1, 11.9]	16.8 (12.5, NR)	61 (52-71); 50	44 (33-56); 24	40 (29-52); 1
RCC	10/34 (29.4) [15.1, 47.5]	9/34 (26.5) [12.9, 44.4]	NR (13.6, NR)	70 (55-86); 22	52 (32-72); 7	52 (32-72); 1

* The efficacy population consists of response-evaluable patients up to March 2013

[a] Objective response rate was calculated as {[CR + PR] ÷ n} x 100.

NR, not reached

NA, not available from July 3, 2012 data cut. The percentage of patients with stable disease for ≥ 36 weeks or ≥ 48 weeks [95% CI], median duration of response (months) and median progression-free survival (95% CI) were N/A.

The following results are drawn from preliminary analyses of BMS-936558 clinical efficacy data as of March 2013. Sustained survival, as reflected by median overall survival and 1- and 2-year landmark overall survival rates, was noted in each of the responding patient populations (Table 2). Median overall survivals of 9.6 months for lung cancer, 16.8 months for melanoma, and greater than 22 months for kidney cancer, were observed. Thirteen patients with melanoma, lung or kidney cancer were characterized as having unconventional response patterns that did not meet RECIST criteria (*e.g.*, persistent reduction in target lesions in the presence of new lesions or following initial

progression) (Wolchok *et al*, 2009).

Table 3. Duration of Objective Responses to BMS-936558*

Tumor Type	Dose (mg/kg)	No. Patients with OR	Duration of Response (months)†
MEL	0.1	4	7.5+, 5.6+, 5.6, 5.6
	0.3	3	3.8+, 2.1+, 1.9+
	1	8	24.9+, 22.9, 20.3+, 19.3+, 18.4+, 7.6+, 5.6+, 5.3+
	3	7	22.4+, 18.3+, 15.2+, 12.9, 11.1, 9.3, 9.2+
	10	4	24.6+, 23.9+, 18.0+, 17.0
NSCLC§	1	1	9.2+
	3	6	30.8+, 7.6+, 5.5+, 3.7+, 1.9+, NA‡
	10	7	14.8+, 7.6+, 7.3+, 6.7, 4.2, 3.7+, 3.7
RCC	1	4	17.5+, 9.2+, 9.2, 5.6+
	10	5	22.3+. 21.7+, 12.9, 12.0, 8.4

*MEL denotes melanoma, NA not applicable, NSCLC non-small-cell lung cancer, RCC renal-cell cancer.

†Time from first response to time of documented progression, death, or for censored data, time to last tumor assessment.

‡One patient was treated beyond an initial evaluation of progressive disease and subsequently had a PR; this patient was classified as a responder for the purposes of calculating response rates by RECIST v1.0 but was not eligible for calculation of duration of response.

EXAMPLE 8

Measurement of Membranous PD-L1 Expression by Standard IHC Assay

IHC staining of PD-L1 was performed on pretreatment formalin-fixed paraffin-embedded (FFPE) tumor specimens using the murine anti-human PD-L1 mAb 5H1 (Dong *et al*., 2002) in a standard IHC protocol (Taube *et al*., 2012; Supp. Materials). Briefly, 5μm-FFPE sections mounted on glass slides were deparaffinized in xylene and antigen retrieval was performed using a Tris-EDTA buffer, pH 9.0 at 120°C for 10 min in a Decloaking Chamber (Biocare Medical). Endogenous peroxidase, biotin and proteins were blocked (CAS system K1500, Dako; Avidin/biotin Blocking Kit, SP-2001, Vector Laboratories; Serotec Block ACE). The primary 5H1 Ab was applied at a concentration of 2 μg/ml and allowed to incubate at 4°C for 20 h. Secondary Ab (biotinylated anti-mouse IgG1, 553441 BD) was applied at a concentration of 1 μg/ml for 30 min at room temperature (RT). The signal was then developed with amplification according to the manufacturer's protocol (CAS system K1500, Dako). Sections were counterstained with hematoxylin, dehydrated in ethanol and cleared in xylene, and a coverslip was applied.

The percentage of tumor cells exhibiting cell surface staining for PD-L1 was scored by two independent pathologists who were blinded to treatment outcomes. PD-L1 positivity was defined per specimen by a 5% expression threshold (Taube *et al.*, 2012; Thompson *et al.*, 2006), and in cases with multiple specimens, if any specimen met this criterion. A Fisher's exact test was applied to assess the association between PD-L1 expression and OR, noting, however, that this analysis was based in part on optional biopsies from a non-random subset of the population and testing of a statistical hypothesis was not pre-specified.

Sixty-one pretreatment tumor specimens from 42 patients (18 MEL, 10 NSCLC, 7 CRC, 5 RCC, and 2 CRPC) were analyzed for tumor cell surface PD-L1 expression (Figure 8). Biopsy specimens from 25 of 42 patients were positive for PD-L1 expression by IHC. A Fisher's exact test was applied to assess the association between PD-L1 expression and OR in a post-hoc analysis. Among the 42 surface-PD-L1^{+} patients, 9 (36%) achieved an OR. Importantly, among 17 patients with PD-L1^{-} tumors, none achieved an OR. Thus, in a subset of patients cell surface expression of PD-L1 on tumor cells in pretreatment biopsies is associated with an increased rate of OR among patients treated with BMS-936558, while no patients with documented PD-L1-negative tumors experienced an OR. These data indicate that tumor PD-L1 expression is a molecular marker that can enable patient selection for anti-PD-1 immunotherapy.

EXAMPLE 9

Isolation of Rabbit mAbs that Detect Membranous hPD-L1 Antigen in FFPE Tissues

Rabbit Abs against human PD-L1 polypeptide were prepared by Epitomics, Inc. (Burlingame, CA) by immunization of rabbits using a recombinant human PD-L1 fusion protein. Antiserum titers were evaluated using standard direct ELISA with the hPD-L1 antigen and cell ELISA using transfected cells overexpressing hPD-L1. These Abs were also screened for their ability to bind to PD-L1 by IHC assay of FFPE tissue sections. The rabbit with the highest Ab titer was selected for splenectomy. Lymphocytes isolated from the spleen were fused to myeloma cells in 40 x 96-well plates, and screened by ELISA against the immunizing PD-L1 antigen and by cell ELISA against cells overexpressing hPD-L1. Positive clones were expanded into 24-well plates, and confirmatory screens were conducted by direct ELISA and cell ELISA. The supernatants (sups) of clones that were specific to the screening antigen were re-screened by IHC.

A set of mouse anti-hPD-L1 mAbs were also produced by immunization of mice

using a protocol similar to that described above for the rabbit mAbs.

Out of a total of 185 multiclones from both rabbit and mouse immunizations screened, only ten rabbit multiclone Abs specifically detected the membranous form of hPD-L1. None of the purified mouse subclones were found to specifically detect cell surface hPD-L1. Sixty subclones from the top five rabbit multiclones (designated Nos. 13, 20, 28, 29 and 49, each comprising 12 subclones) were screened initially by IHC on FFPE low density tissue microarrays (TMAs), followed by confirmation and specificity verification in narrowed 25 subclones. Rabbit IgG was used as a negative isotype control, and mAb 5H1 (Dong *et al.*, 2002) was used as the positive control. Specificity was further verified by antigen preabsorption assay. Through two rounds of IHC, the following 15 purified subclones were selected as the most promising Abs in terms of specificity and intensity of staining: 13-1, 13-3, 13-7, 13-8; 20-5, 20-7, 20-12, 20-6; 28-1, 28-8, 28-12; 49-5, 49-7, 49-9; and 29-8. Immunoreactivity data on these selected Abs are summarized in Table 4.

Additional assays were performed to further characterize the purified Ab clones, including determining binding affinity and cross-competition among the Abs (to identify overlapping versus different epitope regions) by surface plasmon resonance. All the Abs exhibited high binding affinity ($K_D < 10^{-9}$ M). These 15 purified clones were also re-screened by IHC on FFPE low density TMA or regular sections against various cell and tissue types known to be positive or negative for cell surface expression of PD-L1. Rabbit IgG was used as the isotype control, and mAb 5H1 was used as the positive control. At high concentration (10 μg/ml), clones 28-x and 49-x displayed low to moderate levels of background staining in tissues, while clones 13-x exhibited no background staining, which suggests that the 13-x clones have a wider dynamic range. The 20-x clones displayed various degree of background staining which was primarily cytoplasmic and diffuse. The clone with most robust detection specifically of membranous PD-L1, rabbit clone 28-8 (K_D = 100 p M, as determined by SPR), was selected as the lead Ab for subsequent IHC assays. MAbs 28-1, 28-12, 20-12 and 29-8 had K_D values of 130 pM, 94 pM, 160 pM and 1200 pM, respectively. The sequences of the heavy chain variable (V_H) and light (kappa) chain variable (V_κ) regions of mAb 28-8 are set forth in SEQ ID NOs. 35 and 36, respectively. The 28-8 Ab was shown to recognize a different epitope from mouse mAb 5H1, based on SPR analysis. Clones 28-1, 28-12, 29-8 and 20-12 were the next best Abs in terms of robust detection of membranous PD-L1 in FFPE tissues.

Although mAb 13-1 had the best specificity in terms of detection of membranous PD-L1, the maximal detection level was lower than that of the other lead Abs. Western blotting was also performed with plus/minus antigen competition to verify the specificity of the top selected Abs for PD-L1.

Table 4. Immunoreactivity of rabbit anti-hPD-L1 mAbs

	Specific Staining		Non-Specific staining
Antibody Name	Pos. vs Neg. Staining on PD-L1 Cells*	Intensity Range on Tissues† (Very High, High, Medium, Low)	Background Staining on Tissues (High, Medium, Low)
13-1	Pos	Low to Very High	None
13-3	Pos	Low to High	None
13-7	Pos	Low to High	None
13-8	Pos	Low to High	None
20-5	Pos	Low to Very High	High
20-6	Pos	Low to High	Medium
20-7	Pos	Low to High	Medium
20-12	Pos	Low to High	Medium
28-1	Pos	Low to Very High	None
28-8	Pos	Medium to Very High	None
28-10	Pos	Medium to Very High	Low
28-12	Pos	Low to Very High	None
49-5	Pos	Low to High	None
49-7	Pos	Low to High	Low
49-9	Pos	Low to High	None
5H1	Pos	High to Very High	None
Rb IgG	Neg	Neg	None

*PD-L1 stably transfected CHO cells vs. CHO-S control;
†PD-L1 positive tissues included placenta and one non-small cell lung cancer; Detection up to "very high" expression suggests better sensitivity at detecting membranous PD-L1.

The binding of mAbs 5H1 and 28-8 to membranous PD-L1 in FFPE test tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells from different types of tumors was compared. Membranous PD-L1 expression was evaluated using the histoscore method performed by 2 independent pathologists. Four NSCLC, 2 MEL, and 2 RCC tumors were stained with 28-8 at 2 mg/ml and 5H1 at 5 mg/ml. The data are tabulated in Table 5, and shown graphically in Figure 9. The rabbit mAb 28-8 showed better detection (higher histoscores) for 7 out of 10 samples using 2.5-fold less Ab, and in

only one sample was the histoscore for 5H1 slightly higher than for mAb 28-8.

Table 5. Comparison of mAbs 28-8 and 5H1 by histoscore analysis

Tissue	Sample I.D.	Histoscore Average (5H1)	Histoscore Average (28-8)
NSCLC	1080754B	245	261
NSCLC	1080766B	103	130
NSCLC	1080790	40	37
NSCLC	1080993B	12	16
Mel	T030668	98	113
Mel	T980744	98	123
Mel	1168657B	0	0
Mel	T980747	1	9
RCC	1164619B	4	4
RCC	1167809B	108	125

Taube *et al*. (2012) demonstrated by flow cytometry on cultured cells that mAb 5H1 bound to the cell surface, and the specificity of binding to PD-L1 was confirmed using a PD-L1 fusion protein to competitively block binding of the 5H1 mAb to tissue sections. These authors also compared 5H1 with a rabbit polyclonal anti-hPD-L1 Ab, 4059, previously described by Gadiot *et al*. (2011), and found that whereas 5H1 showed a cell surface staining pattern on FFPE samples, Ab 4059 demonstrated diffuse cytoplasmic staining. Further, when 5H1 was compared to Ab 4059 by western blot analysis, Ab 4059 bound to multiple proteins in lysates of melanoma cells in addition to a 50 kDa protein corresponding to the expected mass of glycosylated PD-L1, in contrast to 5H1 which specifically detected the 50 kDa band of glycosylated PD-L1 (Taube *et al*., 2012).

In the present study, an automated IHC assay (*see* Example 10) was used to evaluate the binding of several commercially available anti-PD-L1 Abs and 5H1 (Dong *et al*., 2002) to FFPE tissue samples containing various cells expressing PD-L1. The results, summarized in Table 6, show that none of the commercially available Abs tested specifically recognized membranous PD-L1 expression in human tissues known to express PD-L1, or to clearly distinguish CHO cells expressing PD-L1 versus the untransfected parent CHO cells that did not express PD-L1. The inability of the polyclonal Ab (pAb) 4059 to bind specifically recognized membranous PD-L1 is consistent with the findings of Taube *et al*. (2012). The binding of 28-8 was similar to

that of 5H1 in this assay, though histoscore analysis suggests that 28-8 performs better than 5H1.

Table 6. Binding of mAbs to FFPE Samples Containing PD-L1-expressing cells

Source	Antibody Types	Clone No. (mAb) /Catalog No. (pAb)	Pos. vs. Neg. Staining on PD-L1 Cells*	Human Positive Tissues†
MBL	mAb	27A2	Failed	Failed
BioLegend	mAb	29E.2A3	Failed	Failed
eBiosciences	mAb	M1H1	No Staining	No staining
Collaborator	mAb	5H1	Passed	Passed
ProSci	pAb	4059	Failed	Failed
LifeSpan BioSciences	pAb	LS-B480/0604	Failed	Failed

*PD-L1 stably transfected CHO cells vs. parent CHO-S negative control;
†PD-L1 positive tissues included tonsil and/or thymus;
mAb, mouse mAb; pAb, rabbit polyclonal Ab.

EXAMPLE 10

Development of Automated IHC Protocol for Assessing PD-L1 Expression

An automated IHC protocol was developed to assay PD-L1 expression in FFPE specimens. Tissue sections (4 μm) were mounted on slides, deparaffinized in an autostainer (Leica) by soaking twice for 5 min in xylene, and re-hydrated through soaking twice for 2 min each time in 100% EtOH, twice in 95% (v/v) EtOH, once in 70% (v/v) EtOH, and once in de-ionized water (dH_2O). Antigen retrieval was performed using a decloaking chamber (Biocare Medical Decloaking Chamber Plus) and Dako pH 6 buffer, heated to 110°C (P1) for 10 min, then moved to the next step (P2 FAN ON at 98°C; FAN OFF at 90°C). The slides were cooled at room temperature (RT) for 15 min and rinsed with water for about 1 min.

Reagents were set up on an autostainer (BioGenex i6000), using a pap pen to define the tissue area. The IHC assay, run using the autostainer in research mode, comprised the following steps: neutralizing endogenous peroxidase using the Peroxidase Block (Leica) for 10 min, followed by rinsing 3 times with IHC wash buffer (Dako); applying Protein Block (Leica) to the slides, and incubating for 10 min at RT, followed by washing 3 times with wash buffer; applying the primary Ab to the slides (2 μg/ml) and incubating for 1 h at RT, followed by washing 6 times with wash buffer; adding Post

Primary Block (NovoLink Kit) to the slides and incubating for 30 min, followed by washing 6 times with wash buffer; adding NovoLink Polymer (NovoLink Kit) to the slides and incubating for 30 min, followed by washing 6 times with wash buffer; adding the DAB chromogen substrates (NovoLink Kit) and developing for 3 min, followed by rinsing 5 times with dH_2O at RT; counterstaining with hematoxylin (NovoLink Kit) for 1 min at RT, followed by washing 3 times with dH_2O for 5 times at RT. The primary Ab was selected from the rabbit anti-PD-L1 Abs shown in Table 4; mAb 28-8 was the preferred Ab. As a negative control, rabbit IgG (Dako) was used. The tissue sections were dehydrated using a Leica autostainer by washing once for 2 min in 70% EtOH, twice for 2 min in 95% EtOH, and three times for 2 min in 70% EtOH, then cleared by washing three times for 5 min in xylene. The sections were permanently mounted with permount to the slide, covered with a coverslip, and transferred to a chemical hood to dry.

EXAMPLE 11

Design of Phase I Clinical Study on Anti-PD-L1 Antibody

Study design

A Phase I study was conducted to assess the safety and tolerability of BMS-936559 (also referred to herein and in U.S. Patent No. 7,943,743 as 12A4) in patients with selected advanced solid tumors. Secondary objectives included initial assessment of the antitumor activity of BMS-936559 and pharmacokinetic evaluation. Pharmacodynamic measures were included under exploratory objectives. Patients were treated in 6-week cycles of BMS-936559 administered as a 60-minute intravenous infusion every 2 weeks on days 1, 15, and 29 of each cycle. Patients continued treatment for up to 16 cycles unless they experienced unacceptable toxicity, disease progression, or withdrew consent. In some patients who were clinically stable, treatment beyond initial disease progression was permitted until further progression was confirmed.

Dose escalation

Patients with advanced NSCLC, MEL, CRC, RCC, ovarian (OV), gastric (GC), breast (BC), and pancreatic (PC) carcinoma were eligible to enroll. Using an accelerated titration design, safety was assessed at doses of 0.3, 1, 3, and 10 mg/kg. One patient was enrolled in each successive cohort until there was a $\geq$ grade 2 drug-related AE during cycle 1. Two additional patients were then enrolled at that dose level and the study was transitioned to a standard 3+3 design. Intra-patient dose escalation or de-escalation was

not permitted. The maximum tolerated dose (MTD) was defined as the highest dose where less than one-third of patients had a dose-limiting toxicity.

Cohort expansion

Initially, 5 expansion cohorts (n=16/cohort) were enrolled at 10 mg/kg for patients with NSCLC, MEL, RCC, OV, and CRC. Based on initial signals of activity, additional expansion cohorts (up to n=16/cohort) were enrolled for MEL (at 1.0 and 3.0 mg/kg), NSCLC (squamous or nonsquamous histology cohorts randomized to 1, 3, or 10 mg/kg), and at 10 mg/kg for PC, BC, and GC.

Patients

Patients were required to have documented advanced NSCLC, MEL, RCC, OV, CRC, PC, GC, or BC, and have failed at least one prior tumor-appropriate therapy for advanced/metastatic disease (except for PC or GC patients who could be treatment-naïve). Other inclusion criteria included age $\geq$ 18 years, life expectancy $\geq$ 12 weeks, Eastern Cooperative Oncology Group performance status of $\leq$ 2, measurable disease as defined by RECIST v1.0, and adequate hematologic, hepatic, and renal function. Patients with treated brain metastases were allowed, if stable for at least 8 weeks. Major exclusion criteria included a history of autoimmune disease or other diseases requiring systemic steroids or immunosuppressive medication, prior therapy with T cell- modulating Abs (including anti-PD-1, anti-PD-L1 and anti-CTLA-4), history of HIV, or active hepatitis B or C.

In this ongoing study, 207 patients with NSCLC (n=75), MEL (n=55), CRC (n=18), RCC (n=17), OV (n=17), PC (n=14), GC (n=7), or BC (n=4) were treated with BMS-936559 during a 34-month period and are included in the safety data. Efficacy was characterized in 160 response-evaluable patients. The baseline demographic characteristics of the total and response-evaluable patient populations were very similar (Brahmer *et al.*, 2012). Among treated patients, 86% had received prior chemotherapy and 28% immunologic or biological therapy. Prior therapies by tumor type included immunotherapy (56%) and B-RAF inhibitor (9%) in patients with MEL; platinum-based chemotherapy (95%) and tyrosine kinase inhibitors (TKI; 41%) in patients with NSCLC; and nephrectomy (94%), anti-angiogenic therapy (82%), and immunotherapy (41%) in patients with RCC (*see* Brahmer *et al.*, 2012) for more details on patient pre-treatments).

Statistical analysis

All 207 patients commencing treatment as of the date of analysis were used for summaries of baseline characteristics and AEs. The efficacy population consisted of 160 response-evaluable patients who initiated treatment at least 7 months before the date of analysis. AEs were coded using MedDRA v14.1. Individual best overall responses were derived from radiographic scan measurements according to modified RECIST v1.0. ORs were confirmed by at least one sequential tumor assessment. Additional details regarding statistical methods are provided in Brahmer *et al.*, (2012).

EXAMPLE 12

Safety Evaluations on Patients Treated with Anti-PD-L1 Antibody

Safety evaluations (clinical examination and laboratory assessments) were conducted on all treated patients at baseline and regular intervals (weekly during cycle 1 and biweekly thereafter). AE severity was graded based on the NCI CTCAE, v3.0. Disease assessment via computed tomography (CT) scans or magnetic resonance imaging was performed at baseline and prior to each treatment cycle.

A MTD was not reached up to the highest tested dose of 10 mg/kg of BMS-936559. The median duration of therapy was 12 weeks (range 2.0-111.1 weeks). A relative dose intensity of $\geq$ 90% was achieved in 86% of patients. Twelve of 207 patients (6%) discontinued treatment due to a BMS-936559-related AE (*see* Brahmer *et al.*, 2012, for details).

AEs regardless of causality (any grade) were reported in 188 of 207 patients. Investigator-assessed BMS-936559-related AEs were noted in 126 of 207 (61%) patients. The most common drug-related AEs were fatigue, infusion reactions, diarrhea, arthralgia, rash, nausea, pruritus, and headache. Most events were low grade with BMS-936559-related grade 3-4 events noted in 19 of 207 (9%) patients (Brahmer *et al.*, 2012). The spectrum, frequency, and severity of BMS-936559-related AEs were similar across the dose levels, with the exception of infusion reactions. Drug-related AEOSIs, with potential immune-related etiologies, were observed in 81 of 207 (39%) of the patients and included rash, hypothyroidism, hepatitis, and single cases each of sarcoidosis, endophthalmitis, diabetes mellitus, and myasthenia gravis (Brahmer *et al.*, 2012). These AEs were predominantly grade 1-2 and generally reversible with treatment interruption or discontinuation. Notably, 9 patients were treated with corticosteroids for the management of AEs. AEs improved or resolved in all patients. Furthermore, 4 of these 9 patients

maintained disease control despite treatment with corticosteroids. Endocrine AEs were managed with replacement therapy and patients reinitiated treatment with BMS-936559 at the discretion of the treating physician. Infusion reactions were observed in 21 of 207 (10%) patients, predominantly at 10 mg/kg. They were grade 1-2 with the exception of one grade 3 event at 10 mg/kg. Infusion reactions were generally rapidly reversible with antihistamines and antipyretics and, in some cases, corticosteroids. A prophylactic regimen with antihistamines and antipyretics was implemented during the study. Patients with grade 1-2 infusion reactions were able to continue treatment with BMS-936559 with prophylactic antihistamines and antipyretics and at a reduced infusion rate. BMS-936559-related serious AEs occurred in 11 of 207 (5%) patients. As of the data analysis date, 45 patients (22%) had died (Brahmer *et al*., 2012); no drug-related deaths were observed.

EXAMPLE 13

Pharmacokinetics/Pharmacodynamics Analyses on Anti-PD-L1 Antibody

For PK analyses, serial blood samples were collected and serum concentrations of BMS-936559 were quantified by ELISA. Peripheral blood mononuclear cells were isolated from patients at baseline and following one treatment cycle to assay PD-L1 RO by BMS-936559 on circulating CD3-positive T-cells via flow cytometry (Brahmer *et al*., 2010).

Serum concentrations of BMS-936559 increased in a dose-dependent manner from 1-10 mg/kg (n=131). Geometric mean area under the curve (0-14 days) for the 1, 3, and 10 mg/kg dose levels were 2210, 7750, and 36620 μg/mL•hr (coefficient of variation [CV] 34-59%), respectively; geometric mean peak concentrations at these dose levels were 27, 83, and 272 μg/mL (CV 30–34%), respectively, after the first dose. The half-life of BMS-936559 was estimated from population pharmacokinetic data as approximately 15 days. PD-L1 RO on CD3-positive peripheral blood lymphocytes was assessed in 29 MEL patients at the end of 1 cycle of treatment, at BMS-936559 doses from 1-10 mg/kg. Median RO exceeded 65% for all groups (Brahmer *et al*., 2012).

EXAMPLE 14

Antitumor Efficacy Exhibited by Anti-PD-L1 Antibody

One-hundred and sixty patients out of the 207 treated were evaluable for response and included those with NSCLC, MEL, CRC, RCC, OV, and PC, but not patients with GC or BC. Clinical activity was observed at all doses ≥ 1 mg/kg (Brahmer *et al.*, 2012). ORs (confirmed complete [CR] or partial [PR] responses) were observed in patients with MEL, NSCLC, RCC, and OV (Table 7), as illustrated by representative spider plots and CT scans (Figures 10-13), and many ORs were also durable (Table 8). Four additional patients had a persistent reduction in target lesions in the presence of new lesions, consistent with an "immune-related" pattern of response; however, for the purpose of calculating response rates, they were not categorized as responders. Antitumor responses and/or prolonged stable disease (SD) were observed in patients with a variety of prior therapies received. ORs were observed even in patients with an extensive burden of metastatic disease.

In patients with MEL, there were 9 ORs across the 1, 3, and 10 mg/kg dose levels, with response rates of 6%, 29%, and 19%, respectively. Three MEL patients achieved a CR. All 9 MEL patients who experienced an OR started treatment ≥ 1 year prior to data analysis; of these 5 had a response duration ≥ 1 year. Additionally 14 MEL patients (27%) had SD lasting ≥ 24 weeks. In patients with NSCLC, there were 5 ORs amongst the 3 and 10 mg/kg dose levels, with response rates of 8% and 16%, respectively. There were ORs in patients with non-squamous (n=4) or squamous histology (n=1). All 5 NSCLC responders started treatment ≥ 24 weeks prior to data analysis; of these, 3 had responses lasting ≥ 24 weeks. Six additional NSCLC patients had SD lasting ≥ 24 weeks. There was 1 PR out of 17 patients with OV (6% response rate) and 3 patients (18%) with SD lasting ≥ 24 weeks, all at the 10 mg/kg dose. In patients with RCC, there were ORs in 2 of 17 (12%) patients treated at 10 mg/kg with responses lasting 4 and 18 months, respectively. Seven additional RCC patients had SD lasting ≥ 24 weeks.

Table 7. Clinical Activity of BMS-936559 in 160 Patients, Response Evaluable*

Tumor Type	Dose (mg/kg)	n	ORR§ No. patients (%) [95% CI]	SD ≥ 24 wk No. patients (%) [95% CI]	PFSR** at 24 wk (%) [95% CI]
MEL	0.3	1	0 [0-98]	0 [0-98]	N/A
	1	18	1 (6) [0.1-27]	6 (33) [13-59]	39 [16-61]
	3	17	5 (29)† [10-56]	3 (18) [4-43]	47 [21-72]
	10	16	3 (19)†† [4-46]	5 (31) [11-59]	44 [19-68]
ALL MEL		**52**	**9 (17) (8-30)**	**14 (27) [16-41]**	**42 [28-56]**
NSCLC§	1	11	0 [0-29]	0 [0-29]	N/A
Squamous	1	1	0 [0-98]	0 [0-98]	N/A
Non-Squamous	1	10	0 [0-31]	0 [0-31]	N/A
	3	13	1 (8) [0.2-36]	1 (8) [0.2-36]	34 [7-60]
Squamous	3	4	0 [0-60]	1 (25) [0.6-81]	50 [1-99]
Non-Squamous	3	9	1 (11) [0.3-48]	0 [0-34]	25 [0-55]
	10	25	4 (16) [5-36]	5 (20) [7-41]	46 [25-67]
Squamous	10	8	1 (13) [0.3-53]	2 (25) [3-65]	47 [10-83]
Non-Squamous	10	17	3 (18) [4-43]	3 (18) [4-43]	46 [20-72]
ALL NCSLC		**49**	**5 (10) [3-22]**	**6 (12) [5-25]**	**31 [17-45]**
ALL Squamous		13	1 (8) [0.2-36]	3 (23.1) [5-54]	43 [15-71]
ALL Non-Squamous		36	4 (11) [3-26]	3 (8) [2-23]	26 [10-42]
OV	3	1	0 [0-98]	0 [0-98]	N/A
	10	16	1 (6) [0.2-30]	3 (19) [4-46]	25 [4-46]
ALL OV		**17**	**1 (6) [0.1-29]**	**3 (18) [4-43]**	**22 [2-43]**
RCC	10	17	2 (12) [2-36]	7 (41) [18-67]	53 [29-77]

CI denotes Confidence intervals, MEL melanoma, RCC renal cell carcinoma, NSCLC non-small-cell lung cancer, OV ovarian cancer, RCC renal cell carcinoma, N/A not applicable, ORR objective response rate (complete response + partial response), SD stable disease, and PFSR progression-free survival rate.

*Efficacy population consists of response-evaluable patients who initiated treatment at least 7 months prior to the date of analysis and had measurable disease at a baseline tumor assessment and at least one of the following: an on-study tumor assessment, clinical progression, or death.

†Includes two CRs.

††Includes one CR.

§Objective response rates ({[CR + PR] ÷ n} × 100) are based on confirmed responses only, with confidence intervals calculated using the Clopper-Pearson method.

** Progression-free survival rate was the proportion of patients who did not progress, and were alive at 24 weeks, calculated by the Kaplan-Meier methodology, with confidence intervals using the Greenwood method

Individual patient responses were adjudicated per RECIST v1.0 with modification (see study protocol in Brahmer *et al.* (2012) *N Engl J Med* (submitted) for additional information).

Table 8. Duration of Objective Responses to BMS-936559*

Tumor Type	Dose (mg/kg)	No. Patients with OR	Duration of Response (months)†
MEL	1	1	6.9
	3	5	23.5+, 22.9+, 16.2+, 4.1+, 3.5
	10	4	24.6+, 23.9+, 18.0+, 17.0
NSCLC	1	0	9.2+
	3	1	2.3+
	10	4	16.6+, 12.6+, 9.8, 3.5
RCC	10	2	17, 4
OV	10	1	1.3+

*MEL denotes melanoma, NSCLC non-small-cell lung cancer, RCC renal-cell cancer, OV ovarian cancer.

†Time from first response to time of documented progression, death, or for censored data Denoted by +), time to last tumor assessment.

Sequence Listing Summary

SEQ ID NO:	Description
1	V_H amino acid sequence of 17D8
2	V_H amino acid sequence of 2D3
3	V_H amino acid sequence of 4H1
4	V_H amino acid sequence of 5C4
5	V_H amino acid sequence of 4A11
6	V_H amino acid sequence of 7D3
7	V_H amino acid sequence of 5F4
8	V_L amino acid sequence of 17D8
9	V_L amino acid sequence of 2D3
10	V_L amino acid sequence of 4H1
11	V_L amino acid sequence of 5C4
12	V_L amino acid sequence of 4A11
13	V_L amino acid sequence of 7D3
14	V_L amino acid sequence of 5F4
15	V_H amino acid sequence of 3G10
16	V_H amino acid sequence of 12A4
17	V_H amino acid sequence of 10A5
18	V_H amino acid sequence of 5F8

SEQ ID NO:	Description
19	V_H amino acid sequence of 10H10
20	V_H amino acid sequence of 1B12
21	V_H amino acid sequence of 7H1
22	V_H amino acid sequence of 11E6
23	V_H amino acid sequence of 12B7
24	V_H amino acid sequence of 13G4
25	V_L amino acid sequence of 3G10
26	V_L amino acid sequence of 12A4
27	V_L amino acid sequence of 10A5
28	V_L amino acid sequence of 5F8
29	V_L amino acid sequence of 10H10
30	V_L amino acid sequence of 1B12
31	V_L amino acid sequence of 7H1
32	V_L amino acid sequence of 11E6
33	V_L amino acid sequence of 12B7
34	V_L amino acid sequence of 13G4
35	V_H amino acid sequence of 28-8
36	V_L amino acid sequence of 28-8

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CLAIMS

What is claimed is:

1. A method of treating a subject afflicted with a cancer comprising administering to the subject a therapeutically effective amount of an antibody or an antigen-binding portion thereof that disrupts the interaction between Programmed Death-1 (PD-1) and Programmed Death Ligand-1 (PD-L1).

2. The method of claim 1, wherein the antibody or antigen-binding portion thereof binds specifically to PD-1.

3. The method of claim 1, wherein the antibody or antigen-binding portion thereof binds specifically to PD-L1.

4. The method of claim 1, wherein the subject has been pre-treated for the cancer.

5. The method of claim 1, wherein the cancer is an advanced, metastatic and/or refractory cancer.

6. The method of claim 1, wherein the administration of the antibody or antigen-binding portion thereof induces a durable clinical response in the subject.

7. The method of claim 3, wherein the cancer is selected from the group consisting of melanoma, renal cell carcinoma, squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, colorectal cancer, castration-resistant prostate cancer, ovarian cancer, gastric cancer,), hepatocellular carcinoma, pancreatic carcinoma, squamous cell carcinoma of the head and neck, carcinomas of the esophagus, gastrointestinal tract and breast, and a hematological malignancy.

8. The method of claim 1, wherein the therapeutically effective amount of the antibody or antigen-binding portion thereof comprises a dose ranging from 0.1 to 10.0 mg/kg body weight which is administered at a dosing schedule of once per week, once every two weeks, or once a month.

9. A method for immunotherapy of a subject afflicted with cancer, which method comprises:

   (a) selecting a subject that is a suitable candidate for immunotherapy, the selecting comprising:

(i) providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells;

(ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; and

(iii) selecting the subject as a suitable candidate based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and

(b) administering a composition comprising a therapeutically effective amount of an anti-PD-1 antibody to the selected subject.

10. The method of claim 9, wherein the proportion of cells 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.

11. The method of claim 9, wherein the test tissue sample is a formalin-fixed paraffin-embedded (FFPE) tissue sample.

12. The method of claim 11, wherein the presence of PD-L1 polypeptide is determined using an automated IHC assay.

13. The method of claim 12, wherein the IHC assay is performed using an anti-PD-L1 monoclonal antibody to bind to the PD-L1 polypeptide, wherein the anti-PD-L1 monoclonal antibody is selected from 28-8, 28-1, 28-12, 29-8, and 5H1.

14. The method of claim 9, wherein the predetermined threshold level is 1% of tumor cells or a single tumor-infiltrating inflammatory cell expressing cell surface PD-L1 as determined by automated IHC using mAb 28-8.

15. A method for treatment of a subject afflicted with cancer, which method comprises:

(a) selecting a subject that is not suitable for anti-PD-1 antibody immunotherapy, the selecting comprising:

(i) providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells;

(ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; and

(iii) selecting the subject as not suitable for anti-PD-1 antibody immunotherapy based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is less than a predetermined threshold level; and

(b) administering a standard-of-care therapeutic other than an anti-PD-1 antibody to the selected subject.

16. A monoclonal antibody or an antigen-binding portion thereof that binds specifically to a cell surface-expressed PD-L1 polypeptide in a formalin-fixed, paraffin-embedded (FFPE) tissue sample.

17. The monoclonal antibody or antigen-binding portion thereof of claim 16, which antibody or portion thereof comprises the CDR1, CDR2 and CDR3 regions in a heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 35, and the CDR1, CDR2 and CDR3 regions in a light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 36.

18. The monoclonal antibody or antigen-binding portion thereof of claim 16, which antibody or portion thereof comprises a heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 35, and a light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 36.

CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING

ABSTRACT

The disclosed invention provides a method for immunotherapy of a subject afflicted with cancer, comprises administering to the subject a composition comprising a therapeutically effective amount of an antibody that inhibits signaling from the PD-1/PD-L1 signaling pathway. This invention also provides a method for immunotherapy of a subject afflicted with cancer comprising selecting a subject that is a suitable candidate for immunotherapy based on an assessment that the proportion of cells in a test tissue sample from the subject that express PD-L1 on the cell surface exceeds a predetermined threshold level, and administering a therapeutically effective amount of an anti-PD-1 antibody to the selected subject. The invention additionally provides rabbit mAbs that bind specifically to a cell surface-expressed PD-L1 antigen in a FFPE tissue sample, and an automated IHC method for assessing cell surface expression in FFPE tissues using the provided anti-PD-L1 Abs.

Application Data Sheet 37 CFR 1.76

Application Information

Title of the Invention	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
Attorney Docket Number	11981USPSP2
Large Entity Status Claimed	YES
Application Type	PROVISIONAL
Subject Matter	UTILITY
Total Number of Drawing Sheets (if any)	24
Sequence Submissions (if any)	YES

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Application Data Sheet 37 CFR 1.76

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Application Data Sheet 37 CFR 1.76

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Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

Application No	Continuity Type	Parent Application No./Status	Parent Filing Date
This Application			

Foreign Priority Information

This section allows for the applicant to claim benefit of foreign priority and to identify any prior foreign application for which priority is not claimed. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(a).

Application No	Country	Filing Date	Priority Claimed

Authorization to Permit Access

☑ Authorization to Permit Access to the Instant Application by the Participating Offices

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO),the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO),and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date of filing this Authorization.

Application Data Sheet 37 CFR 1.76

Applicant Information

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Participating Office			
Applicant 1: Bristol-Myers Squibb Company			
If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.			
☐ Assignee		☐ Legal Representative under 35 U.S.C 117	
☒ Person to whom the inventor is obligated to assign		☐ Person who shows sufficient proprietary interest	
If applicant is the legal representative, indicate the authority to file the patent application, the inventor is: ☐ Deceased ☐ Legally incapacitated			
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If the Applicant is an Organization check here ☑			
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Signature:

Signature	/Ashton J. Delauney/
Date	March 15, 2013
Attorney/Agent Name	ASHTON J. DELAUNEY
Registration Number	64,286
Telephone Number	609-252-4560

FIG. 1A

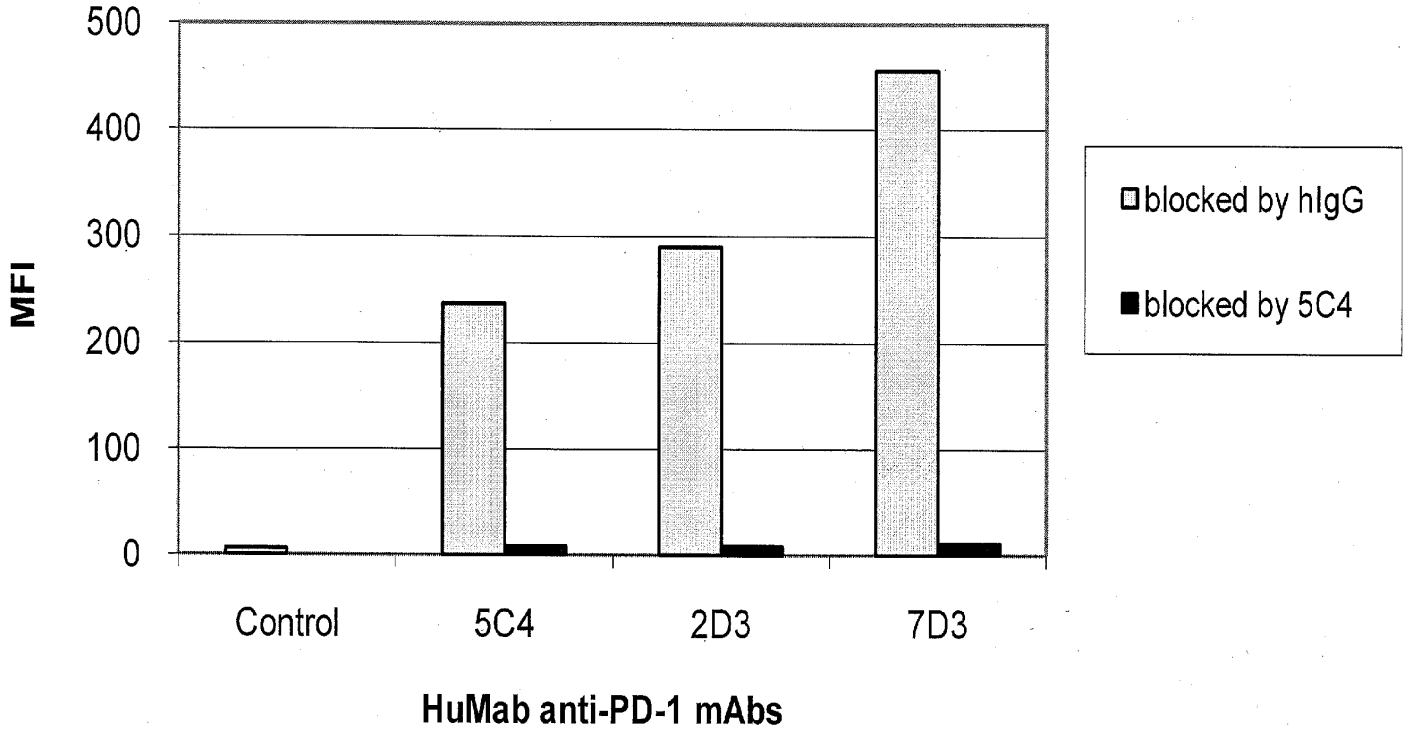

500
400
300
200
100
0
MFI
Control
5C4
2D3
7D3
HuMab anti-PD-1 mAbs
blocked by hIgG
blocked by 5C4

FIG. 1B

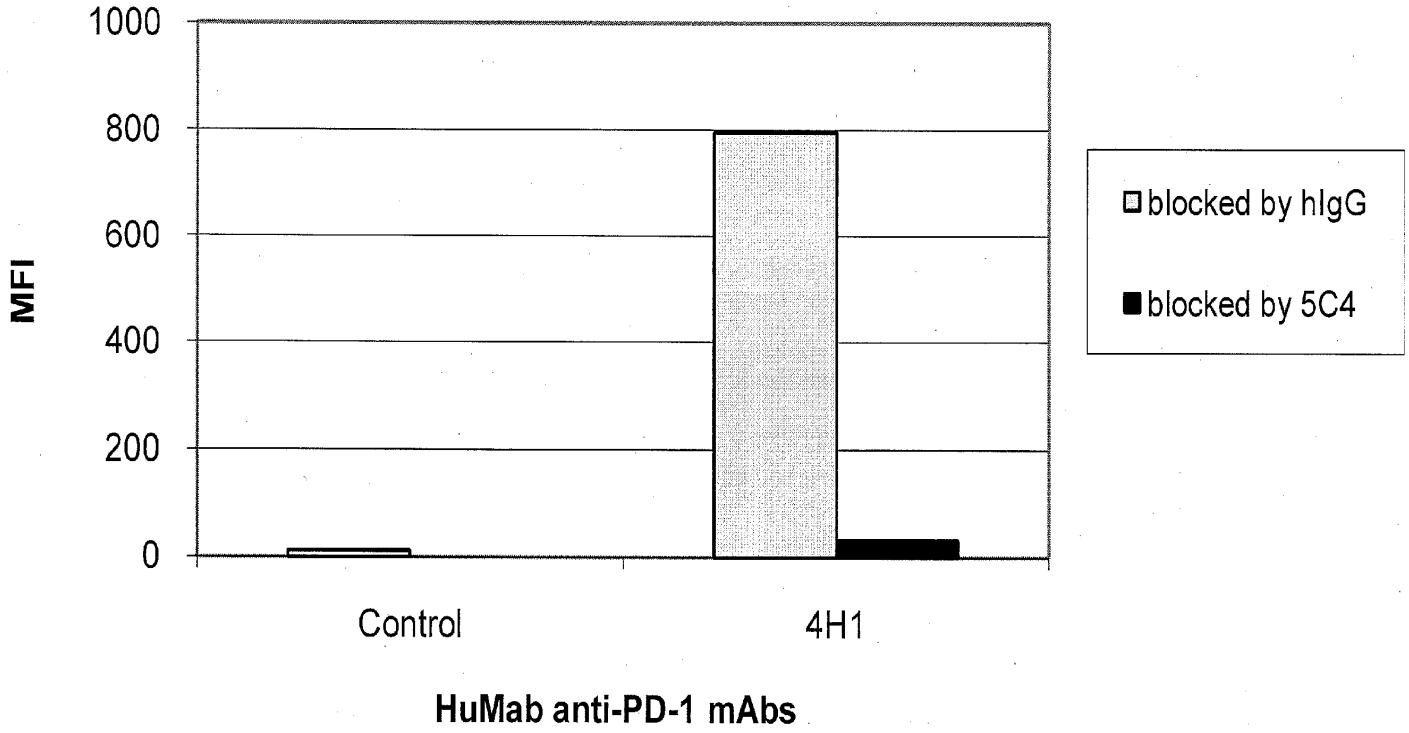

1000
800
600
400
200
0
MFI
Control
4H1
HuMab anti-PD-1 mAbs
blocked by hIgG
blocked by 5C4

FIG. 1C

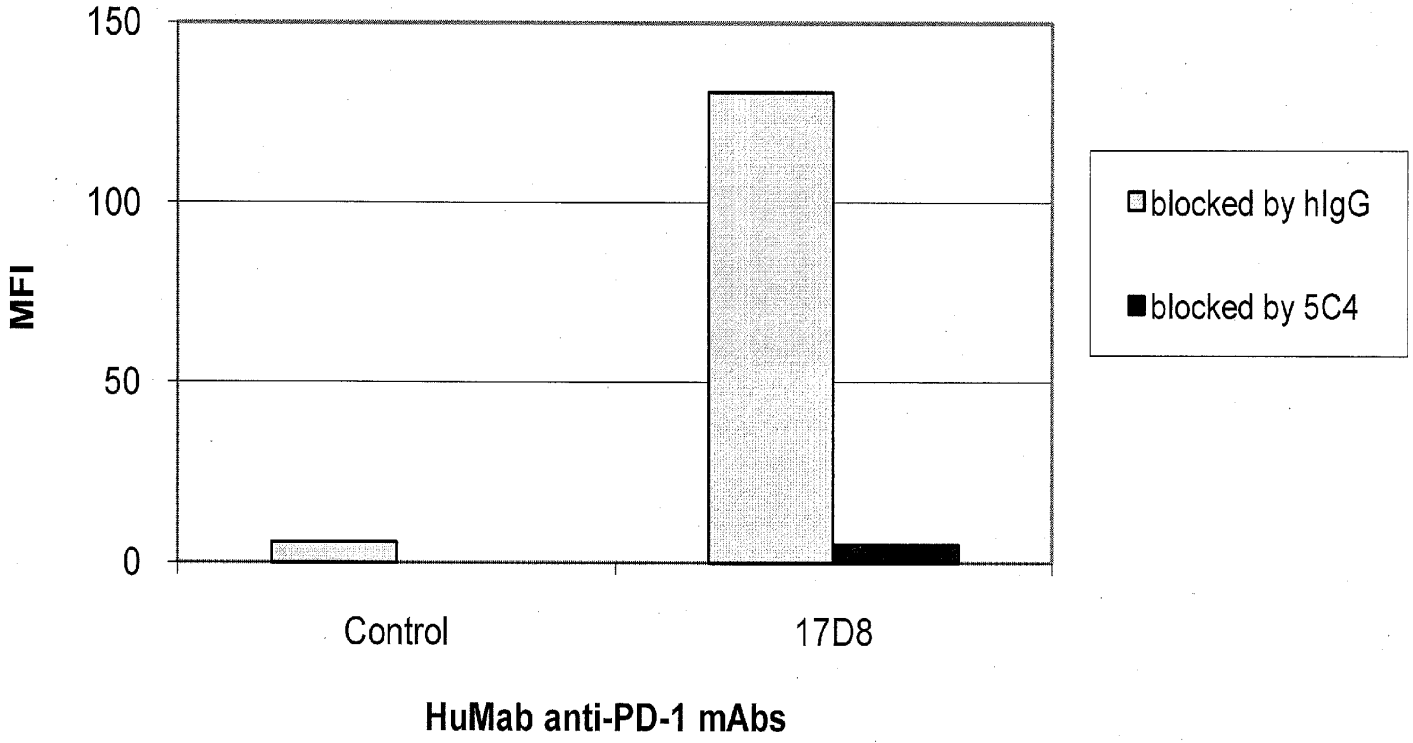

150
100
50
0
MFI
Control
17D8
HuMab anti-PD-1 mAbs
blocked by hIgG
blocked by 5C4

FIG. 2A

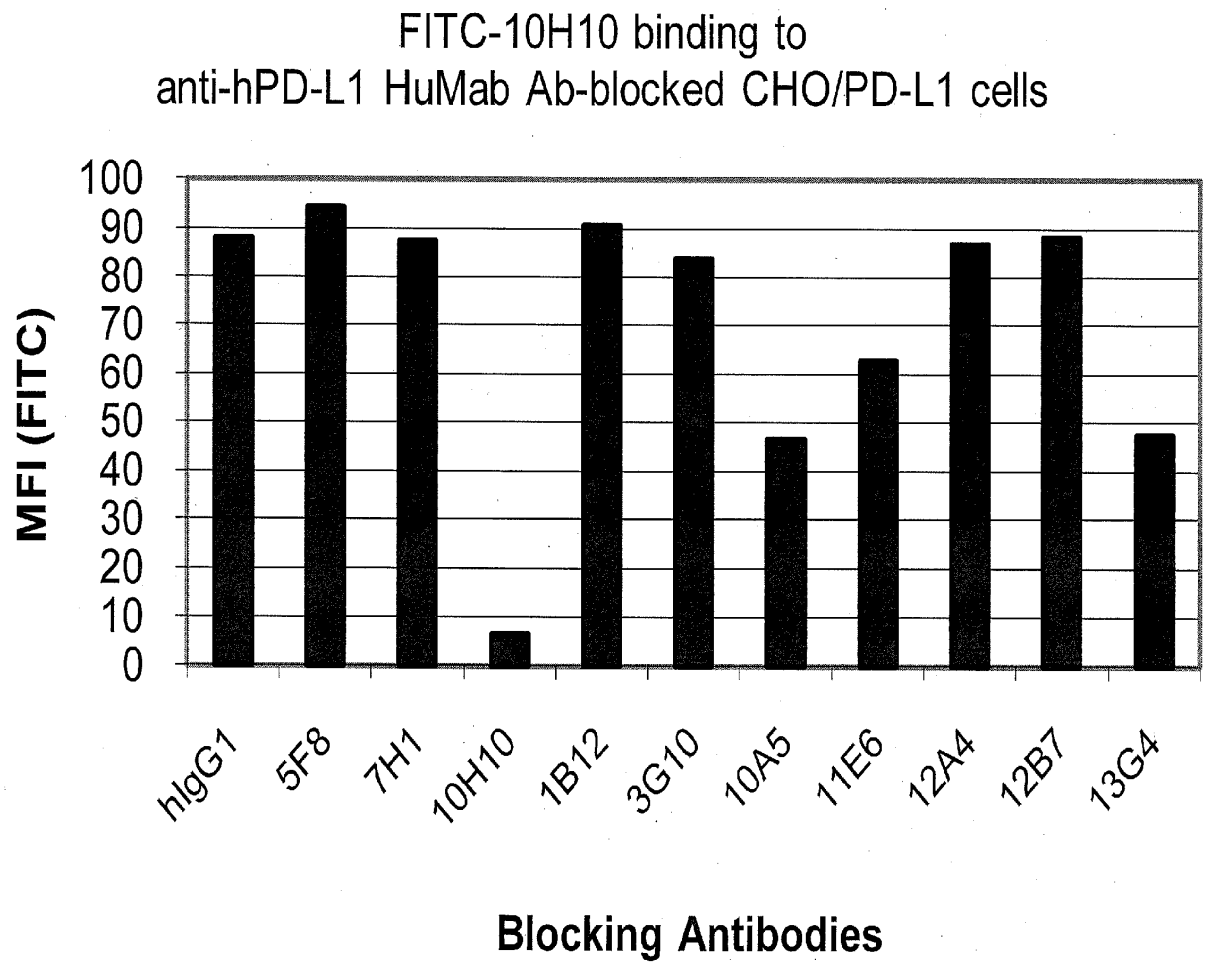

FITC-10H10 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
100
90
80
70
60
50
40
30
20
10
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2B

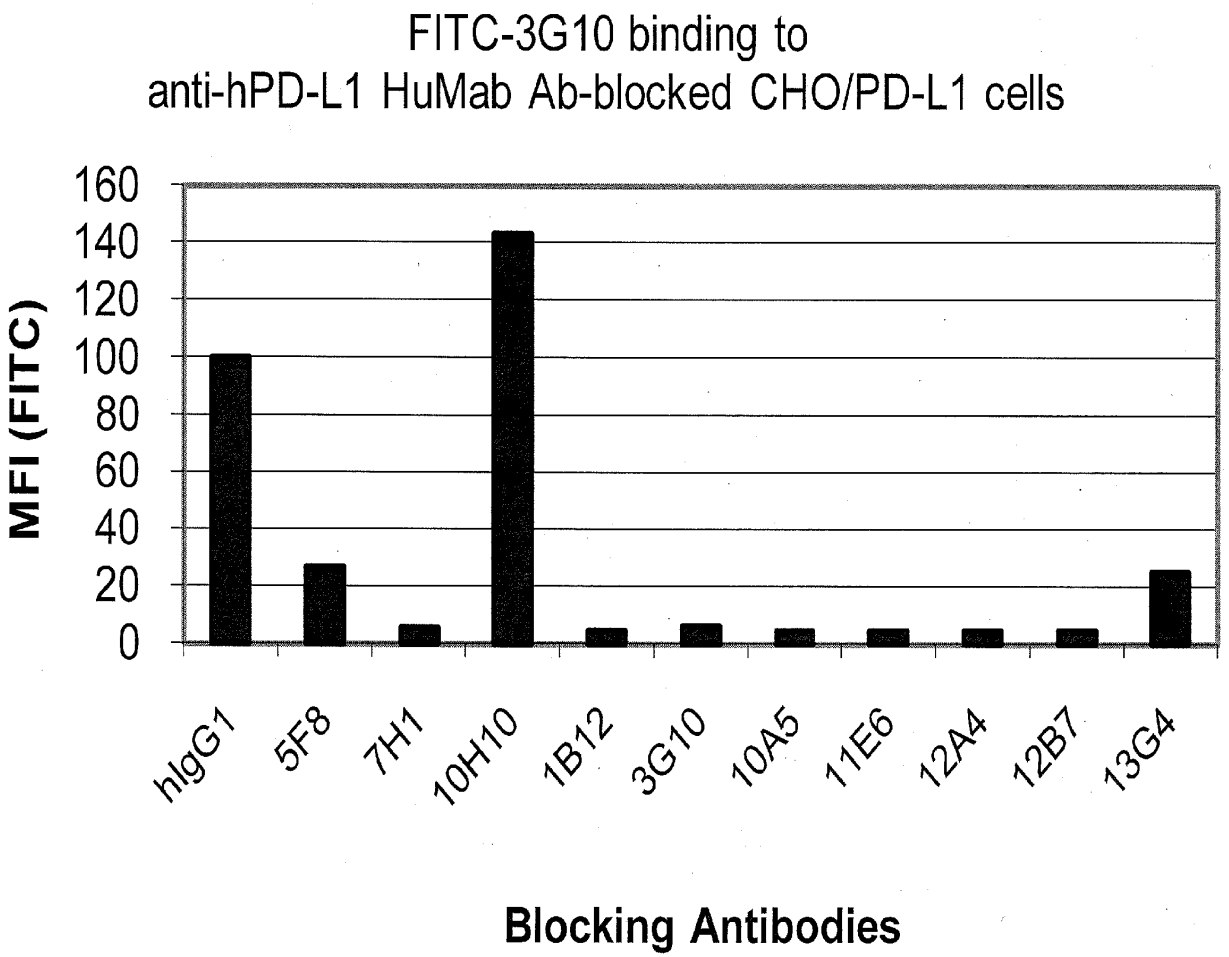

FITC-3G10 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
160
140
120
100
80
60
40
20
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2C

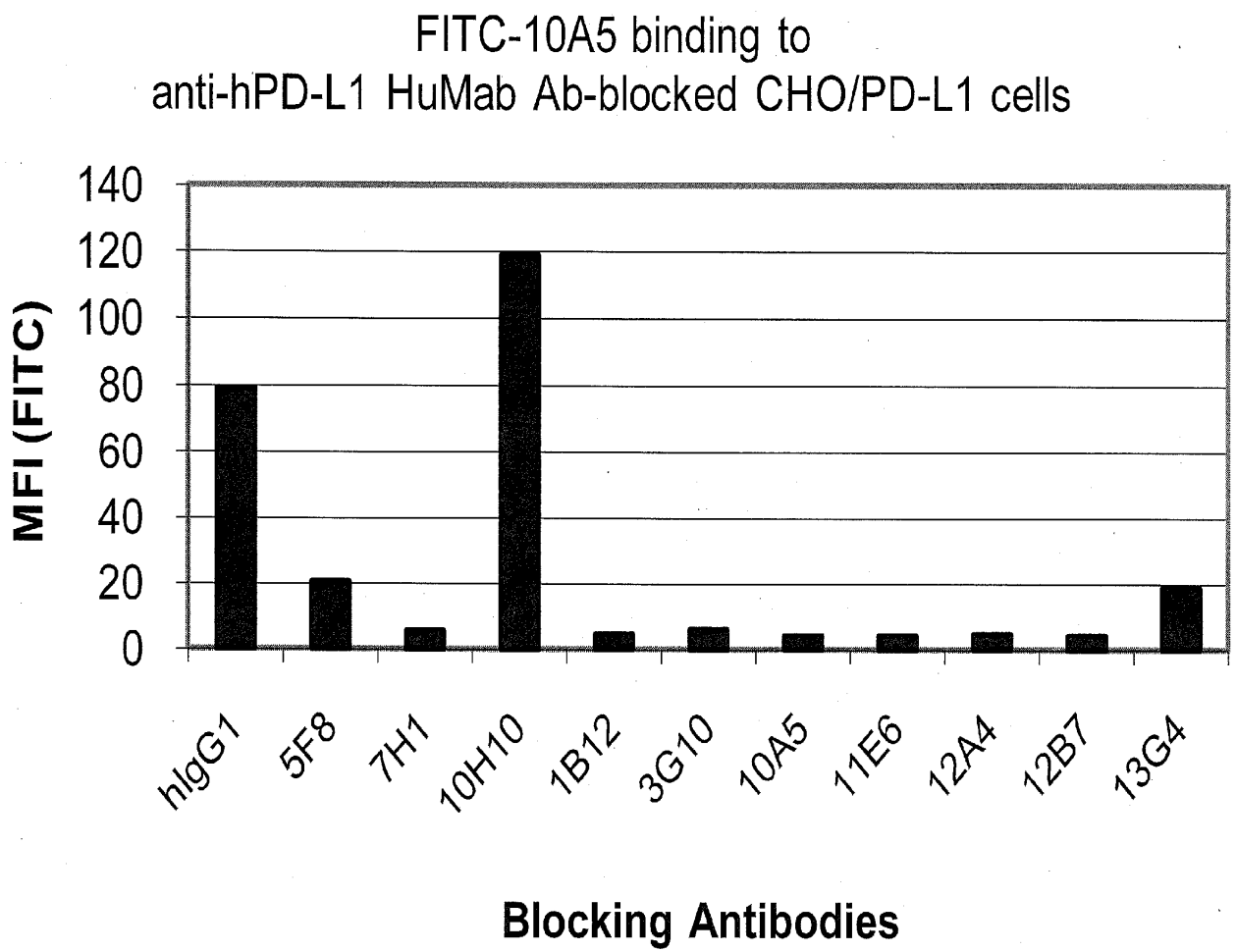

FITC-10A5 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
140
120
100
80
60
40
20
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2D

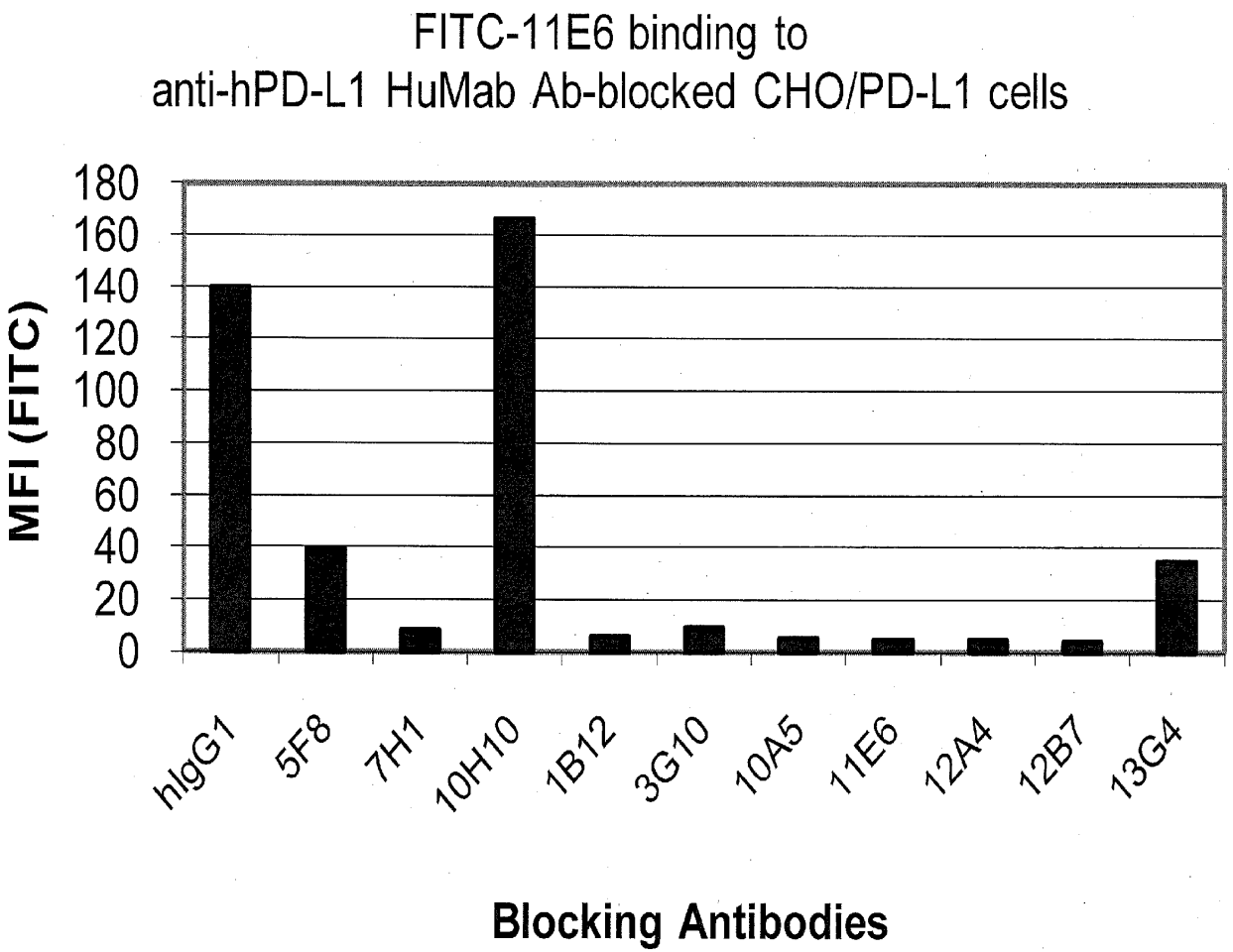

FITC-11E6 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
180
160
140
120
100
80
60
40
20
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2E

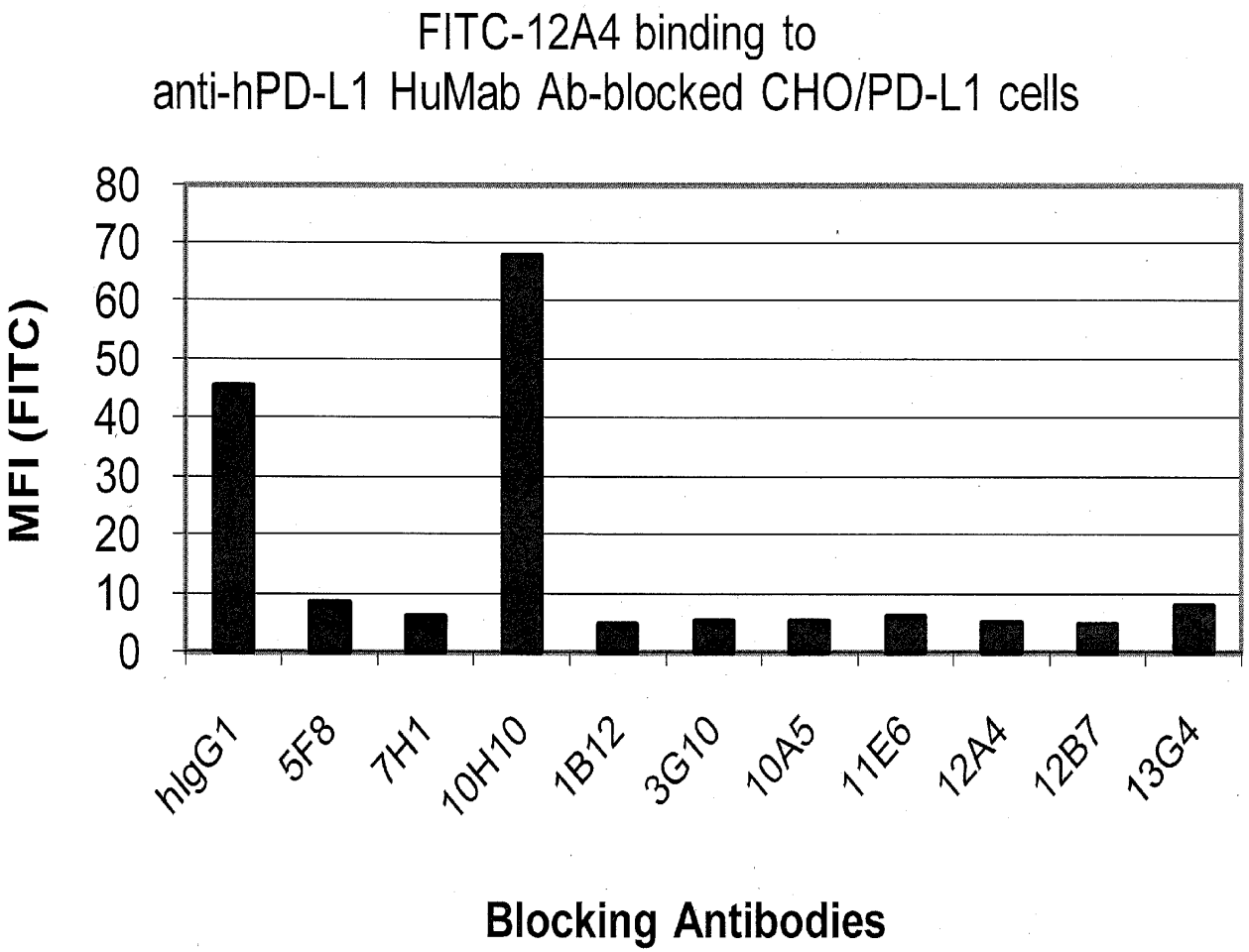

FITC-12A4 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
80
70
60
50
40
30
20
10
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2F

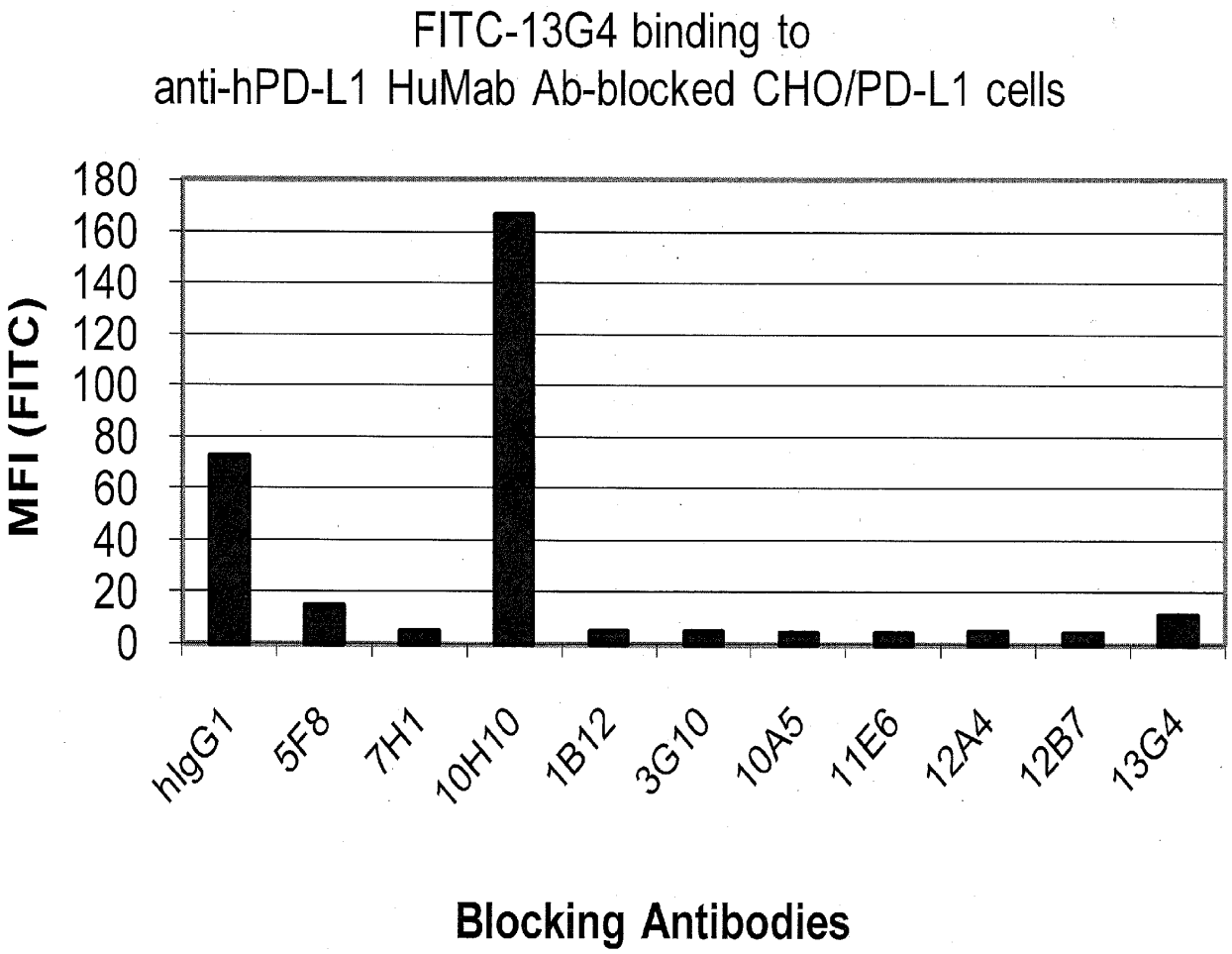

FITC-13G4 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
180
160
140
120
100
80
60
40
20
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 3

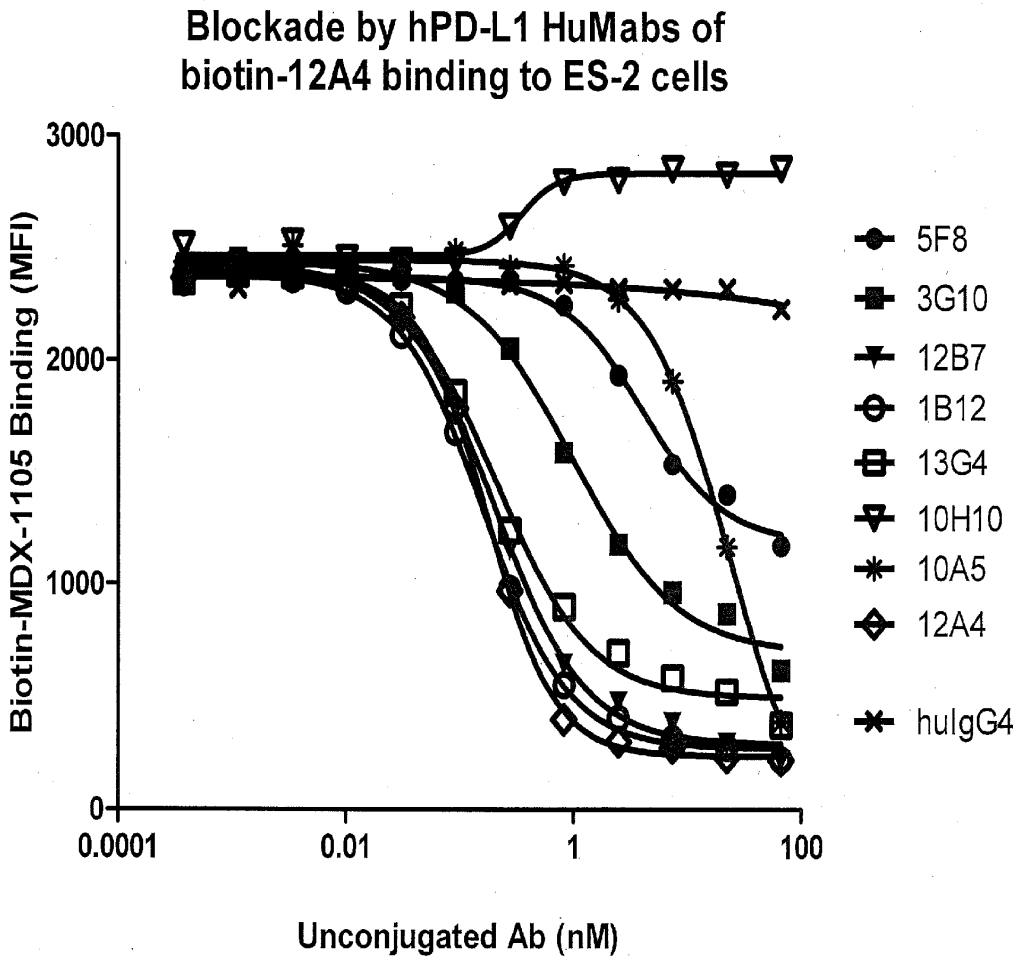

Blockade by hPD-L1 HuMabs of
biotin-12A4 binding to ES-2 cells
Biotin-MDX-1105 Binding (MFI)
3000
2000
1000
0
0.0001
0.01
1
100
Unconjugated Ab (nM)
5F8
3G10
12B7
1B12
13G4
10H10
10A5
12A4
huIgG4

FIG. 4

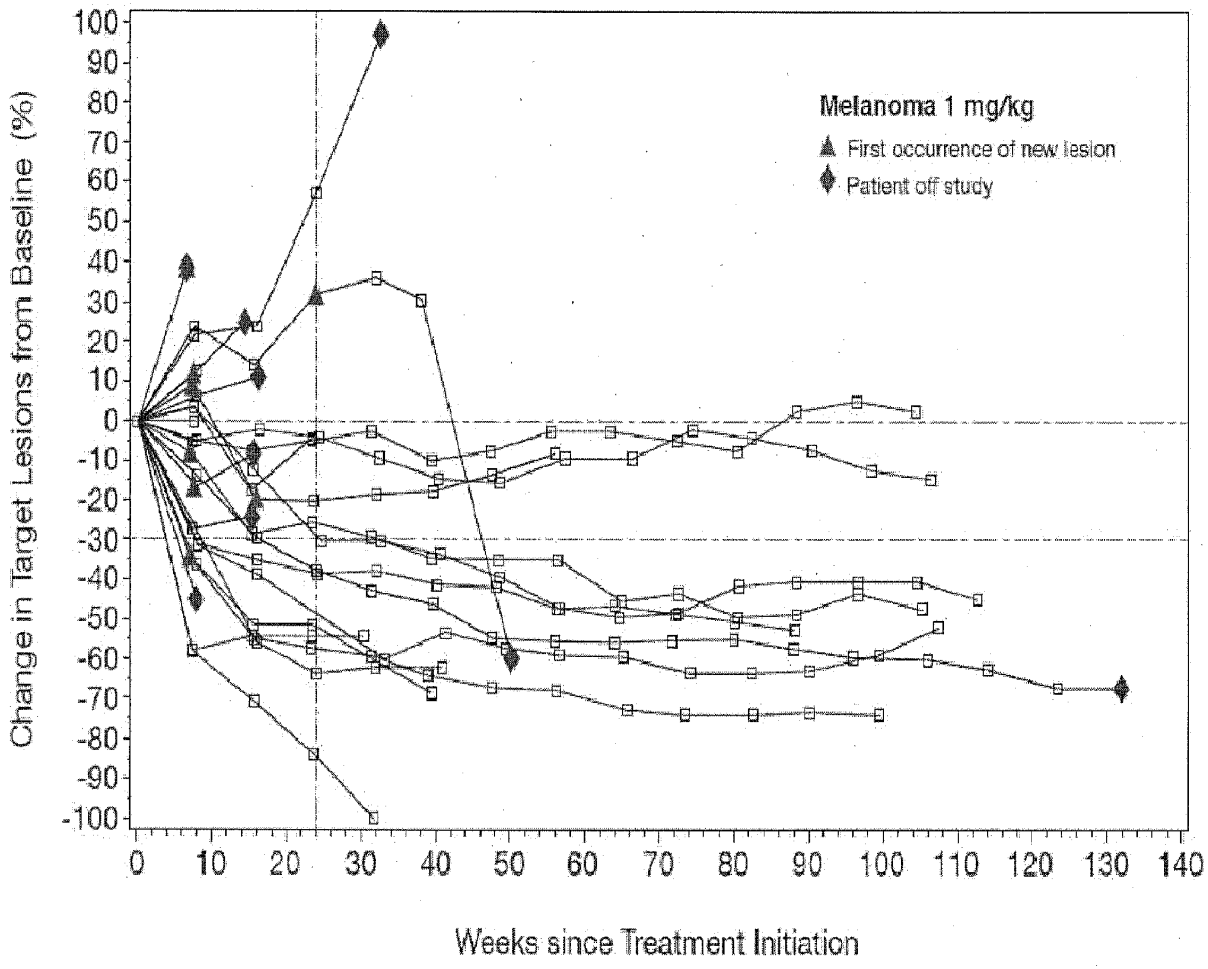

Melanoma 1 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
Weeks since Treatment Initiation

FIG. 5

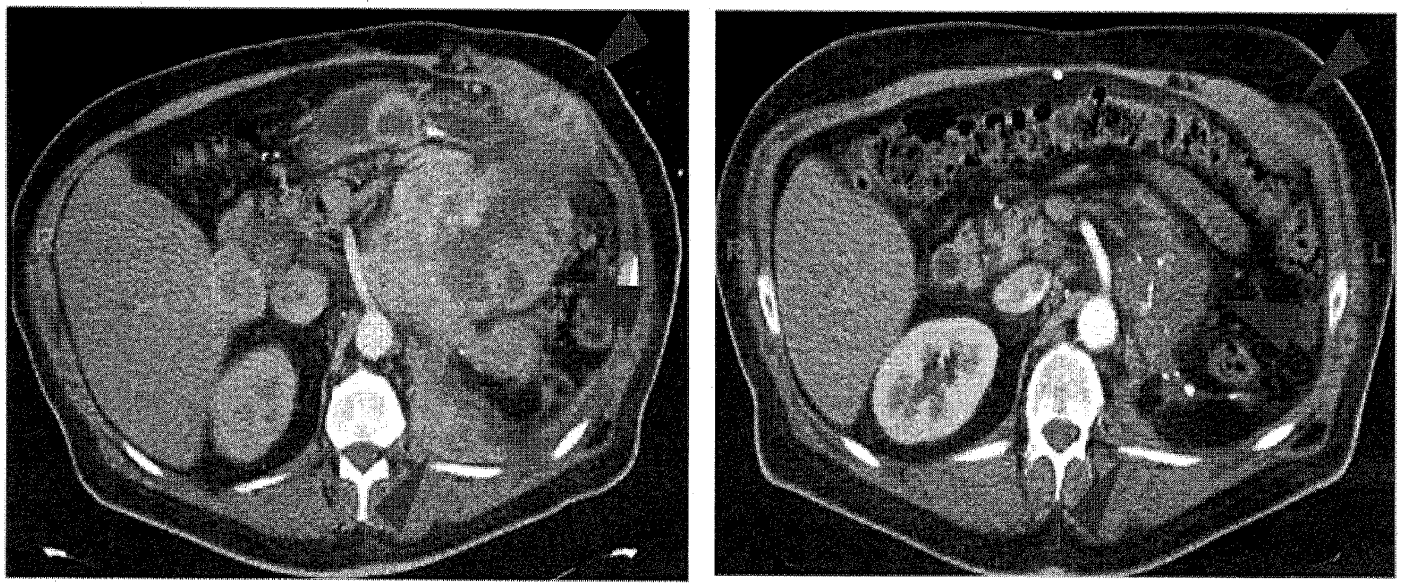


FIG. 6

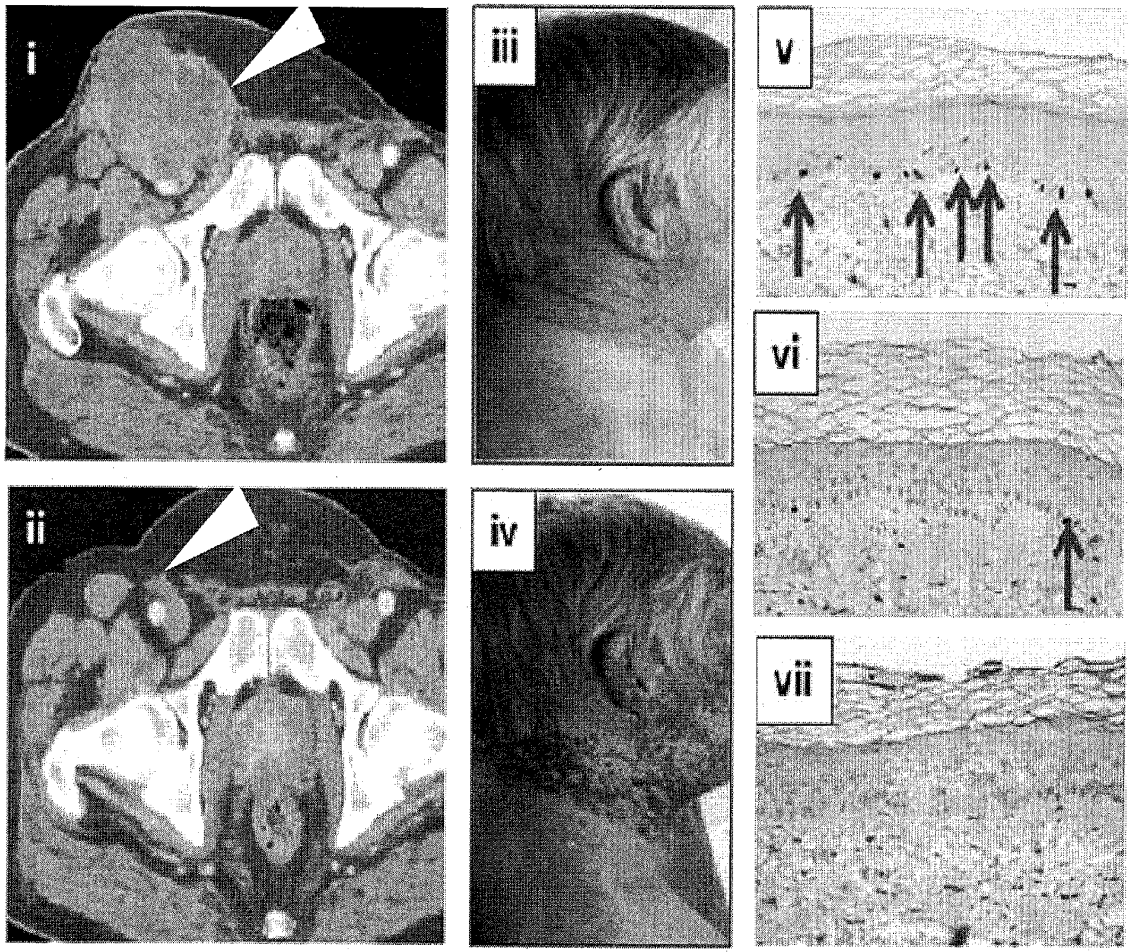


FIG. 7

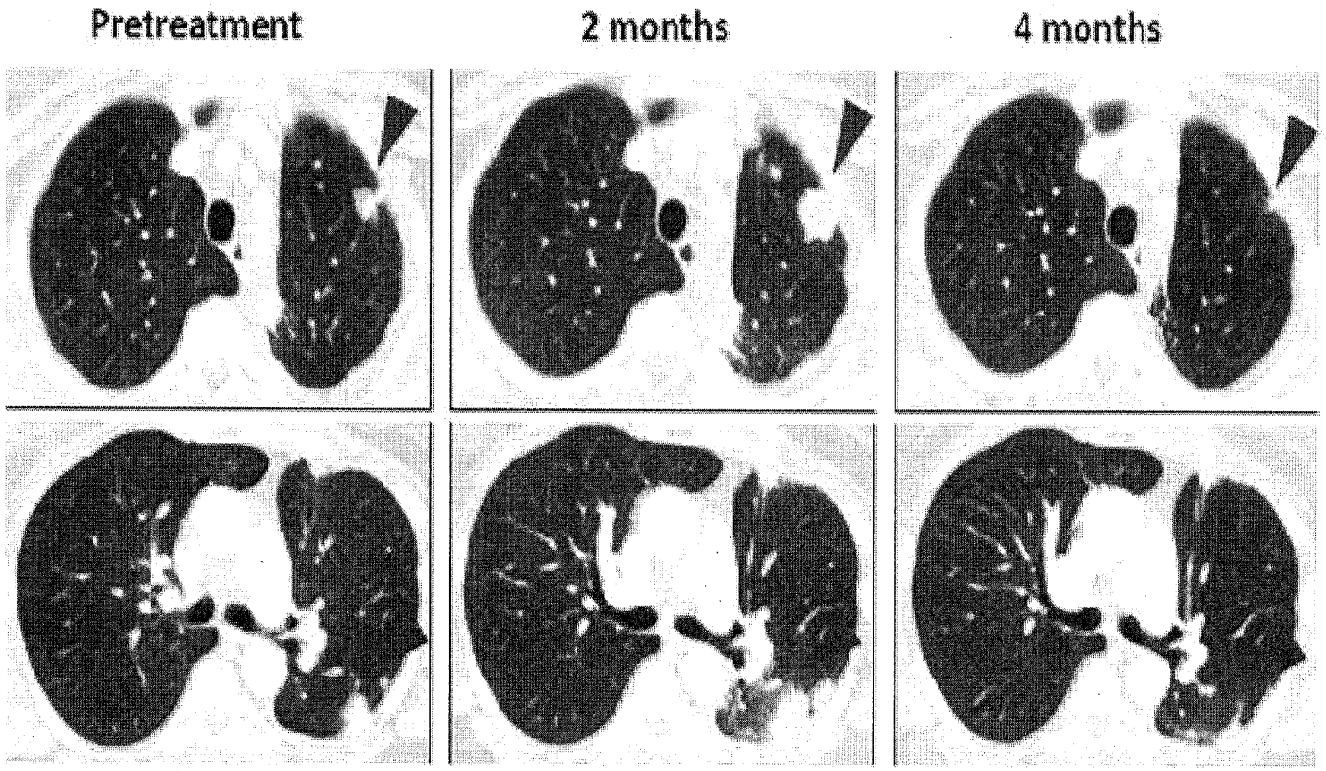
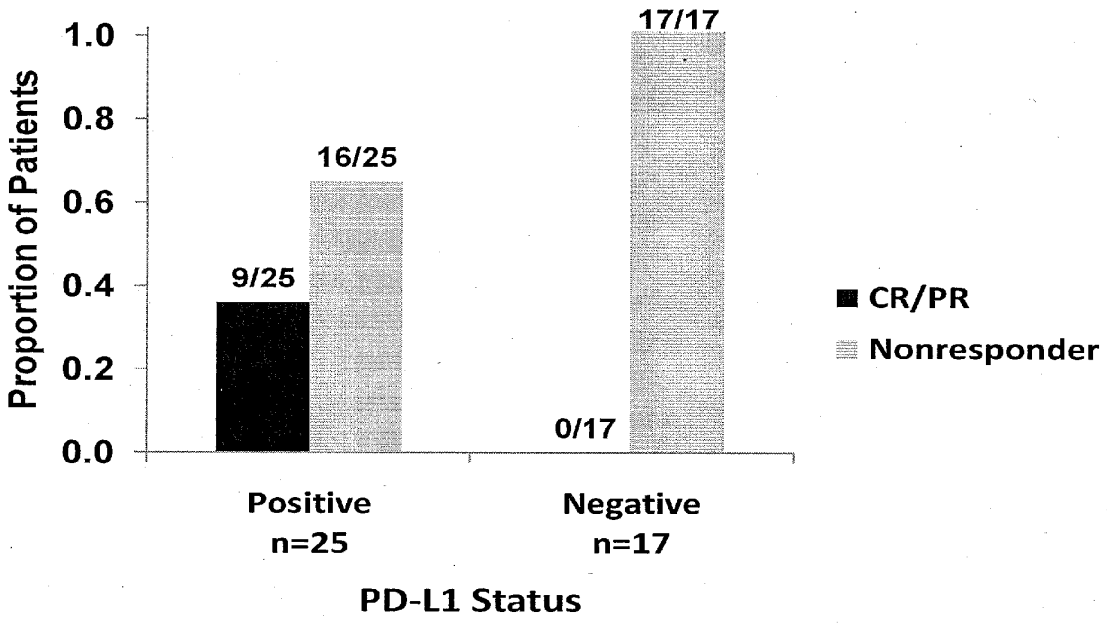


FIG. 8A



Association Between Pretreatment Tumor PD-L1 Expression and Clinical Response			
Response Status	**PD-L1-Positive no. (%)**	**PD-L1-Negative no. (%)**	**Total no. (%)**
CR/PR	9 (36)	0	9 (21)
Nonresponder	16* (64)	17 (100)	33 (79)
All Patients	25	17	42

Fisher's exact test for association P=0.006.

***Two patients categorized as nonresponders at the time of data analysis are still under evaluation.**

FIG. 8B

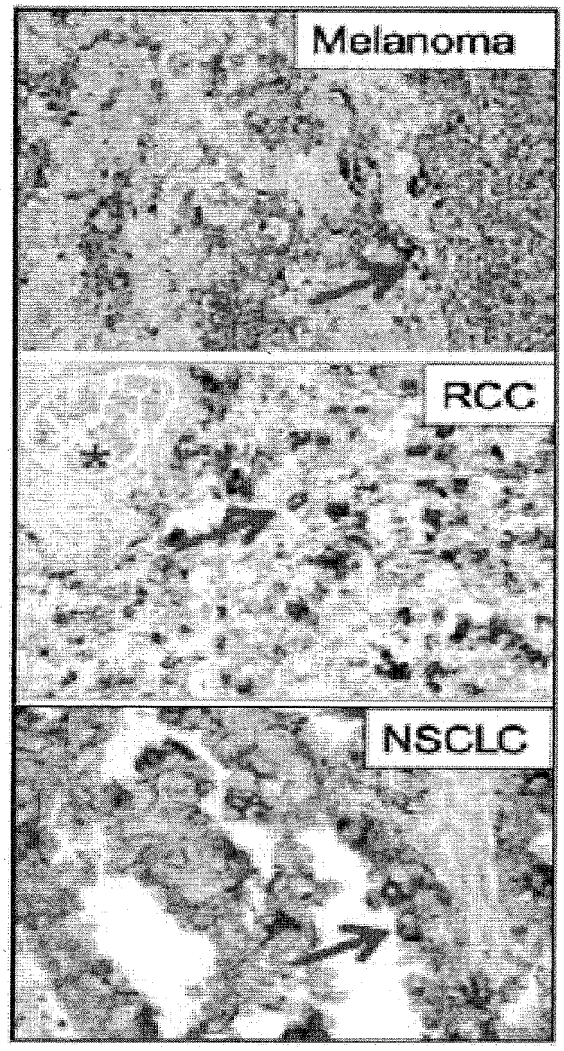


FIG. 9

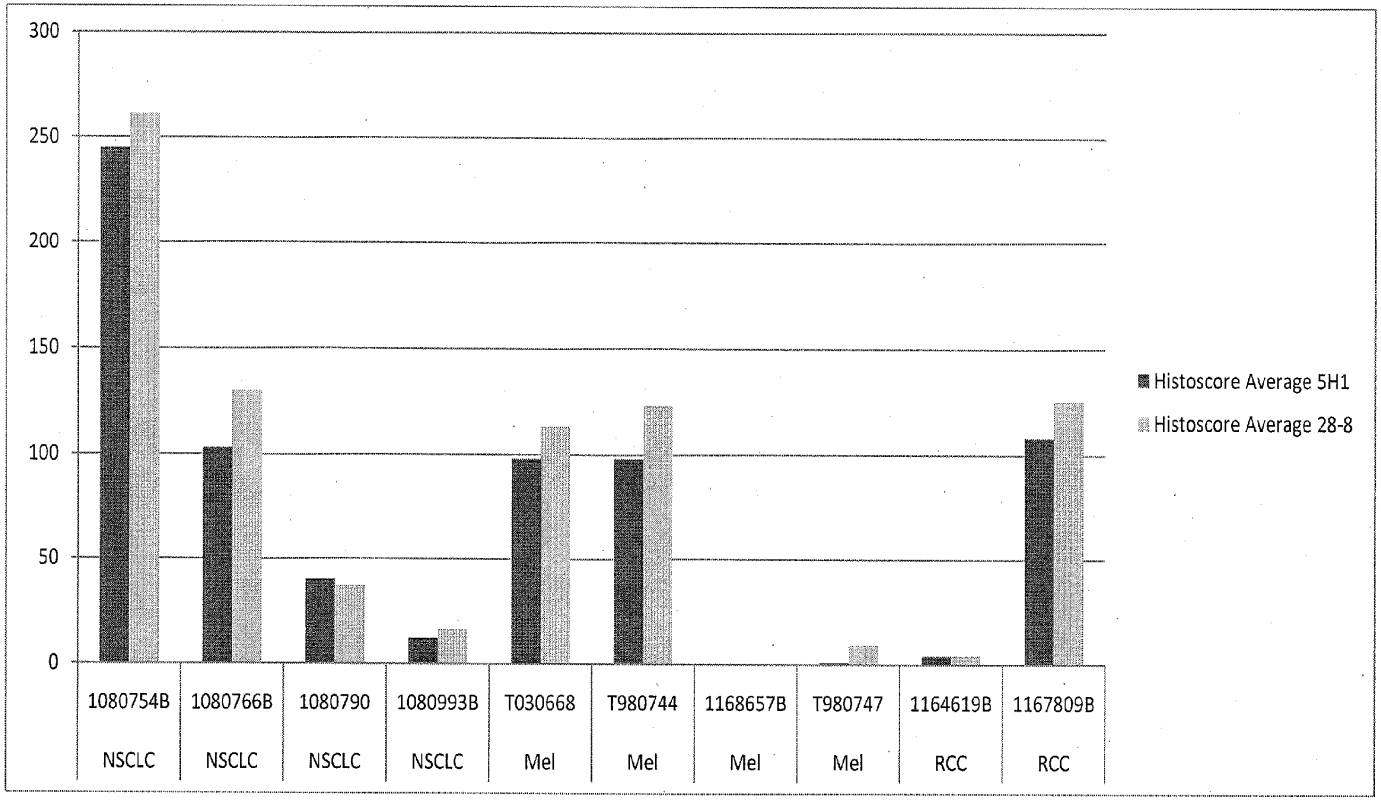

300
250
200
150
100
50
0
1080754B NSCLC
1080766B NSCLC
1080790 NSCLC
1080993B NSCLC
T030668 Mel
T980744 Mel
1168657B Mel
T980747 Mel
1164619B RCC
1167809B RCC
Histoscore Average 5H1
Histoscore Average 28-8

FIG. 10A

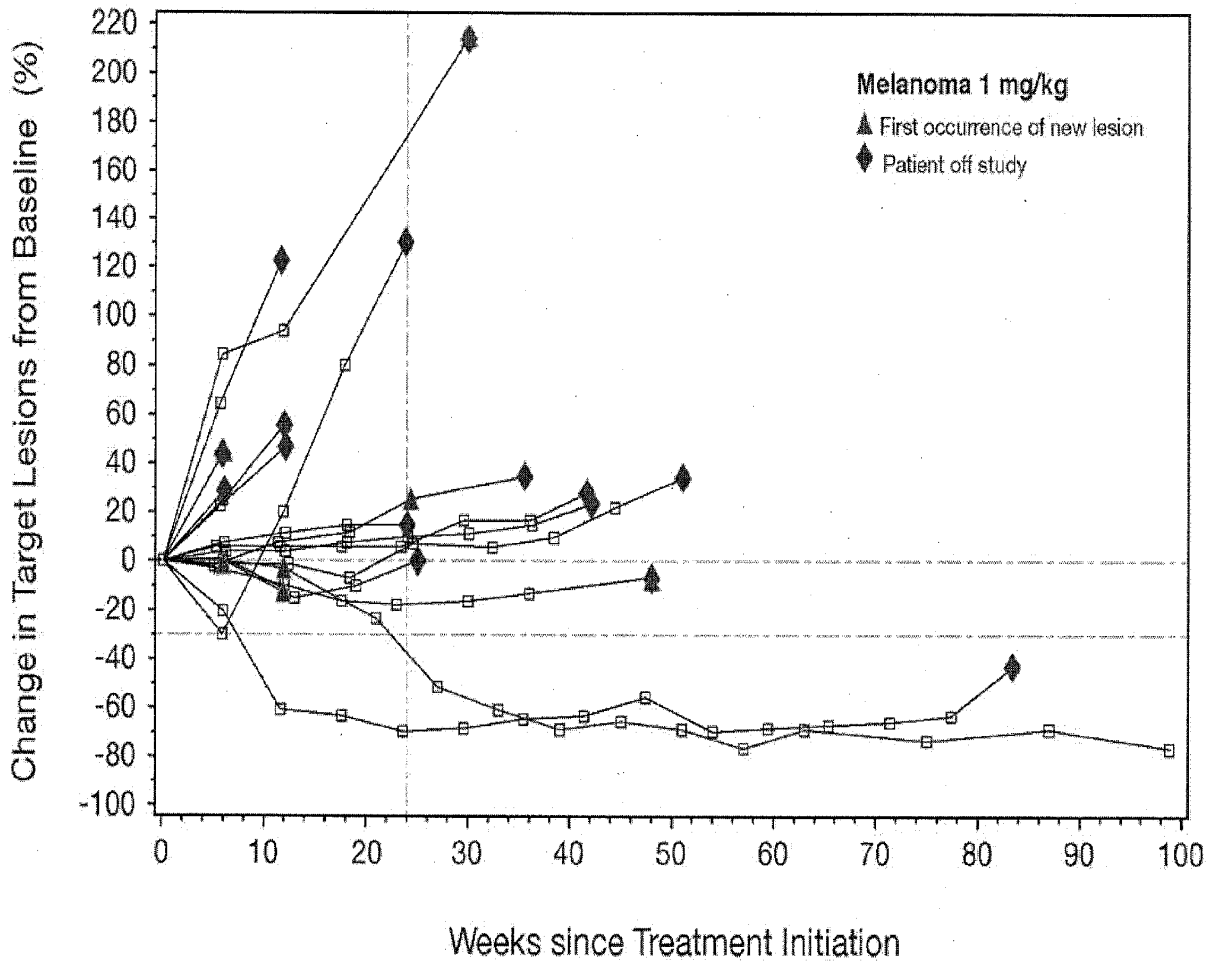

Melanoma 1 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
220
200
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
Weeks since Treatment Initiation

FIG. 10B

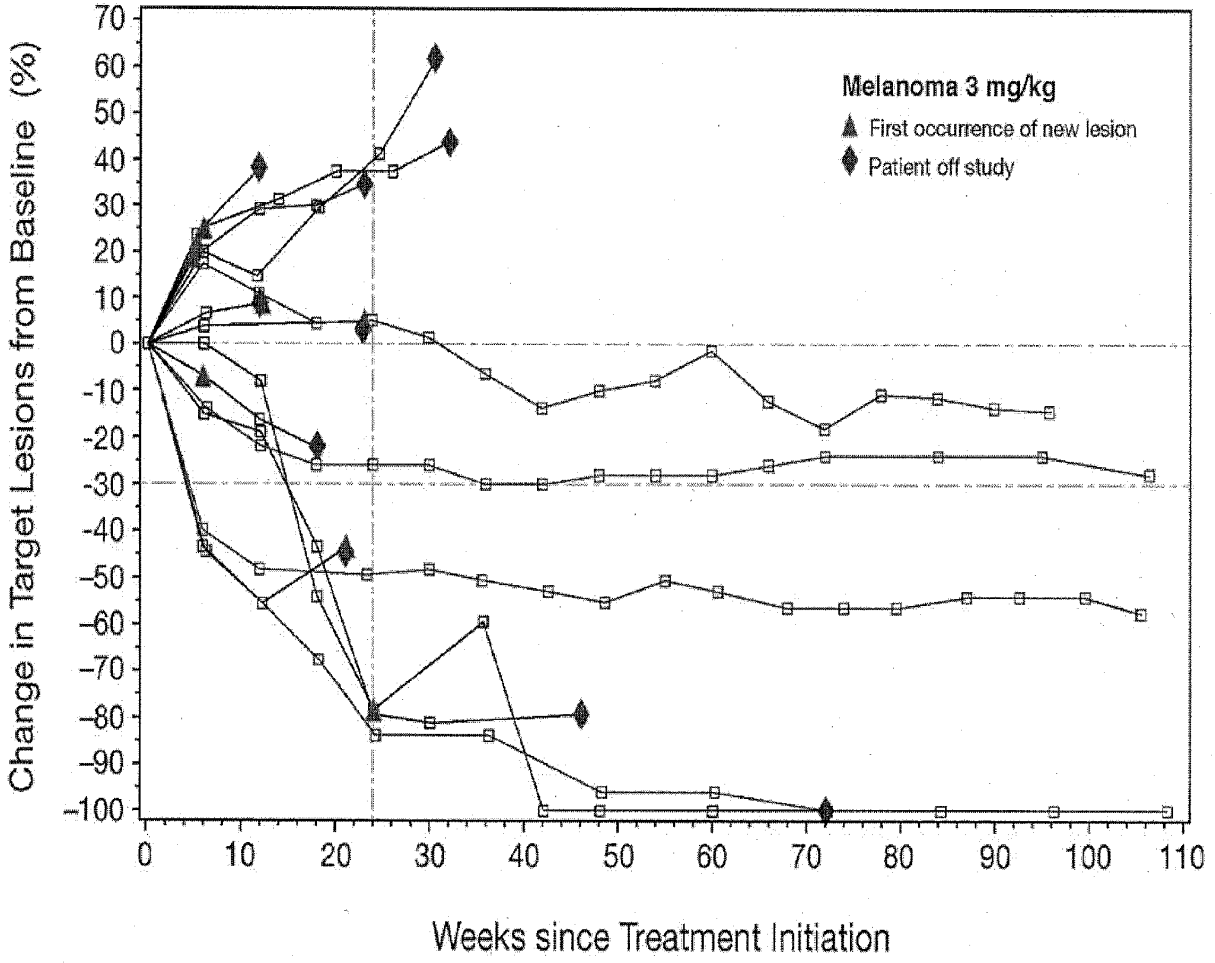

Melanoma 3 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
10
20
30
40
50
60
70
80
90
100
110
Weeks since Treatment Initiation

FIG. 10C

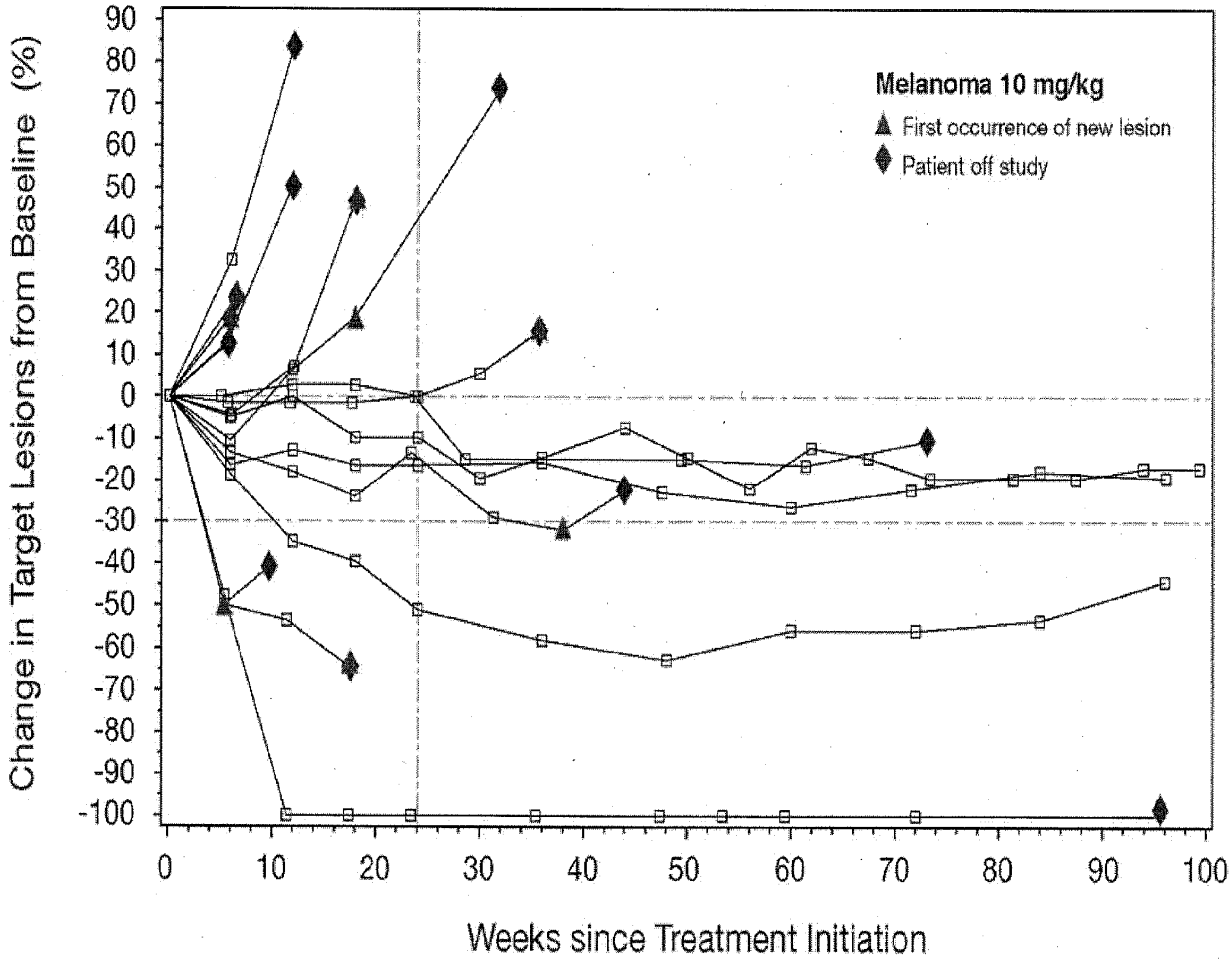

Melanoma 10 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
10
20
30
40
50
60
70
80
90
100
Weeks since Treatment Initiation

FIG. 10D

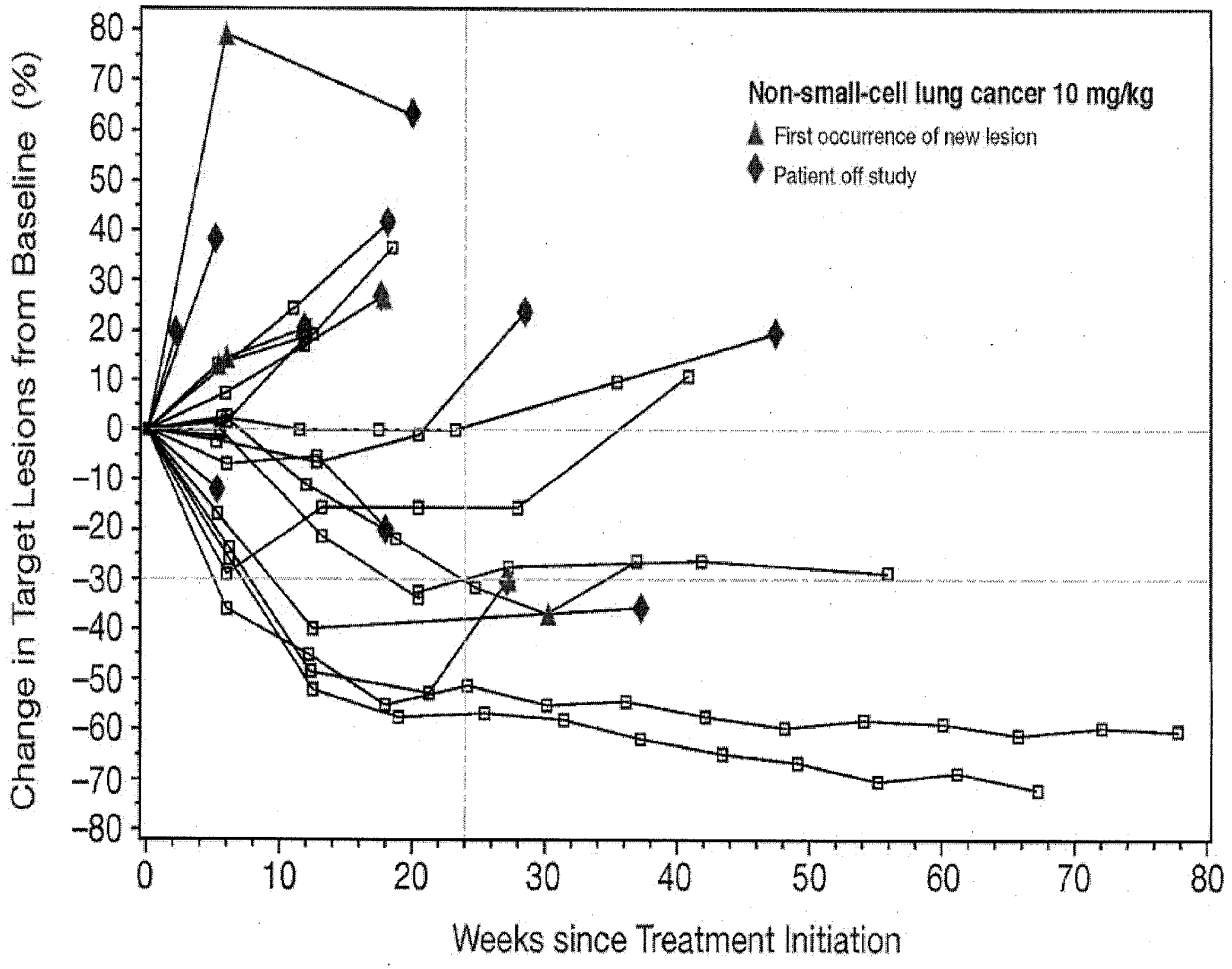

Non-small-cell lung cancer 10 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
0
10
20
30
40
50
60
70
80
Weeks since Treatment Initiation

FIG. 11

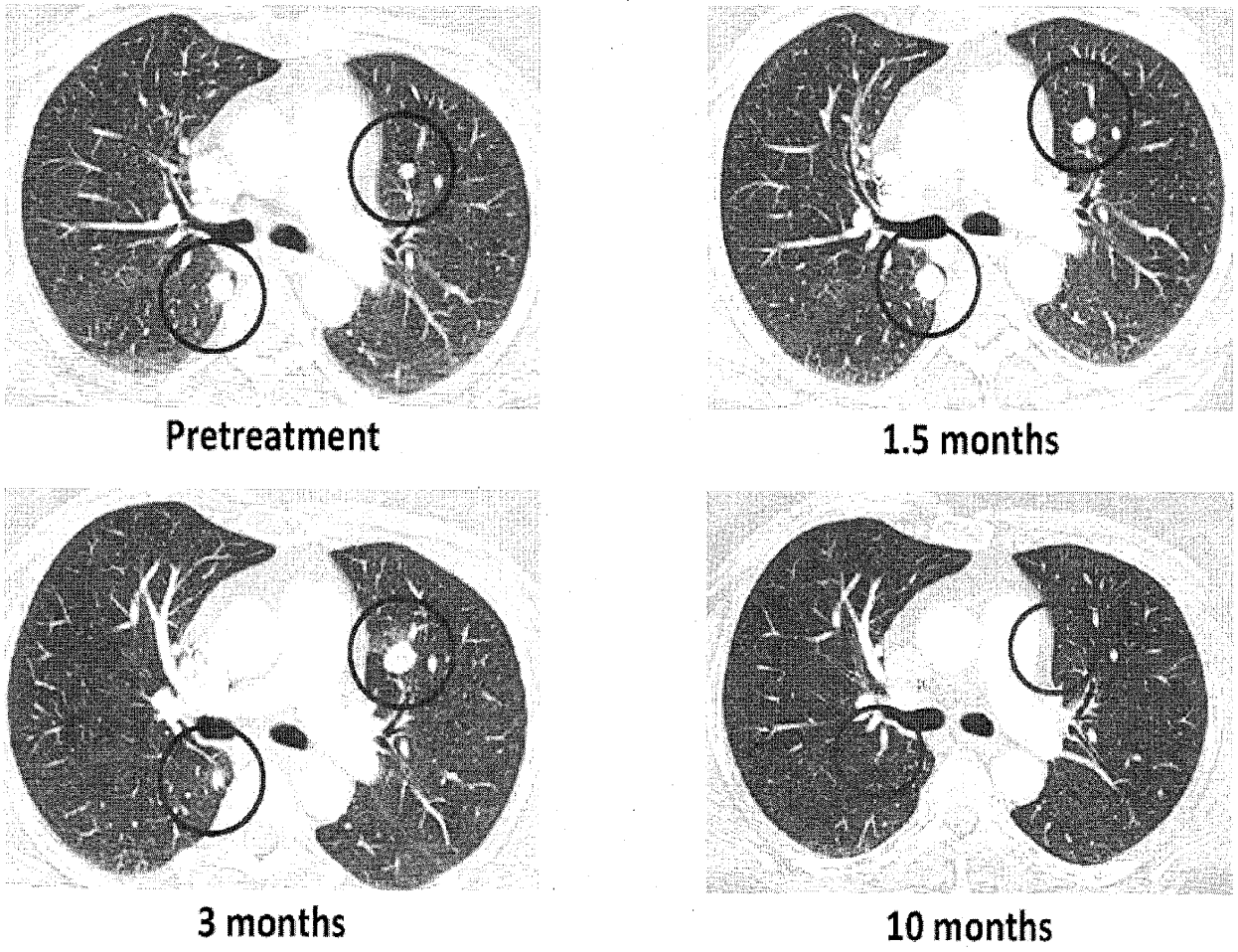


FIG. 12

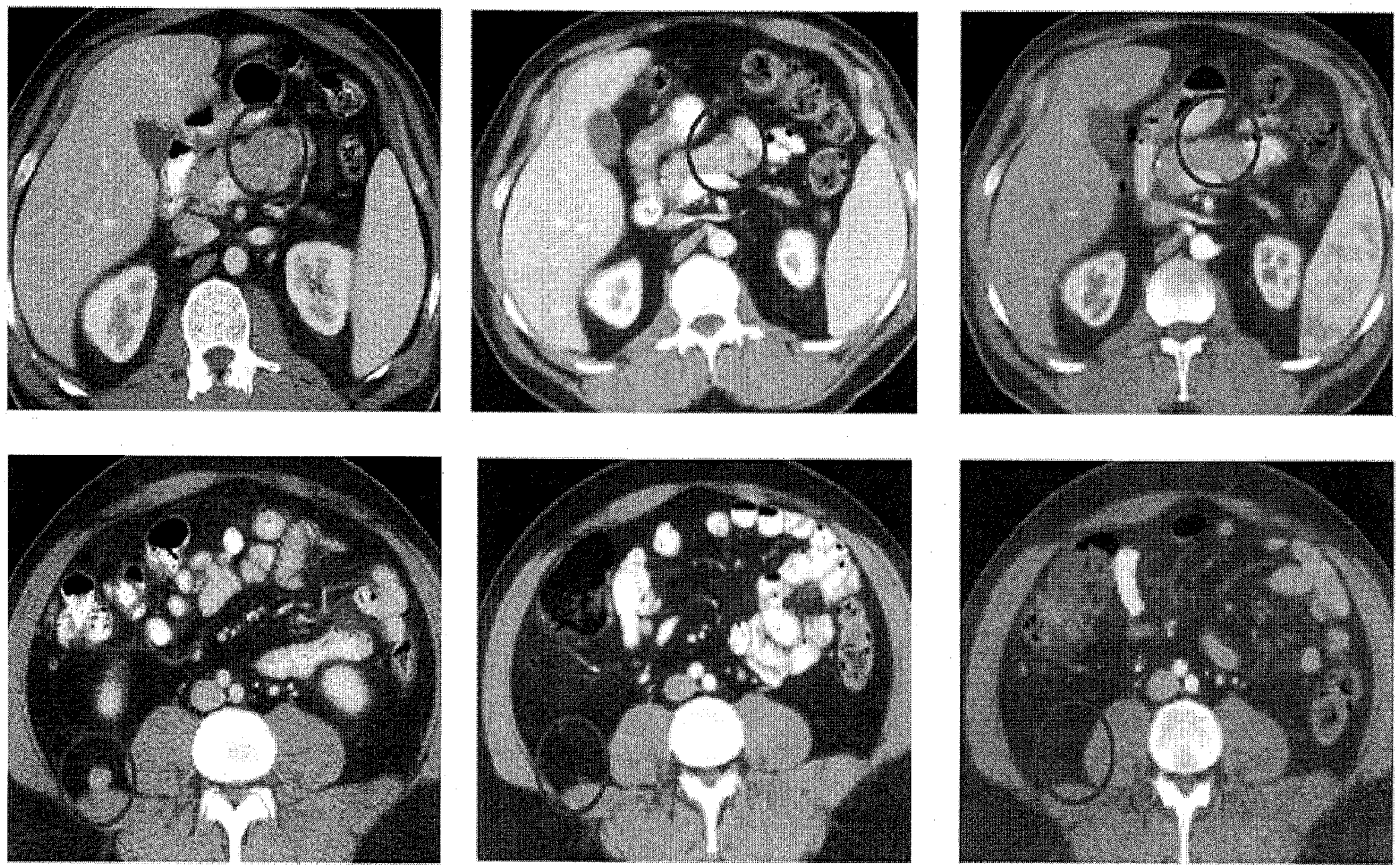
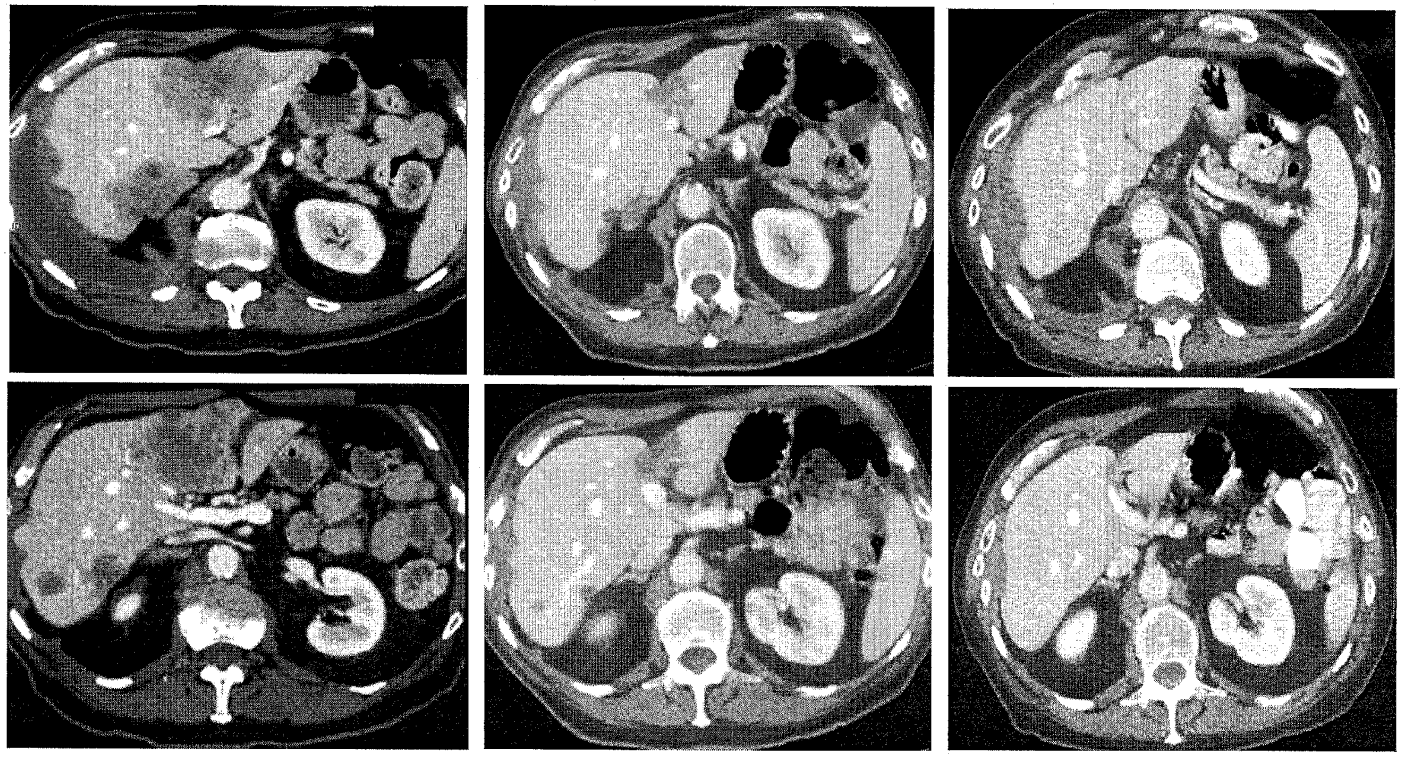


FIG. 13



Electronic Acknowledgement Receipt	
EFS ID:	15255512
Application Number:	61790747
International Application Number:	
Confirmation Number:	8988
Title of Invention:	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-LI SIGNALING
First Named Inventor/Applicant Name:	John Cogswell
Customer Number:	23914
Filer:	Ashton J. Delauney/Lisa Swidra
Filer Authorized By:	Ashton J. Delauney
Attorney Docket Number:	11981USPSP2
Receipt Date:	15-MAR-2013
Filing Date:	
Time Stamp:	15:25:58
Application Type:	Provisional

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$250
RAM confirmation Number	5678
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Application Data Sheet	20130315_ADS_11981USPSP2.pdf	70426 aa3bfac292c372148e86b9bbcfc02718dee426e2	no	4
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
2		20130315_SPEC_11981USPSP_2.pdf	465225 ce4bd3343f6c075ac31d2f8639a3f855866526f3	yes	105

Multipart Description/PDF files in .zip description

Document Description	Start	End
Specification	1	101
Claims	102	104
Abstract	105	105

Warnings:					
Information:					
3	Drawings-only black and white line drawings	20130315_DRW_11981USPSP2.pdf	6491557 8f7dab9725e7ca3f738d404ebd3ee776f621bRa9	no	24
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Information:					
4	Sequence Listing (Text File)	20120515_SEQ_11981USPSP2.txt	37595	no	0
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Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	29721 d85f1c6d0fee54a064aa7f04f11cda5046685d33	no	2
Warnings:					
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International application number:		**PCT/US2013/040764**
International filing date:		**13 May 2013 (13.05.2013)**
Document type:		**Certified copy of priority document**
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	Number:	**61/790,747**
	Filing date:	**15 March 2013 (15.03.2013)**
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CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING

This application claims the benefit of U.S. Provisional Patent Application No. 61/647,442, filed May 15, 2012, and U.S. Provisional Patent Application No. 61/790,747, filed March 15, 2013, the contents of which are hereby incorporated herein by reference in their entirety.

Throughout this application, various other publications are referenced in parentheses by author name and date, or by Patent No. or Patent Publication No. Full citations for these publications may be found at the end of the specification immediately preceding the claims. 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

This invention relates to methods for immunotherapy of a cancer patient comprising administering to the patient antibodies that disrupt the PD-1/PD-L1 signaling pathway. A biomarker may be used as part of this treatment for identifying suitable patients for immunotherapy and for predicting the efficacy of anti-PD-1 treatment.

BACKGROUND OF THE INVENTION

Human cancers harbor numerous genetic and epigenetic alterations, generating neoantigens potentially recognizable by the immune system (Sjoblom *et al.*, 2006). 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. However, although an endogenous immune response to cancer is observed in preclinical models and patients, this response is ineffective, and established cancers are viewed as "self" and tolerated by the immune system. Contributing to this state of tolerance, tumors may exploit several distinct mechanisms to actively subvert anti-tumor immunity. These mechanisms include dysfunctional T-cell signaling (Mizoguchi *et al.*, 1992), suppressive regulatory cells (Facciabene *et al.*, 2012), and the co-opting of endogenous "immune

checkpoints," which serve to down-modulate the intensity of adaptive immune responses and protect normal tissues from collateral damage, by tumors to evade immune destruction (Topalian *et al.*, 2011; Mellman *et al.*, 2011).

Until recently, cancer immunotherapy had focused substantial effort on approaches that enhance anti-tumor immune responses by adoptive-transfer of activated effector cells, immunization against relevant antigens, or providing non-specific immune-stimulatory agents such as cytokines. In the past decade, however, intensive efforts to develop specific 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 (Hodi *et al.*, 2010) and, as described herein, the development of Abs that block the inhibitory PD-1 pathway.

Programmed Death-1 (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 downregulate T cell activation and cytokine secretion upon binding to PD-1 (Freeman *et al.*, 2000; Latchman *et al.*, 2001). Unlike CTLA-4, PD-1 primarily functions in peripheral tissues where activated T-cells may encounter the immunosuppressive PD-L1 (B7-H1) and PD-L2 (B7-DC) ligands expressed by tumor and/or stromal cells (Flies *et al.*, 2011; Topalian *et al.*, 2012a). 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), and the use of Ab inhibitors of the PD-1/PD-L1 interaction for treating cancer has entered clinical trials (Brahmer *et al.*, 2010; Topalian *et al.*, 2012b; Brahmer *et al.*, 2012; Flies *et al.*, 2011; Pardoll, 2012; Hamid and Carvajal, 2013).

The promise of the emerging field of personalized medicine is that advances in pharmacogenomics will increasing 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. Advances in validating PD-L1 expression as a biomarker for screening patients for anti-PD-1 immunotherapy are described herein.

SUMMARY OF THE INVENTION

The present disclosure provides a method for immunotherapy of a subject afflicted with cancer, which method comprises administering to the subject a composition comprising a therapeutically effective amount of an agent that disrupts, reduces or suppresses signaling from an inhibitory immunoregulator. In preferred embodiments, the agent is an Ab. In other preferred embodiments, the inhibitory immunoregulator is a component of the PD-1/PD-L1 signaling pathway. In further preferred embodiments, the Ab disrupts the interaction between PD-1 and PD-L1. In certain embodiments, the Ab is an anti-PD-1 Ab of the invention or an anti-PD-L1 Ab of the invention. In preferred embodiments, the anti-PD-1 Ab of the invention is nivolumab (BMS-936558) and the anti-PD-L1 Ab of the invention is BMS-936559. In certain embodiments, the subject has been pre-treated for the cancer. In other embodiments, the cancer is an advanced, metastatic and/or refractory cancer. In preferred embodiments, the administration of the Ab or antigen-binding portion to the subject thereof induces a durable clinical response in the subject.

This disclosure also provides a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is a suitable candidate for immunotherapy, the selecting comprising (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells, (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface, and (iii) selecting the subject as a suitable candidate based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level;

and (b) administering a composition comprising a therapeutically effective amount of an agent that disrupts signaling from the PD-1/PD-L1 pathway, for example, an anti-PD-1 or an anti-PD-L1 Ab, to the selected subject.

The disclosure further provides a method for treatment of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is not suitable for immunotherapy with an agent that disrupts signaling from the PD-1/PD-L1 pathway, for example, an anti-PD-1 or an anti-PD-L1 Ab, the selecting comprising (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; and (iii) selecting the subject as not suitable for said immunotherapy based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is less than a predetermined threshold level; and (b) administering a standard-of-care therapeutic other than an agent that disrupts signaling from the PD-1/PD-L1 pathway to the selected subject.

In addition, the disclosure provides a method for selecting a cancer patient for immunotherapy with an agent that disrupts signaling from the PD-1/PD-L1 pathway, for example, an anti-PD-1 or an anti-PD-L1 Ab, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold proportion; and (d) selecting the patient for immunotherapy based on an assessment that PD-L1 is expressed in cells of the test tissue sample.

This disclosure further provides a method for predicting the therapeutic effectiveness of an agent that disrupts signaling from the PD-1/PD-L1 pathway, for example, an anti-PD-1 or an anti-PD-L1 Ab, for treating a cancer patient, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold value; and (d)

predicting the therapeutic effectiveness of the agent, wherein if the proportion of cells that express PD-L1 on the cell surface exceeds the threshold proportion the agent is predicted to be effective in treating the patient, and wherein if the proportion of cells that express PD-L1 on the cell surface is below the threshold proportion the agent is predicted to not be effective in treating the patient.

The present disclosure also provides a method for determining an immunotherapeutic regimen comprising an agent that disrupts signaling from the PD-1/PD-L1 pathway, for example, an anti-PD-1 or an anti-PD-L1 Ab, for treating a cancer patient, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold proportion; and (d) determining an immunotherapeutic regimen comprising the agent based on the determination that the proportion of cells that express PD-L1 on the cell surface exceeds the predetermined threshold proportion.

In certain embodiments of the methods described herein, the test tissue sample is a formalin-fixed and paraffin-embedded (FFPE) sample. In certain other embodiments, assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is achieved by immunohistochemical (IHC) staining of the FFPE sample. In preferred embodiments, the mAb 28-8 or 5H1 is used in an automated IHC assay to bind to PD-L1 on the surface of cells in the test tissue sample. In preferred embodiments of any of the methods disclosed herein, the cancer is melanoma (MEL), renal cell carcinoma (RCC), squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, colorectal cancer (CRC), castration-resistant prostate cancer (CRPC), hepatocellular carcinoma (HCC), squamous cell carcinoma of the head and neck, carcinomas of the esophagus, ovary, gastrointestinal tract and breast, or a hematologic malignancy such as multiple myeloma, B-cell lymphoma, T-cell lymphoma, Hodgkin's lymphoma/primary mediastinal B-cell lymphoma, and chronic myelogenous leukemia.

This invention additionally provides a mAb or antigen-binding portion thereof that binds specifically to a cell surface-expressed human PD-L1 antigen in a FFPE tissue sample. In preferred embodiments, the mAb or antigen-binding portion thereof does not bind to a cytoplasmic PD-L1 antigen in the FFPE tissue sample. In other preferred

embodiments, the monoclonal Ab (mAb) is the rabbit mAb designated 28-8, 28-1, 28-12, 29-8 or 20-12, or the mouse mAb designated 5H1.

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 cited references, including scientific articles, newspaper reports, GenBank entries, patents and patent applications cited throughout this application are expressly incorporated herein by reference.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1C. Cross-competition between 5C4 and other HuMab anti-PD-1 mAbs for binding to human PD-1 (hPD-1) expressed on CHO cells. A, the 5C4 Fab fragment substantially blocked the binding of mAbs 5C4 itself, as well as the binding of 2D3 and 7D3; B, the 5C4 Fab fragment substantially blocked the binding of mAb 4H1; C, the 5C4 mAb substantially blocked the binding of mAb 17D8.

Figures 2A-2F. Cross-competition of FITC-conjugated human anti-hPD-L1 mAbs for binding to human PDF-L1 (hPD-L1) expressed on CHO cells. A, Binding of labeled 10H10 was partially blocked by 10A5, 11E6 and 13G4 and was significantly blocked by itself; B, Binding of labeled 3G10 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10; C, Binding of labeled 10A5 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10; D, Binding of labeled 11E6 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10; and E, Binding of labeled 12A4 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10; and F, Binding of labeled 13G4 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10.

Figure 3. Cross-competitive inhibition of binding of biotinylated mAb 12A4 to ES-2 cells by human anti-hPD-L1 mAbs. Fluorescence of bound biotin-12A4 is plotted against the concentration of unlabeled hPD-L1 HuMabs.

Figure 4. Spider plot showing activity of anti-PD-1 mAb in patients with treatment-refractory melanoma (MEL). A representative plot of changes in tumor burden over time demonstrates the time course of change in the sum of the longest diameters of target lesions, compared with baseline, in 27 MEL patients treated with 5C4 at a dose of 1.0 mg/kg. In the majority of patients who achieved an objective response (OR), responses were durable and evident by the end of cycle 3 (6 months) of treatment (vertical dashed line). Tumor regressions followed conventional as well as "immune-

related" patterns of response, such as prolonged reduction in tumor burden in the presence of new lesions.

Figure 5. Activity of anti-PD-1 mAb in patient with metastatic RCC. Partial regression of metastatic RCC in a 57-year-old patient treated with 5C4 at 1 mg/kg is illustrated. This patient had previously undergone radical surgery and had developed progressive disease after receiving sunitinib, temsirolimus, sorafenib, and pazopanib. Arrows show regression of recurrent tumor in the operative field.

Figures 6. Activity of anti-PD-1 mAb in patient with metastatic MEL. A complete response of metastatic MEL is illustrated in a 62-year-old patient treated with 5C4 at 3 mg/kg, associated with vitiligo. (i) Pretreatment CT scan, inguinal lymph node metastasis (arrow); (ii) after 13 months of treatment. Numerous metastases in the subcutaneous tissue and retroperitoneum also regressed completely (not shown). Vitiligo developed after 6 months of treatment; photos taken at 9 months under visible light (iii) and ultraviolet light (iv). Skin biopsies with immunohistochemistry for microphthalmia-associated transcription factor (MITF) show melanocytes (arrows) at the epidermal-dermal junction in normal skin (v), and scarce (vi) or absent (vii) melanocytes in skin partially or fully affected by vitiligo.

Figure 7. Activity of anti-PD-1 mAb in patient with metastatic NSCLC. A partial response is illustrated in a patient with metastatic NSCLC (nonsquamous histology) treated with 5C4 at 10 mg/kg. Arrows show initial progression in pulmonary lesions followed by regression ("immune-related" pattern of response).

Figures 8A and 8B. Correlation between tumor PD-L1 expression and anti-PD-1 clinical response. Pretreatment tumor cell surface expression of PD-L1, as determined by IHC on formalin-fixed paraffin-embedded specimens, correlates with OR to PD-1 blockade. Forty-two subjects with advanced cancers including melanoma, non-small cell lung cancer, colorectal cancer, renal cell cancer, and castration-resistant prostate cancer (n=18, 10, 7, 5, and 2, respectively) were studied. A, There was a significant correlation of tumor cell surface PD-L1 expression with objective clinical response. No patients with PD-L1 negative tumors experienced an OR. B, Examples of IHC analysis with the anti-PD-L1 mAb 5H1 are shown in a melanoma lymph node metastasis (top), a renal cell cancer nephrectomy specimen (middle), and a lung adenocarcinoma brain metastasis (bottom). All 400X original magnification. Arrows indicate one of many tumor cells in each specimen with surface membrane staining for PD-L1. Asterisk indicates a normal

glomerulus in the nephrectomy specimen, which is negative for PD-L1 staining.

Figure 9. Graphical comparison of the binding of mAbs 28-8 and 5H1 to PD-L1 antigen in tumor tissues by histoscore analysis. The rabbit mAb 28-8 showed higher histoscores in 7 out of 10 samples tested.

Figures 10A-10D. Spider plot showing activity of anti-PD-L1 mAb in patients with treatment-refractory MEL and NSCLC. Representative plots demonstrate the time course of target lesion tumor burden over time in patients with MEL treated with BMS-936559 at doses of 1 (A), 3 (B), 10 mg/kg (C) and in patients with NSCLC treated at 10 mg/kg (D). In the majority of patients who achieved ORs, responses were durable and were evident by the end of cycle 2 (3 months) of treatment, irrespective of dose or tumor type. Tumor regressions followed conventional as well as "immune-related" patterns of response.

Figure 11. Complete response in a patient with melanoma treated with BMS-936559 at 3 mg/kg. Circles indicate an initial increase in the size of pulmonary nodules at 6 weeks and 3 months followed by complete regression at 10 months ("immune-related" pattern of response).

Figure 12. Complete response in a patient with melanoma treated with BMS-936559 at 1 mg/kg. This patient developed an isolated brain metastasis 3 months after initiation of treatment that was successfully treated with stereotactic radiosurgery. A partial response in abdominal disease (circled) was noted at 8 months, with no evidence of disease at 15 months.

Figure 13. Partial response in a patient with NSCLC (non-squamous histology) treated with BMS-936559 at 10 mg/kg. Note the response in disease in right lung pleura and liver.

Figures 14A and 14B. Clinical activity of MEL patients who received a concurrent regimen of nivolumab and ipilimumab. A, Representative spider plots show changes from baseline in the tumor burden, measured as the sum of products of perpendicular diameters of all target lesions, in patients who received the concurrent regimen of 1 mg/kg nivolumab + 3 mg/kg ipilimumab, the MTD. Triangles indicate the first occurrence of a new lesion. B, A representative waterfall plot shows maximum percentage response in baseline target lesions in patients who received the concurrent regimen.

Figure 15. Tumor regressions of a 52-year old MEL patient who received a

concurrent regimen of 1 mg/kg nivolumab + 3 mg/kg ipilimumab. This patient presented with extensive neck, mediastinal, axillary, abdominal and pelvic lymphadenopathy, bilateral pulmonary nodules, small bowel metastasis, peritoneal implants and diffuse subcutaneous nodules. Baseline lactate dehydrogenase (LDH) was 2.25 x upper limit of normal, hemoglobin was 9.7 g/dL, and symptoms included nausea and vomiting. Within 4 weeks of treatment the LDH normalized, symptoms improved (appetite increased, nausea decreased), and cutaneous lesions were regressing. At week-12 scans, there was marked reduction in all areas of disease. Arrows denote location of metastatic disease.

Figures 16A-16C. Clinical activity of MEL patients who received various concurrent regimens of nivolumab and ipilimumab. Representative spider plots show changes from baseline in the tumor burden, measured as the sum of products of perpendicular diameters of all target lesions, in patients who received a concurrent regimen of 0.3 mg/kg nivolumab + 3 mg/kg ipilimumab (A), 3 mg/kg nivolumab + 1 mg/kg ipilimumab (B), or 3 mg/kg nivolumab + 3 mg/kg ipilimumab (C). Triangles indicate the first occurrence of a new lesion.

Figure 17. Tumor regressions of a 61-year old MEL patient who received a concurrent regimen of 0.3 mg/kg nivolumab + 3 mg/kg ipilimumab. The patient presented with stage IV (M1c) MEL of an unknown primary site, metastatic to the stomach and the mesentery. Lactate dehydrogenase was 225 and hemoglobin was 9.6 g/dL after a recent transfusion. Twelve weeks after the initiation of study treatment, a CT scan showed an 86% reduction in the bulky disease burden.

Figures 18A-18C. Clinical activity of MEL patients who received a sequenced regimen of nivolumab and ipilimumab. Representative spider plots show changes from baseline in the tumor burden, measured as the sum of products of perpendicular diameters of all target lesions, in patients who received a sequenced regimen of 1 mg/kg nivolumab (A) or 3 mg/kg nivolumab (B) after prior ipilimumab therapy. Triangles indicate the first occurrence of a new lesion. C, A representative waterfall plot shows maximum percentage response in baseline target lesions in patients who received a sequenced regimen; "*" denotes patients who had radiographic progression with prior ipilimumab treatment.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods for immunotherapy of a subject afflicted with diseases such as cancer or an infectious disease, which methods comprise

administering to the subject a composition comprising a therapeutically effective amount of a compound or agent that potentiates an endogenous immune response, either stimulating the activation of the endogenous response or inhibiting the suppression of the endogenous response. More specifically, this disclosure provides methods for potentiating an endogenous immune response in a subject afflicted with cancer so as to thereby treat the patient, which method comprises administering to the subject a therapeutically effective amount of an agent, such as an Ab or an antigen-binding portion thereof, that disrupts or inhibits signaling from an inhibitory immunoregulator. In certain embodiments, the inhibitory immunoregulator is a component of the PD-1/PD-L1 signaling pathway. Accordingly, certain embodiments of the invention provide methods for immunotherapy of a subject afflicted with cancer, which methods comprise administering to the subject a therapeutically effective amount of an Ab or an antigen-binding portion thereof that disrupts the interaction between the PD-1 receptor and its ligand, PD-L1. In certain preferred embodiments, the Ab or antigen-binding portion thereof binds specifically to PD-1. In other preferred embodiments, the Ab or antigen-binding portion thereof binds specifically to PD-L1. Certain embodiments comprise the use of an anti-PD-1 Ab is in combination with another anti-cancer agent, preferably an anti-CTLA-4 Ab, to treat cancer. In certain other embodiments, the subject is selected as suitable for immunotherapy in a method comprising measuring the surface expression of PD-L1 in a test tissue sample obtained from a patient with cancer of the tissue, for example, determining the proportion of cells in the test tissue sample that express PD-L1 on the cell surface, and selecting the patient for immunotherapy based on an assessment that PD-L1 is expressed on the surface of cells in the test tissue sample.

Terms

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.

"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. Preferred routes of administration for Abs of the invention 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. Alternatively, an Ab 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. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

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.

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 Abs 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.

Antibodies typically bind specifically to their cognate antigen with high affinity, reflected by a dissociation constant (K_D) of 10^{-5} to 10^{-11} M^{-1} or less. Any K_D greater than about 10^{-4} M^{-1} is generally considered to indicate nonspecific binding. As used herein, an Ab that "binds specifically" to an antigen refers to an Ab that binds to the antigen and substantially identical antigens with high affinity, which means having a K_D of 10^{-7} M or less, preferably 10^{-8} M or less, even more preferably 5 x 10^{-9} M or less, and most preferably between 10^{-8} M and 10^{-10} M or less, but does not bind with high affinity to unrelated antigens. An antigen is "substantially identical" to a given antigen if it exhibits a high degree of sequence identity to the given antigen, for example, if it exhibits at least 80%, at least 90%, preferably at least 95%, more preferably at least 97%, or even more preferably at least 99% sequence identity to the sequence of the given antigen. By way of example, an Ab that binds specifically to human PD-1 may also have cross-reactivity with PD-1 antigens from certain primate species but may not cross-react with PD-1 antigens from certain rodent species or with an antigen other than PD-1, *e.g.*, a human PD-L1 antigen.

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 Ab 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 Abs; monoclonal and polyclonal Abs; chimeric and humanized Abs; human or nonhuman Abs; wholly synthetic Abs; and single chain Abs. A nonhuman Ab 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 Ab.

An "isolated antibody" refers to an Ab that is substantially free of other Abs having different antigenic specificities (*e.g.*, an isolated Ab that binds specifically to PD-1 is substantially free of Abs that bind specifically to antigens other than PD-1). An isolated

Ab that binds specifically to PD-1 may, however, have cross-reactivity to other antigens, such as PD-1 molecules from different species. Moreover, an isolated Ab may be substantially free of other cellular material and/or chemicals. By comparison, an "isolated" nucleic acid refers to a nucleic acid composition of matter that is markedly different, *i.e.*, has a distinctive chemical identity, nature and utility, from nucleic acids as they exist in nature. For example, an isolated DNA, unlike native DNA, is a free-standing portion of a native DNA and not an integral part of a larger structural complex, the chromosome, found in nature. Further, an isolated DNA, unlike native genomic DNA, can typically be used in applications or methods for which native genomic DNA is unsuited, *e.*g., as a PCR primer or a hybridization probe for, among other things, measuring gene expression and detecting biomarker genes or mutations for diagnosing disease or assessing the efficacy of a therapeutic. An isolated nucleic acid may be purified so as to be substantially free of other cellular components or other contaminants, *e.g.*, other cellular nucleic acids or proteins, using standard techniques well known in the art. Examples of isolated nucleic acids include fragments of genomic DNA, PCR-amplified DNA, cDNA and RNA.

The term "monoclonal antibody" ("mAb") refers to a preparation of Ab molecules of single molecular composition, *i.e.*, Ab 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 Ab. MAbs may be produced by hybridoma, recombinant, transgenic or other techniques known to those skilled in the art.

A "human" antibody (HuMAb) refers to an Ab having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the Ab contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The human Abs 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 Abs 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" Abs and "fully human" Abs and are used synonymously.

A "humanized" antibody refers to an Ab in which some, most or all of the amino

acids outside the CDR domains of a non-human Ab are replaced with corresponding amino acids derived from human immunoglobulins. In one embodiment of a humanized form of an Ab, 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 Ab to bind to a particular antigen. A "humanized" Ab retains an antigenic specificity similar to that of the original Ab.

A "chimeric antibody" refers to an Ab in which the variable regions are derived from one species and the constant regions are derived from another species, such as an Ab in which the variable regions are derived from a mouse Ab and the constant regions are derived from a human Ab.

An "antigen-binding portion" of an Ab (also called an "antigen-binding fragment") refers to one or more fragments of an Ab that retain the ability to bind specifically to the antigen bound by the whole Ab.

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 divide and grow 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.

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 Abs, 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.

An "immunoregulator" refers to a substance, an agent, a signaling pathway or a component thereof that regulates an immune response. "Regulating," "modifying" or "modulating" an immune response refers to any alteration in a cell of the immune system or in the activity of such cell. Such regulation includes stimulation or suppression of the immune system which may be manifested by an increase or decrease in the number of

various cell types, an increase or decrease in the activity of these cells, or any other changes which can occur within the immune system. Both inhibitory and stimulatory immunoregulators have been identified, some of which may have enhanced function in the cancer microenvironment.

The term "immunotherapy" refers to the treatment of a subject afflicted with, or at risk of contracting or suffering a recurrence of, a disease by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response. "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, condition or biochemical indicia associated with a disease.

"Potentiating an endogenous immune response" means increasing the effectiveness or potency of an existing immune response in a subject. This increase in effectiveness and potency may be achieved, for example, by overcoming mechanisms that suppress the endogenous host immune response or by stimulating mechanisms that enhance the endogenous host immune response.

A "predetermined threshold value," 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 being positive for cell surface PD-L1 expression. For cell surface expression assayed by IHC with the mAb 28-8, the predetermined threshold value for cells expressing PD-L1 on the cell surface ranges from at least about 0.01% to at least about 20% of the total number of cells. In preferred embodiments, the predetermined threshold value for cells expressing PD-L1 on the cell surface ranges from at least about 0.1% to at least about 10% of the total number of cells. More preferably, the predetermined threshold value is at least about 5%. Even more preferably, the predetermined threshold value is at least about 1%, or in the range of 1-5%.

The "Programmed Death-1 (PD-1)" receptor 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.

"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 analogs having at least one common epitope with hPD-L1. The complete hPD-L1 sequence can be found under GenBank Accession No. Q9NZQ7.

A "signal transduction pathway" or "signaling 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 the cell. A "cell surface receptor" includes, for example, molecules and complexes of molecules that are located on the surface of a cell and are capable of receiving a signal and transmitting 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, which is located on the surface of activated T cells, activated B cells and myeloid cells, and transmits a signal that results in a decrease in tumor-infiltrating lymphocytes and a decrease in T cell proliferation. An "inhibitor" of signaling refers to a compound or agent that antagonizes or reduces the initiation, reception or transmission of a signal, be that signal stimulatory or inhibitory, by any component of a signaling pathway such as a receptor or its ligand.

A "subject" includes any human or nonhuman animal. The term "nonhuman animal" includes, but is not limited to, vertebrates such as nonhuman primates, sheep, dogs, cats, rabbits and ferrets, rodents such as mice, rats and guinea pigs, avian species such as chickens, amphibians, and reptiles. In preferred embodiments, the subject is a mammal such as a nonhuman primate, sheep, dog, cat, rabbit, ferret or rodent. In more preferred embodiments, the subject is a human. The terms, "subject," "patient" and "individual" are used interchangeably herein.

A "therapeutically effective amount" or "therapeutically effective dosage" of a drug or therapeutic agent, such as an Ab of the invention, 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.

By way of example, an anti-cancer agent promotes cancer regression in a subject. In preferred embodiments, a therapeutically effective amount of the drug promotes cancer regression to the point of eliminating the cancer. "Promoting cancer regression" means that administering an effective amount of the drug, alone or in combination with an anti-neoplastic agent, results in a reduction in tumor growth or size, necrosis of the tumor, a decrease in severity of at least one disease symptom, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. In addition, the terms "effective" and "effectiveness" with regard to a treatment includes both pharmacological effectiveness and physiological safety. Pharmacological effectiveness refers to the ability of the drug to promote cancer regression in the patient. Physiological safety refers to the level of toxicity, or other adverse physiological effects at the cellular, organ and/or organism level (adverse effects) resulting from administration of the drug.

By way of example for the treatment of tumors, a therapeutically effective amount of the drug 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. In other preferred embodiments of the invention, tumor regression may be observed and continue for a period of at least about 20 days, more preferably at least about 40 days, or even more preferably at least about 60 days. Notwithstanding these ultimate measurements of therapeutic effectiveness, evaluation of immunotherapeutic drugs must also make allowance for "immune-related" response patterns.

An "immune-related" response pattern refers to a clinical response pattern often observed in cancer patients treated with immunotherapeutic agents that produce antitumor effects by inducing cancer-specific immune responses or by modifying native immune processes. This response pattern is characterized by a beneficial therapeutic effect that follows an initial increase in tumor burden or the appearance of new lesions, which in the evaluation of traditional chemotherapeutic agents would be classified as disease progression and would be synonymous with drug failure. Accordingly, proper evaluation of immunotherapeutic agents may require long-term monitoring of the effects of these

agents on the target disease.

A therapeutically effective amount of a drug includes a "prophylactically effective amount," which is any amount of the drug that, when administered alone or in combination with an anti-neoplastic agent to a subject at risk of developing a cancer (*e.g.*, a subject having a pre-malignant condition) or of suffering a recurrence of cancer, inhibits the development or recurrence of the cancer. In preferred embodiments, the prophylactically effective amount prevents the development or recurrence of the cancer entirely. "Inhibiting" the development or recurrence of a cancer means either lessening the likelihood of the cancer's development or recurrence, or preventing the development or recurrence of the cancer entirely.

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.

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.

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 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.

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.

Various aspects of the invention are described in further detail in the following subsections.

Antibodies of the Invention

Abs of the present invention include a variety of Abs having structural and functional properties described herein, including high-affinity binding to PD-1 or PD-L1, respectively. These Abs may be used, for example, as therapeutic Abs to treat subjects afflicted with disease or as reagents in diagnostic assays to detect their cognate antigens. Human mAbs (HuMAbs) that 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) with high affinity have been disclosed in U.S. Patent No. 8,008,449, and HuMAbs that bind specifically to PD-L1 with high affinity have been disclosed in U.S. Patent No. 7,943,743. The Abs of the invention include, but are not limited to, all of the anti-PD-1 and anti-PD-L1 Abs disclosed in U.S. Patent Nos. 8,008,449 and 7,943,743, respectively. Other anti-PD-1 mAbs have been described in, for example, U.S. Patent Nos. 6,808,710, 7,488,802 and 8,168,757, and PCT Publication No. WO 2012/145493, and anti-PD-L1 mAbs have been 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. To the extent these anti-PD-1 and anti-PD-L1 mAbs exhibit the structural and functional properties disclosed herein for antibodies of the invention, they too are included as antibodies of the invention.

Anti-PD-1 Antibodies of the Invention

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 Ab responses; and (j) inhibits tumor cell growth *in vivo*. Anti-PD-1 Abs of the present invention include mAbs that bind specifically to human PD-1

and exhibit at least one, preferably at least five, of the preceding characteristics.

U.S. Patent No. 8,008,449 exemplifies seven anti-PD-1 HuMAbs: 17D8, 2D3, 4H1, 5C4 (also referred to herein as nivolumab or BMS-936558), 4A11, 7D3 and 5F4. Isolated DNA molecules encoding the heavy and light chain variable regions of these Abs have been sequenced, from which the amino acid sequences of the variable regions were deduced. The V_H amino acid sequences of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are provided herein as 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 provided herein as SEQ ID NOs. 8, 9, 10, 11, 12, 13 and 14, respectively.

Preferred anti-PD-1 Abs of the present invention include the anti-PD-1 HuMAbs 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4. These preferred Abs bind specifically to human PD-1 and comprise: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 1 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 8; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 2 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 9; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 3 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 10; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 5 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 12; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 6 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 13; or (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 7 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 14.

Given that each of these Abs can bind to PD-1, the V_H and V_L sequences can be "mixed and matched" to create other anti-PD-1 Abs of the invention. PD-1 binding of such "mixed and matched" Abs can be tested using binding assays, *e.g.*, enzyme-linked immunosorbent assays (ELISAs), western blots, radioimmunoassays and Biacore analysis that are well known in the art (*see, e.g.*, U.S. Patent No. 8,008,449). 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, anti-PD-1 Abs of the invention include an isolated mAb 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 Ab specifically binds PD-1, preferably human PD-1.

The CDR domains of the above Abs have been delineated using the Kabat system, and these Abs may also be defined by combinations of their 3 heavy chain and 3 light chain CDRs (*see* U.S. Patent No. 8,008,449). Since each of these Abs can bind to PD-1 and antigen-binding specificity is provided primarily by the CDR1, CDR2, and CDR3 regions, the V_H CDR1, CDR2, and CDR3 sequences and V_κ CDR1, CDR2, and CDR3 sequences can be "mixed and matched" (*i.e.*, CDRs from different Abs can be mixed and match, although each Ab must contain a V_H CDR1, CDR2, and CDR3 and a V_κ CDR1, CDR2, and CDR3) to create other anti-PD-1 Abs that also constitute Abs of the invention. PD-1 binding of such "mixed and matched" Abs can be tested using the binding assays described above (*e.g.*, ELISAs, western blots, radioimmunoassays and Biacore analysis).

Abs of the invention also include isolated Abs that bind specifically to PD-1 and comprise a heavy chain variable region derived from a particular germline heavy chain immunoglobulin and/or a light chain variable region derived from a particular germline light chain immunoglobulin. Specifically, in certain embodiments, Abs of the invention include isolated Abs comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-33 or 4-39 germline sequence, and/or a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6, or L15 germline sequence. The amino acid sequences of the V_H and V_κ regions encoded by the V_H 3-33,

V_H 4-39, V_κ L6 and V_κ L15 germline genes are provided in U.S. Patent No. 8,008,449.

As used herein, an Ab can be identified as comprising a heavy or a light chain variable region that is "derived from" a particular human germline immunoglobulin by comparing the amino acid sequence of the human Ab to the amino acid sequences encoded by human germline immunoglobulin genes, and selecting the human germline immunoglobulin sequence that is closest in sequence (*i.e.*, greatest percentage of sequence identity) to the sequence of the human Ab. A human Ab that is "derived from" a particular human germline immunoglobulin 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 Ab 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 Ab 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 Ab 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, the sequence of a human Ab 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 other embodiments, the human Ab may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid differences from the amino acid sequence encoded by the germline immunoglobulin gene.

Preferred Abs of the invention also include isolated Abs or antigen-binding portions thereof comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-33 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6 germline sequence; or (b) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 4-39 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L15 germline sequence. Examples of Abs having a V_H and a V_κ derived from V_H 3-33 and V_κ L6 germline sequences, respectively, include 17D8, 2D3, 4H1, 5C4, and

7D3. Examples of Abs having V_H and V_κ regions derived from V_H 4-39 and V_κ L15 germline sequences, respectively, include 4A11 and 5F4.

In yet other embodiments, anti-PD-1 Abs of the invention comprise heavy and light chain variable regions having amino acid sequences that are highly similar or homologous to the amino acid sequences of the preferred anti-PD-1 Abs described herein, wherein the Ab retains the functional properties of the preferred anti-PD-1 Abs of the invention. For example, Abs of the invention include mAbs comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises consecutively linked amino acids having a sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs. 1, 2, 3, 4, 5, 6 and 7, and the light chain variable region comprises consecutively linked amino acids having a sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs. 8, 9, 10, 11, 12, 13 and 14. In other embodiments, the V_H and/or V_L amino acid sequences may exhibit at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the sequences set forth above.

As used herein, the percent sequence identity (also referred to as the percent sequence homology) between two sequences (amino acid or nucleotide sequences) is a function of the number of identical positions shared by the sequences relative to the length of the sequences compared (*i.e.*, % identity = number of identical positions/total number of positions being compared x 100), taking into account the number of any gaps, and the length of each such gap, introduced to maximize the degree of sequence identity between the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using mathematical algorithms that are well know to those of ordinary skill in the art (*see, e.g.*, U.S. Patent No. 8,008,449).

Antibodies having very similar amino acid sequences are likely to have essentially the same functional properties where the sequence differences are conservative modifications. As used herein, "conservative sequence modifications" refer to amino acid modifications that do not significantly affect the binding characteristics of the Ab containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Conservative amino acid substitutions are substitutions in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Thus, for example, one or more amino acid residues within the CDR regions of an Ab of the invention can be replaced with other amino acid residues

from the same side chain family and the altered Ab can be tested for retained function using functional assays that are well known in the art. Accordingly, certain embodiments of the anti-PD-1 Abs of the invention comprise heavy and light chain variable regions each comprising CDR1, CDR2 and CDR3 domains, wherein one or more of these CDR domains comprise consecutively linked amino acids having sequences that are the same as the CDR sequences of the preferred anti-PD-1 Abs described herein (*e.g.*, 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4), or conservative modifications thereof, and wherein the Abs retain the desired functional properties of the preferred anti-PD-1 Abs of the invention.

Further, it is well known in the art that the heavy chain CDR3 is the primary determinant of binding specificity and affinity of an Ab, and that multiple Abs can predictably be generated having the same binding characteristics based on a common CDR3 sequence (*see, e.g.*, Klimka *et al.*, 2000; Beiboer *et al.*, 2000; Rader *et al.*, 1998; Barbas *et al.*, 1994; Barbas *et al.*, 1995; Ditzel *et al.*, 1996; Berezov *et al.*, 2001; Igarashi *et al.*, 1995; Bourgeois *et al.*, 1998; Levi *et al.*, 1993; Polymenis and Stoller, 1994; and Xu and Davis, 2000). The foregoing publications demonstrate that, in general, once the heavy chain CDR3 sequence of a given Ab is defined, variability in the other five CDR sequences does not greatly affect the binding specificity of that Ab. Thus, Abs of the invention comprising 6 CDRs can be defined by specifying the sequence of the heavy chain CDR3 domain.

Anti-PD-1 Abs of the invention also include isolated Abs that bind specifically to human PD-1 and cross-compete for binding to human PD-1 with any of HuMAbs 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4. Thus, anti-PD-1 Abs of the invention include isolated Abs or antigen-binding portions thereof that cross-compete for binding to PD-1 with a reference Ab or a reference antigen-binding portion thereof comprising: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 1 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 8; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 2 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 9; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 3 and a human light chain

variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 10; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 5 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 12; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 6 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 13; or (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 7 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 14.

The ability of Abs to cross-compete for binding to an antigen indicates that these Abs bind to the same epitope region (*i.e.*, the same or an overlapping or adjacent epitope) of the antigen and sterically hinder the binding of other cross-competing Abs to that particular epitope region. Thus, the ability of a test Ab to competitively inhibit the binding of, for example, 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4, to human PD-1 demonstrates that the test Ab binds to the same epitope region of human PD-1 as 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4, respectively. All isolated Abs that bind to the same epitope region of human PD-1 as does HuMAb 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4 are included among the Abs of the invention. These cross-competing Abs are expected to have very similar functional properties by virtue of their binding to the same epitope region of PD-1. For example, cross-competing anti-PD-1 mAbs 5C4, 2D3, 7D3, 4H1 and 17D8 have been shown to have similar functional properties (*see* U.S. Patent No. 8,008,449 at Examples 3-7). The higher the degree of cross-competition, the more similar will the functional properties be. Further, cross-competing Abs can be readily 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 Abs of the invention (*see, e.g.*, Examples 1 and 2).

In certain embodiments, the Abs that cross-compete for binding to human PD-1

with, or bind to the same epitope region of human PD-1 as, 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4 are mAbs. For administration to human patients, these cross-competing Abs are preferably chimeric Abs, or more preferably humanized or human Abs. Such human mAbs can be prepared and isolated as described in U.S. Patent No. 8,008,449. Data provided in Example 1 show that 5C4 or a Fab fragment thereof cross-competes with each of 2D3, 7D3, 4H1 or 17D8 for binding to hPD-1 expressed on the surface of a cell, indicating that all five anti-PD-1 mAbs bind to the same epitope region of hPD-1 (Figures 1A-1C).

An anti-PD-1 Ab of the invention further can be prepared using an Ab having one or more of the V_H and/or V_L sequences disclosed herein as starting material to engineer a modified Ab, which modified Ab may have altered properties from the starting Ab. An Ab 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 Ab can be engineered by modifying residues within the constant region(s), for example, to alter the effector function(s) of the Ab. Specific modifications to Abs include CDR grafting, site-specific mutation of amino acid residues within the V_H and/or V_κ CDR1, CDR2 and/or CDR3 regions to thereby improve one or more binding properties (*e.g.*, affinity) of the Ab, site-specific mutation of amino acid residues within the V_H and/or V_κ framework regions to decrease the immunogenicity of the Ab, modifications within the Fc region, typically to alter one or more functional properties of the Ab, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity, and chemical modification such as pegylation or alteration in glycosylation patterns to increase or decrease the biological (*e.g.*, serum) half life of the Ab. Specific examples of such modifications and methods of engineering Abs are described in detail in U.S. Patent No. 8,008,449. Anti-PD-1 Abs of the invention include all such engineered Abs that bind specifically to human PD-1 and are obtained by modification of any of the above-described anti-PD-1 Abs.

Anti-PD-1 Abs of the invention also include antigen-binding portions of the above Abs. It has been amply demonstrated that the antigen-binding function of an Ab can be performed by fragments of a full-length Ab. Examples of binding fragments encompassed within the term "antigen-binding portion" of an Ab 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 Ab.

These fragments, obtained initially through proteolysis with enzymes such as papain and pepsin, have been subsequently engineered into monovalent and multivalent antigen-binding fragments. For example, 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 peptide 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 variable fragments (scFv). Divalent or bivalent scFvs (di-scFvs or bi-scFvs) can be engineered by linking two scFvs in within a single peptide chain known as a tandem scFv which contains two V_H and two V_L regions. ScFv dimers and higher multimers can also be created using linker peptides of fewer than 10 amino acids that are too short for the two variable regions to fold together, which forces the scFvs to dimerize and produce diabodies or form other multimers. Diabodies have been shown to bind to their cognate antigen with much higher affinity than the corresponding scFvs, having dissociation constants up to 40-fold lower than the K_D values for the scFvs. Very short linkers ($\leq$ 3 amino acids) lead to the formation of trivalent triabodies or tetravalent tetrabodies that exhibit even higher affinities for to their antigens than diabodies. Other variants include minibodies, which are scFv-C_{H3} dimers, and larger scFv-Fc fragments (scFv-C_{H2}-C_{H3} dimers), and even an isolated CDR may exhibit antigen-binding function. These Ab fragments are engineered using conventional recombinant techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are intact Abs. All of the above proteolytic and engineered fragments of Abs and related variants (*see* Hollinger and Hudson, 2005; Olafsen and Wu, 2010, for further details) are intended to be encompassed within the term "antigen-binding portion" of an Ab.

Anti-PD-L1 Antibodies of the Invention

Each of the anti-PD-L1 HuMAbs disclosed in U.S. Patent No. 7,943,743 has been demonstrated to exhibit one or more of the following characteristics (a) binds to human PD-L1 with a K_D of 1 x 10^{-7} M or less; (b) increases T-cell proliferation in a Mixed Lymphocyte Reaction (MLR) assay; (c) increase interferon-γ production in an MLR assay; (d) increase IL-2 secretion in an MLR assay; (e) stimulates Ab responses; (f) inhibits the binding of PD-L1 to PD-1; and (g) reverses the suppressive effect of T

regulatory cells on T cell effector cells and/or dendritic cells. Anti-PD-L1 Abs of the present invention include mAbs that bind specifically to human PD-L1 and exhibit at least one, preferably at least four, of the preceding characteristics.

U.S. Patent No. 7,943,743 exemplifies ten anti-PD-1 HuMAbs: 3G10, 12A4 (also referred to herein as BMS-936559), 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4. Isolated DNA molecules encoding the heavy and light chain variable regions of these Abs have been sequenced, from which the amino acid sequences of the variable regions were deduced. The V_H amino acid sequences of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4 are shown in SEQ ID NOs. 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24, respectively, whereas their V_L amino acid sequences are shown in SEQ ID NOs. 25, 26, 27, 28, 29, 30, 31, 32, 33 and 34, respectively.

Preferred anti-PD-L1 Abs of the present invention include the anti-PD-L1 HuMAbs 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4. These preferred Abs bind specifically to human PD-L1 and comprise: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 15 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 25; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 17 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 27; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 18 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 28; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 19 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 29; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 20 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 30; (g) a human heavy chain variable

region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 21 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 31; (h) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 22 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 32; (i) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 23 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 33; or (j) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 24 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 34.

Given that each of these Abs can bind to PD-L1, the V_H and V_L sequences can be "mixed and matched" to create other anti-PD-L1 Abs of the invention. PD-L1 binding of such "mixed and matched" Abs can be tested using binding assays *e.g.*, ELISAs, western blots, radioimmunoassays and Biacore analysis that are well known in the art (*see, e.g.*, U.S. Patent No. 7,943,743). 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, Abs of the invention also include a mAb, or antigen binding portion thereof, comprising a heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in any of SEQ ID NOs. 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24, and a light chain variable region comprising consecutively linked amino acids having the sequence set forth in any of SEQ ID NOs. 25, 26, 27, 28, 29, 30, 31, 32, 33 or 34, wherein the Ab binds specifically to PD-L1, preferably human PD-L1.

The CDR domains of the above anti-PD-L1 HuMAbs have been delineated using the Kabat system, and these Abs may also be defined by combinations of their 3 heavy chain and 3 light chain CDRs (*see* U.S. Patent No. 7,943,743). Since each of these Abs can bind to PD-L1 and antigen-binding specificity is provided primarily by the CDR1, CDR2, and CDR3 regions, the V_H CDR1, CDR2, and CDR3 sequences and V_κ CDR1, CDR2, and CDR3 sequences can be "mixed and matched" (*i.e.*, CDRs from different Abs

can be mixed and match, although each Ab must contain a V_H CDR1, CDR2, and CDR3 and a V_κ CDR1, CDR2, and CDR3) to create other anti-PD-1 Abs that also constitute Abs of the invention. PD-L1 binding of such "mixed and matched" Abs can be tested using, for example, ELISAs, western blots, radioimmunoassays and Biacore analysis.

Antibodies of the invention also include Abs that bind specifically to PD-L1 and comprise a heavy chain variable region derived from a particular germline heavy chain immunoglobulin and/or a light chain variable region derived from a particular germline light chain immunoglobulin. Specifically, in certain embodiments, Abs of the invention include Abs comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-18, 1-69, 1-3 or 3-9 germline sequence, and/or a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6, L15, A27 or L18 germline sequence. The amino acid sequences of the V_H and V_κ regions encoded by the V_H 1-18, V_H 1-3, V_H 1-69, V_H 3-9, V_κ L6, V_κ L15 and V_κ A27 germline genes are provided in U.S. Patent No. 7,943,743.

Preferred Abs of the invention include isolated Abs or antigen-binding portions thereof comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-18 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6 germline sequence; (b) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-69 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6 germline sequence; (c) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-3 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L15 germline sequence; (d) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-69 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ A27 germline sequence; (e) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-9 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a

sequence derived from a human V_κ L15germline sequence; or (f) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-9 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L18germline sequence.

An example of an Ab having a V_H and a V_κ derived from V_H 1-18 and V_κ L6 germline sequences, respectively, is 3G10. Examples of Abs having V_H and V_κ regions derived from V_H 1-69 and V_κ L6 germline sequences, respectively, include 12A4, 1B12, 7H1 and 12B7. An example of an Ab having a V_H and a V_κ derived from V_H 1-3 and V_κ L15 germline sequences, respectively, is 10A5. Examples of Abs having V_H and V_κ regions derived from V_H 1-69 and V_κ A27 germline sequences, respectively, include 5F8, 11E6 and 11E6a. An example of an Ab having a V_H and a V_K derived from V_H 3-9 and V_κ L15 germline sequences, respectively, is 10H10. An example of an Ab having a V_H and a V_κ derived from V_H 1-3 and V_κ L15 germline sequences, respectively, is 10A5. An example of an Ab having a V_H and a V_κ derived from V_H 3-9 and V_κ L18 germline sequences, respectively, is 13G4.

In certain embodiments, anti-PD-L1 Abs of the invention comprise heavy and light chain variable regions having amino acid sequences that are highly similar or homologous to the amino acid sequences of the preferred anti-PD-L1 Abs described herein, wherein the Ab retains the functional properties of the aforementioned anti-PD-L1 Abs of the invention. For example, Abs of the invention include mAbs comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises consecutively linked amino acids having a sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs. 15, 16, 17, 18, 19, 20, 21, 22, 23, and 24, and the light chain variable region comprises consecutively linked amino acids having a sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs. 25, 26, 27, 28, 29, 30, 31, 32, 33, and 34. In other embodiments, the V_H and/or V_L amino acid sequences may exhibit at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the sequences set forth above.

Certain embodiments of the anti-PD-L1 Abs of the invention comprise heavy and light chain variable regions each comprising CDR1, CDR2 and CDR3 domains, wherein one or more of these CDR domains comprise consecutively linked amino acids having

sequences that are the same as the CDR sequences of the preferred anti-PD-L1 Abs described herein (*e.g.*, 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 and 13G4), or conservative modifications thereof, and wherein the Abs retain the desired functional properties of the preferred anti-PD-L1 Abs of the invention.

On the basis of the evidence that the heavy chain CDR3 is the primary determinant of binding specificity and affinity of an Ab, it is generally true that once the heavy chain CDR3 sequence of a given Ab is defined, variability in the other five CDR sequences will not greatly affect the binding specificity of that Ab. Accordingly, anti-PD-L1 Abs of the invention include isolated Abs comprising 6 CDRs, wherein the Abs are defined by specifying the sequence of the heavy chain CDR3 domain.

Anti-PD-L1 Abs of the invention also include isolated Abs that bind specifically to human PD-L1 and cross-compete for binding to human PD-L1 with any of HuMAbs 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 and 13G4. Thus, anti-PD-L1 Abs of the invention include isolated Abs or antigen-binding portions thereof that cross-compete for binding to PD-L1 with a reference Ab or a reference antigen-binding portion thereof comprising: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 15 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 25; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 17 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 27; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 18 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 28; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 19 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 29; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 20 and a human light chain variable region

comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 30; (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 21 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 31; (h) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 22 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 32; (i) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 23 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 33; or (j) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 24 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 34.

The ability of an Ab to cross-compete with any of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 and 13G4 for binding to human PD-L1 demonstrates that such Ab binds to the same epitope region of each of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 and 13G4, respectively. All isolated Abs that bind to the same epitope region of human PD-L1 as does HuMAb 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 or 13G4 are included among the Abs of the invention. These cross-competing Abs are expected to have very similar functional properties by virtue of their binding to the same epitope region of PD-L1. For example, cross-competing anti-PD-L1 mAbs 3G10, 1B12, 13G4, 12A4 (BMS-936559), 10A5, 12B7, 11E6 and 5F8 have been shown to have similar functional properties (*see* U.S. Patent No. 7,943,743 at Examples 3-11), whereas mAb 10H10, which binds to a different epitope region, behaves differently (U.S. Patent No. 7,943,743 at Example 11). The higher the degree of cross-competition, the more similar will the functional properties be. Further, cross-competing Abs can be identified in standard PD-L1 binding assays, *e.g.*, Biacore analysis, ELISA assays or flow cytometry, that are well known to persons skilled in the art. In preferred embodiments, the Abs that cross-compete for binding to human PD-1 with, or bind to the same epitope region of human PD-L1 as, 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 or 13G4 are mAbs, preferably chimeric Abs, or more preferably humanized or human Abs.. Such human mAbs can be prepared and isolated as described in U.S.

Patent No. 7,943,743.

Data provided in Example 2 show that each of the anti-PD-L1 HuMAbs 5F8, 7H1, 1B12, 3G10, 10A5, 11E6, 12A4, 12B7 and 13G4, *i.e.*, all of the HuMAbs tested except 10H10, substantially blocked binding of mAbs 3G10, 10A5, 11E6, 12A4 and 13G4 to Chinese Hamster Ovary (CHO) cells expressing PD-L1 cells. HuMAb 10H10 substantially blocked the binding only of itself to CHO/PD-L1 cells. These data show that 3G10, 10A5, 11E6, 12A4 and 13G4 cross-compete with all of the HuMAbs tested, except for 10H10, for binding to the same epitope region of human PD-L1 (Figures 2A-F).

Data provided in Example 3 show that the binding of HuMAb 12A4 to ES-2 ovarian carcinoma cells expressing PD-L1 cells was substantially blocked by 12A4 itself and by 1B12 and 12B7, and was moderately to significantly blocked by mAbs 5F8, 10A5, 13G4 and 3G10, but was not blocked by mAb 10H10. These data, largely consistent with the data in Example 2, show that 12A4 itself, and 2 other HuMabs, 12B7 and 1B12, substantially cross-compete with 12A4 for binding to the same epitope region, possibly the same epitope, of human PD-L1; 5F8, 10A5, 13G4 and 3G10, exhibit a significant but lower level of cross-competition with 12A4, suggesting that these mAbs may bind to epitopes that overlap the 12A4 epitope; whereas 10H10 does not cross-compete at all with 12A4 (Figure 3), suggesting that this mAb binds to a different epitope region from 12A4.

Anti-PD-L1 Abs of the invention also include Abs engineered starting from Abs having one or more of the V_H and/or V_L sequences disclosed herein, which engineered Abs may have altered properties from the starting Abs. An anti-PD-L1 Ab can be engineered by a variety of modifications as described above for the engineering of modified anti-PD-1 Abs of the invention.

Anti-PD-L1 Abs of the invention also include isolated Abs 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. Yet, studies on measuring PD-L1 expression have often been conducted on frozen specimens because of the difficulty in isolating anti-human PD-L1 Abs that can be used to stain PD-L1 in FFPE specimens by IHC in general (Hamanishi *et al.*, 2007) and, in particular, Abs that bind specifically to membranous PD-L1 in these tissues. The use of different Abs to stain PD-L1 in frozen versus FFPE tissues, and the ability of certain Abs

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*., 2007; Gadiot et al., 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.

Rabbit and mouse anti-hPD-L1 mAbs were produced as described in Example 9. Out of almost 200 Ab multiclones screened, only ten rabbit multiclone Abs were found to specifically detect the membranous form of PD-L1, and the top five multiclones (designated Nos. 13, 20, 28, 29 and 49) were subsequently subcloned. The clone that produced the most robust detection specifically of membranous PD-L1, rabbit clone 28-8, was selected for the IHC assays. The sequences of the variable regions of mAb 28-8 are set forth in SEQ ID NOs. 35 and 36, 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. 37-42. Rabbit clones 28-1, 28-12, 29-8 and 20-12 were the next best mAbs in terms of robust detection of membranous PD-L1 in FFPE tissues.

Anti-PD-L1 Abs of the invention also include antigen-binding portions of the above Abs, including Fab, F(ab')$_2$ Fd, Fv, and scFv, di-scFv or bi-scFv, and scFv-Fc fragments, diabodies, triabodies, tetrabodies, and isolated CDRs (*see* Hollinger and Hudson, 2005; Olafsen and Wu, 2010, for further details).

Nucleic Acid Molecules Encoding Antibodies of the Invention

Another aspect of the present disclosure pertains to isolated nucleic acid molecules that encode any of the Abs of the invention. These nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. 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.

Nucleic acids of the invention can be obtained using standard molecular biology techniques. For Abs 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 Ab made by the hybridoma can be obtained by standard PCR amplification or cDNA cloning techniques. Nucleic acids encoding Abs obtained from an immunoglobulin gene library (*e.g.*, using phage display techniques) can be recovered from the library.

Preferred nucleic acids molecules of the invention are those encoding the V_H and

V_κ sequences of the anti-PD-1 HuMAbs, 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 (disclosed in U.S. Patent No. 8,008,449), and those encoding the V_H and V_κ sequences of the anti-PD-L1 HuMAbs, 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4 (disclosed in U.S. Patent No. 7,943,743). An isolated DNA encoding the V_H region can be converted to a full-length heavy chain gene by operatively linking the V_H-encoding DNA to another DNA molecule encoding heavy chain constant regions (C_{H1}, C_{H2} and C_{H3}), the sequences of which are known in the art and 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 is preferably an IgG1 or IgG4 constant region. Similarly, an isolated DNA encoding the V_L region can be converted to a full-length light chain gene by operatively linking the V_L-encoding DNA to another DNA molecule encoding the light chain constant region (C_L), the sequence of which is known in the art and 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.

Pharmaceutical Compositions

Antibodies of the present invention may be constituted in a composition, *e.g.*, a pharmaceutical composition, containing one Ab or a combination of Abs, or an antigen-binding portion(s) thereof, and a pharmaceutically acceptable carrier. As used herein, a "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). A pharmaceutical composition of the invention may include one or more pharmaceutically acceptable salts, anti-oxidant, aqueous and nonaqueous carriers, and/or adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents.

Dosage regimens are adjusted to provide the optimum desired response, *e.g.*, a therapeutic response or minimal adverse effects. For administration of an anti-PD-1 or anti-PD-L1 Ab, the dosage ranges from about 0.0001 to about 100 mg/kg, usually from about 0.001 to about 20 mg/kg, and more usually from about 0.01 to about 10 mg/kg, of the subject's body weight. Preferably, the dosage is within the range of 0.1-10 mg/kg body weight. For example, dosages can be 0.1, 0.3, 1, 3, 5 or 10 mg/kg body weight, and

more preferably, 0.3, 1, 3, or 10 mg/kg body weight. The dosing schedule is typically designed to achieve exposures that result in sustained receptor occupancy (RO) based on typical pharmacokinetic properties of an Ab. 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. The dosage and scheduling may change during a course of treatment. For example, dosing schedule may comprise administering the Ab: (i) every two weeks in 6-week cycles; (ii) every four weeks for six dosages, then every three months; (iii) every three weeks; (iv) 3-10 mg/kg body weight once followed by 1 mg/kg body weight every 2-3 weeks. Considering that an IgG4 Ab typically has a half-life of 2-3 weeks, a preferred dosage regimen for an anti-PD-1 or anti-PD-L1 Ab of the invention comprises 0.3-10 mg/kg body weight, preferably 3-10 mg/kg body weight, more preferably 3 mg/kg body weight via intravenous administration, with the Ab being given every 14 days in up to 6-week or 12-week cycles until complete response or confirmed progressive disease.

In some methods, two or more mAbs with different binding specificities are administered simultaneously, in which case the dosage of each Ab administered falls within the ranges indicated. Antibody is usually administered on multiple occasions. Intervals between single dosages can be, for example, weekly, every 2 weeks, every 3 weeks, monthly, every three months or yearly. Intervals can also be irregular as indicated by measuring blood levels of Ab to the target antigen in the patient. In some methods, dosage is adjusted to achieve a plasma Ab concentration of about 1-1000 μg/ml and in some methods about 25-300 μg/ml.

Alternatively, the Ab 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 Ab in the patient. In general, human Abs show the longest half-life, followed by humanized Abs, chimeric Abs, and nonhuman Abs. 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 typically 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 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.

Uses and Methods of the Invention

The Abs, Ab compositions, nucleic acids and methods of the present invention have numerous *in vitro* and *in vivo* utilities including, for example, methods to determine and quantify the expression of PD-1 or PD-L1 comprising binding of the Abs to the target polypeptides or measuring the amount of nucleic acid encoding these polypeptides, and a method for immunotherapy of a subject afflicted with a disease comprising administering to the subject a composition comprising a therapeutically effective amount of a therapeutic agent that inhibits signaling from an inhibitory immunoregulator. In preferred embodiments of the latter method, the inhibitory immunoregulator is a component of the PD-1/PD-L1 signaling pathway, and the therapeutic agent disrupts signaling of this pathway. More preferably, the therapeutic agent is an Ab that interferes with the interaction between PD-1 and PD-L1. In certain preferred embodiments of this method, the Ab binds specifically to PD-1 and blocks the interaction of PD-1 with PD-L1 and/or PD-L2. In other preferred embodiments, the therapeutic agent is an Ab that binds specifically to PD-L1 and blocks the interaction of PD-L1 with PD-1 and/or B7-1 (CD80). Thus, this disclosure provides methods for potentiating an immune response in a subject comprising administering an anti- PD-1 and/or an anti-PD-L1 Ab in order to disrupt the interaction between PD-1 and PD-L1, and methods of treating diseases

mediated by such a potentiation of the immune response. When Abs to PD-1 and PD-L1 are administered together, the two can be administered sequentially in either order or simultaneously. In certain aspects, this disclosure provides methods of modifying an immune response in a subject comprising administering to the subject an anti-PD-1 and/or an anti-PD-L1 Ab of the invention, or antigen-binding portion thereof, such that the immune response in the subject is modified. Preferably, the immune response is potentiated, enhanced, stimulated or up-regulated. In preferred embodiments, the Abs of the present invention are human Abs.

Preferred subjects include human patients in need of enhancement of an immune response. The immunotherapeutic methods disclosed herein are particularly suitable for treating human patients having a disorder that can be treated by potentiating a T-cell mediated immune response. In certain embodiments, the methods are employed for treatment of subjects afflicted with a disease caused by an infectious agent. In preferred embodiments, the methods are employed for treatment of subjects afflicted with, or at risk of being afflicted with, a cancer.

Cancer Immunotherapy

Blockade of PD-1/PD-L1 interaction has been shown to potentiate immune responses *in vitro* (U.S. Patent Nos. 8,008,449 and 7,943,743; Fife *et al.*, 2009) and mediate preclinical antitumor activity (Dong *et al.*, 2002; Iwai *et al.*, 2002). However, the molecular interactions potentially blocked by these two Abs are not identical: anti-PD-1 Abs of the invention disrupt PD-1/PD-L1 and potentially PD-1/PD-L2 interactions; in contrast, whereas anti-PD-L1 Abs of the invention also disrupt PD-1/PD-L1 interactions, they do not block PD-1/PD-L2 interactions but instead may disrupt the PD-1-independent PD-L1/CD80 interaction, which has also been shown to down-modulate T-cell responses *in vitro* and *in vivo* (Park *et al.*, 2010; Paterson *et al.*, 2011; Yang *et al.*, 2011; Butte *et al.*, 2007; Butte *et al.*, 2008). Thus, it is possible that among these varied ligand-receptor pairings, different interactions may dominate in different cancer types, contributing to dissimilar activity profiles for the two Abs.

Disruption of the PD-1/PD-L1 interaction by antagonistic Abs can enhance the immune response to cancerous cells in a patient. PD-L1 is not expressed in normal human cells, but is abundant in a variety of human cancers (Dong *et al.*, 2002). The interaction between PD-1 and PD-L1 impairs T cell responses as manifested by a decrease in tumor-infiltrating lymphocytes (TILs) and a decrease in T-cell receptor-

mediated proliferation, resulting in T cell anergy, exhaustion or apoptosis, and immune evasion by the cancerous cells (Zou and Chen, 2008; Blank *et al.*, 2005; Konishi *et al.*, 2004; Dong *et al.*, 2003; Iwai *et al.*, 2002). Immune suppression can be reversed by inhibiting the local interaction between PD-L1 and PD-1 using an anti-PD-1 and/or an anti-PD-L1 Ab. These Abs may be used alone or in combination to inhibit the growth of cancerous tumors. In addition, either or both of these Abs may be used in conjunction with other immunogenic and/or anti-cancer agents including cytokines, standard cancer chemotherapies, vaccines, radiation, surgery, or other Abs.

Immunotherapy of Cancer Patients Using an Anti-PD-1 Antibody

This disclosure provides a method for immunotherapy of a subject afflicted with cancer, which method comprises administering to the subject a composition comprising a therapeutically effective amount of an Ab or an antigen-binding portion thereof that disrupts the interaction of PD-1 with PD-L1 and/or PD-L2. This disclosure also provides a method of inhibiting growth of tumor cells in a subject, comprising administering to the subject an Ab or an antigen-binding portion thereof that disrupts the interaction of PD-1 with PD-L1 and/or PD-L2 in an amount effective to inhibit growth of the tumor cells. In preferred embodiments, the subject is a human. In other preferred embodiments, the Ab or antigen-binding portion thereof is an anti-PD-1 Ab of the invention or an antigen-binding portion thereof. In certain embodiments, the Ab or antigen-binding portion thereof is of an IgG1 or IgG4 isotype. In other embodiments, the Ab or antigen-binding portion thereof is a mAb or an antigen-binding portion thereof. In further embodiments, the Ab or antigen-binding portion thereof is a chimeric, humanized or human Ab or an antigen-binding portion thereof. In preferred embodiments for treating a human patient, the Ab or antigen-binding portion thereof is a human Ab or an antigen-binding portion thereof.

The clinical trials described in the Examples employed the anti-PD-1 HuMAb, nivolumab (designated 5C4 in U.S. Patent No. 8,008,449), to treat cancer. While 5C4 was selected as the lead Ab for entering the clinic, it is notable that several anti-PD-1 Abs of the invention share with 5C4 functional properties that are important to the therapeutic activity of 5C4, including high affinity binding specifically to human PD-1, increasing T-cell proliferation, IL-2 secretion and interferon-γ production in an MLR assay, inhibiting the binding of PD-L1 and/or PD-L2 to PD-1, and inhibiting tumor cell growth *in vivo*. Moreover, certain of the anti-PD-1 Abs of the invention, 17D8, 2D3, 4H1 and 7D3 are

structurally related to 5C4 in comprising V_H and V_κ regions that have sequences derived from V_H 3-33 and V_κ L6 germline sequences, respectively. In addition, 5C4, 2D3, 7D3, 4H1 and 17D8 all cross-compete for binding to the same epitope region of hPD-1 (Example 1). Thus, the preclinical characterization of nivolumab and other anti-PD-1 HuMabs indicate that the methods of treating cancer provided herein may be performed using different Abs selected from the broad genus of anti-PD-1 Abs of the invention.

Accordingly, certain embodiments of the immunotherapy methods disclosed herein comprise administering to a patient an anti-PD-1 Ab or antigen-binding portion thereof comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-33 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6 germline sequence, or (b) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 4-39 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L15 germline sequence.

In certain other embodiments, the Ab or antigen-binding portion thereof that is administered to the patient cross-competes for binding to PD-1 with a reference Ab or a reference antigen-binding portion thereof comprising: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 1 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 8; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 2 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 9; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 3 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 10; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 5 and a human light chain variable region comprising consecutively linked amino

acids having the sequence set forth in SEQ ID NO: 12; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 6 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 13; or (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 7 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 14. In preferred embodiments, the Ab or antigen-binding portion thereof cross-competes for binding to PD-1 with nivolumab.

In certain embodiments of the immunotherapy methods disclosed herein, the anti-PD-1 Ab or antigen-binding portion thereof administered to the patient comprises: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 1 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 8; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 2 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 9; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 3 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 10; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 5 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 12; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 6 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 13; or (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 7 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 14. In preferred

embodiments, the anti-PD-1 Ab or antigen-binding portion comprises a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11. In other preferred embodiments, the anti-PD-1 Ab is nivolumab.

In certain embodiments of the present methods, the anti-PD-1 Ab is formulated for intravenous administration. In preferred embodiments, the Ab is administered intravenously at a dose of 3 mg/kg over 60 minutes every 2 weeks. Typically, treatment is continued as long as clinical benefit is observed or until unmanageable toxicity or disease progression occurs.

In the clinical trials of anti-PD-1 immunotherapy described in the Examples below, intriguing ORs with durable clinical responses, even in heavily pretreated patients, were observed across multiple tumor types including a substantial proportion of NSCLC, MEL, and RCC patients and in various sites of metastasis including liver, lung, lymph nodes, and bone. MEL and RCC are considered to be immunogenic neoplasms, having previously been demonstrated to be responsive to cancer immunotherapy, *e.g.*, interferon-alfa and interleukin-2 in both MEL and RCC (Eton *et al.*., 2002; Coppin *et al.*, 2005; McDermott and Atkins, 2006) and anti-CTLA-4 Ab in MEL (Hodi *et al.*, 2010). In MEL patients, significant ORRs were observed with nivolumab across different doses of 0.1, 0.3, 1, 3, or 10 mg/kg with an overall response rate of about 31%, and similarly comparable ORRs of about 30% were observed in RCC patients treated with nivolumab doses of 1 or 10 mg/kg (*see* Example 7). In contrast, immunotherapy has historically had minimal success in lung cancer. This lack of success has been attributed to NSCLC being "non-immunogenic" (*see, e.g.*, Holt and Disis, 2008; Holt *et al.*, 2011). The ability of lung cancer to thwart the immune system results from multiple factors, including the secretion of immunosuppressive cytokines, loss of major histocompatibility complex antigen expression, and co-opting of T cell inhibitory pathways (Dasanu *et al.*, 2012; Marincola *et al.*, 2000; Brahmer *et al*, 2012).

Despite the limited efficacy of immunotherapy historically seen in lung cancer and the diverse mechanisms employed by lung cancer cells to evade immune system attack, various tumor cell vaccines (*e.g.*, belagenpumatucel-L, a whole-cell-based vaccine that blocks the action of TGF-β2) and antigen-based vaccines (*e.g.*, a humoral EGF vaccine and vaccines incorporating a MAGE-A3 fusion protein or portions of the tumor-

associated MUC1 antigen) that augment antigen-specific antitumor immunity are being evaluated in clinical trials (Holt *et al.*, 2011; Shepherd *et al.*, 2011; Dasanu *et al.*, 2012; Brahmer *et al*, 2012). However, while such antigen-specific vaccines have shown some promise for the treatment of NSCLC, Holt *et al.* (2011) noted that trials involving nonspecific immunotherapeutic interventions had failed to improve outcomes in NSCLC, which indicated a need to combine them with antigen-specific vaccines. These authors expressed the view that truly efficacious immunotherapy of NSCLC would only result from implementation of strategies to both augment antitumor immunity and counteract tumor-mediated immunosuppression, and concluded that the nonexistent or weak nascent immune responses in NSCLC patients make it unlikely that nonspecific stimulation of the immune system or removal of immunosuppression would create changes in clinical outcomes.

Thus, the results reported herein with NSCLC are particularly striking, unexpected and surprising. In NSCLC patients, ORs were observed at nivolumab doses of 1, 3, or 10 mg/kg with response rates of 3%, 24%, and 20%, respectively (*see* Example 7). ORs were observed across NSCLC histologies: 9 responders of 54 squamous (17%), and 13 of 74 nonsquamous (18%). This level of activity seen with nivolumab in NSCLC patients with significant prior therapy (54% with 3 lines of previous therapy) and across histologies is unprecedented, especially in the squamous histology patients (*cf.* Gridelli *et al.*, 2008; Miller, 2006), and provides a very favorable benefit/risk dynamic regarding efficacy and safety compared to existing standard-of-care.

In certain embodiments of the present immunotherapy methods, the anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic squamous or non-squamous NSCLC after platinum based therapy (regardless of maintenance) and one other treatment. In other embodiments, the anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic squamous or non-squamous NSCLC after failure of platinum-based therapy and one other prior chemotherapy regimen. In further embodiments, the anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic squamous or non-squamous NSCLC after failure of two lines of therapy one of which must include platinum based regimen. In yet further embodiments, the anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic squamous or non-squamous NSCLC after at least one prior chemotherapy, after failure of at least one prior platinum based therapy, or after progression on at least one platinum doublet therapy. In

all of the immunotherapy methods disclosed herein, treatment may be continued as long as clinical benefit is observed or until unmanageable toxicity or disease progression occurs.

Durability of clinical responses to anti-PD-1 in heavily-pretreated cancer patients

The critical role of the PD-1 pathway in suppressing anti-tumor immunity, first revealed in laboratory models, has now been validated in clinical studies. As described herein, monotherapy with drugs blocking PD-1 (nivolumab) or its major ligand PD-L1 (BMS-936559) can mediate regression in patients with treatment-refractory advanced cancers. The objective response rate (ORR) in heavily pretreated NSCLC patients receiving anti-PD-1 Ab, including patients with squamous histology, is surprising and unexpected, as standard salvage therapies historically show modest benefit in these patients (Scagliotti *et al.*, 2011). As measured by standard RECIST in this study, ORs were long-lasting, with median response durations of about 18 months in 22 of 129 responders ((*see* Example 7). In addition, patterns of tumor regression consistent with immune-related patterns of response were observed.

These findings have established the PD-1 pathway as a new therapeutic focus in oncology (Pardoll, 2012; Topalian *et al.*, 2012c; Hamid and Carvajal, 2013). In the current study, in which 54% of patients had progressive disease following 3 or more prior systemic regimens, preliminary analysis has been conducted up to March 2013. This updated analysis has supported and reinforced the data obtained and conclusions reached from the earlier February 2012 analyses. Thus, conventional ORs or prolonged disease stabilization were documented in patients with NSCLC (17% and 10%, respectively), MEL (31%, 7%) and RCC (29%, 27%), across all doses tested (*see* Example 7). Additionally, 13 patients (4%) manifested unconventional, "immune-related" response patterns as previously described with anti-CTLA-4 therapy, several of which were sustained (Sharma *et al.*, 2011).

The durability of ORs across multiple cancer types in patients treated with the anti-PD-1 Ab is particularly notable. The updated analyses again underscored the durability of clinical activity in nivolumab-treated patients, which has not generally been observed with chemotherapy or small molecule inhibitors to date, but has been observed in some patients with advanced melanoma receiving immunotherapies, including ipilimumab and high-dose interleukin-2 (Topalian *et al.*, 2011; Hodi *et al.*, 2010). The persistence of partial tumor regressions following drug discontinuation suggests that PD-1

blockade has reset the immune equilibrium between tumor and host, and the OS benefit may ultimately prove to be significantly longer than has been measured to date. Further follow-up is needed to determine the ultimate durability of tumor regression and disease stabilization in patients on these clinical trials.

Significantly, the durable objective tumor regression and disease stabilization induced by nivolumab in heavily-pretreated patients with advanced NSCLC, MEL, and RCC translate to survival outcomes that surpass historical data for these patient populations treated with conventional chemotherapy and/or tyrosine-kinase inhibitor (TKI) treatments. In NSCLC, 1- and 2-year survival rates were 42% and 14%, respectively, with median OS of 9.2 and 10.1 months in patients with squamous and non-squamous cancers, respectively (*see* Example 7). This high level of efficacy is especially impressive since 54% of these patients had received 3 or more prior therapies. Moreover, because follow-up of many lung cancer patients was relatively limited, these figures may change as data mature. Historically, 2L chemotherapeutics for lung cancer (*i.e.*, docetaxel and pemetrexed) have achieved a median OS of 7.5-8.3 months, and one-year survival rates of approximately 30% (Shepherd *et al.*, 2000; Hanna *et al.*, 2004). In a 2L/3L population, erlotinib-treated patients had a median survival of 6.7 months, versus 4.7 months in placebo-treated patients (Shepherd *et al.*, 2005). No therapy is currently approved for use in lung cancer beyond the 3L setting, and minimal data exist to benchmark survival in this patient population, except in retrospective reviews with reported median survivals of 5.8-6.5 months and 1-year survivals of 25% (Girard *et al.*, 2009; Scartozi *et al.*, 2010).

In nivolumab-treated MEL patients, median OS of 16.8 months was achieved, with landmark survival rates of 62% (1-year) and 43% (2-year) (*see* Example 7). Survival outcomes in pretreated melanoma patients supported the recent FDA approvals of ipilimumab and vemurafenib. In a recent phase 3 trial enrolling melanoma patients with at least one prior treatment for metastatic disease, ipilimumab increased median OS from 6.4 to 10.1 months, compared to a gp100 peptide vaccine (Hodi *et al.*, 2010). In phase 2 trials of ipilimumab in previously treated patients, 2-year survival rates ranged from 24.2-32.8% (Lebbe *et al.*, 2012). Median OS in previously treated patients with BRAF-mutant melanoma enrolled on a large phase 2 of vemurafenib was 15.9 months, and 1-year survival was 58% (Sosman *et al.*, 2012).

Accordingly, in certain embodiments, immunotherapy with anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic MEL after therapy with dacarbazine (regardless of maintenance) and one other treatment. In other embodiments, the anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic MEL after failure of dacarbazine-based therapy. In further embodiments, the anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic MEL after failure of two lines of therapy one of which must include a dacarbazine-based regimen. In yet further embodiments, the anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic MEL after at least one prior chemotherapy, after failure of at least one prior dacarbazine-based therapy, or after progression on at least dacarbazine therapy. In all of the immunotherapy methods disclosed herein, treatment may be continued as long as clinical benefit is observed or until unmanageable toxicity or disease progression occurs.

In nivolumab-treated patients with RCC, among whom 45% received 3 or more prior therapies and 71% received prior anti-angiogenic therapy, median OS was reached at 22 months (as of the March 2013 date of analysis). Landmark survival rates of 70% (1-year) and 50% (2-year) were observed (*see* Example 7). In a recent Phase 3 trial enrolling kidney cancer patients whose disease progressed following anti-angiogenic therapy, everolimus was compared with placebo: median OS was 14.8 versus 14.4 months, respectively (Motzer *et al.*, 2008; Motzer *et al.*, 2010). A recent Phase 3 trial comparing sorafenib to temsirolimus in a sunitinib-refractory kidney cancer population yielded median OS of 16.6 and12.3 months, respectively (Hutson *et al.*, 2012). Thus, as with NSCLC and MEL, treatment of a heavily-pretreated RCC patient population with nivolumab has yielded a considerably longer median OS (> 22 months) than treatment of a less refractory population with standard-of-care therapies. Controlled Phase 3 trials with prospective survival endpoints are underway in NSCLC, MEL and RCC (NCT01673867, NCT01721772, NCT01642004, NCT01668784, and NCT01721746 (*see* Clinical Trials Website, http://www.clinicaltrials.gov). The results from these trials are expected to further demonstrate the high efficacy of, and durability of responses to, nivolumab in these cancers compared standard-of-care therapies.

In certain embodiments, immunotherapy with anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic RCC after therapy with an anti-angiogenic TKI or a mTOR inhibitor (regardless of maintenance) and one other treatment. In other embodiments, the anti-PD-1 Ab is indicated as monotherapy for

locally advanced or metastatic RCC after failure of therapy with an anti-angiogenic TKI or a mTOR inhibitor. In further embodiments, the anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic RCC after failure of two lines of therapy one of which must include an anti-angiogenic TKI or a mTOR inhibitor. In yet further embodiments, the anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic RCC after at least one prior chemotherapy, after failure of at least one prior anti-angiogenic TKI- or mTOR inhibitor-based therapy, or after progression on at least an anti-angiogenic TKI or a mTOR inhibitor therapy. Anti-PD-1 immunotherapy may be continued as long as clinical benefit is observed or until unmanageable toxicity or disease progression occurs.

Notably, OS in lung cancer, melanoma and kidney cancer patients receiving nivolumab was considerably longer than PFS. These results mirror those reported for ipilimumab (Hodi *et al.*, 2010), and reflect the observation that early tumor enlargement or the appearance of new lesions in some patients receiving immune checkpoint blockade can evolve to disease stabilization or regression. These findings suggest that progression-free survival may not be an optimal endpoint for determining the efficacy of nivolumab and other agents in this class.

The data disclosed herein demonstrating the high efficacy, durability and broad applicability of anti-PD-1 immunotherapy for treating cancer has led to nivolumab being tested for additional types of cancer. For example, on the basis that increased PD-L1 expression has been reported with various hematologic malignancies and may prevent the host immune response from exerting a beneficial impact on the malignant cells, a trial to confirm the ability of nivolumab to mediate antitumor activity in patients with hematologic malignancies (multiple myeloma, B-cell lymphoma, T-cell lymphoma, Hodgkin's lymphoma/primary mediastinal B-cell lymphoma, and chronic myelogenous leukemia) has been initiated (NCT01592370). Nivolumab is also being tested as a monotherapy in advanced hepatocellular carcinoma (NCT01658878).

In summary, the results of anti-PD-1 immunotherapy disclosed herein are remarkable in at least the following three respects. First, the efficacy of anti-PD-1 has been shown to surpass historical efficacy data for patients on standard-of-care treatments for cancer. Notably, this efficacy has been demonstrated in patient in heavily pretreated populations in which about half of the patients had progressive disease following 3 or more prior systemic regimens. Such patients, afflicted with advanced, metastatic and/or

refractory cancers, are notoriously difficult to treat. Accordingly, this disclosure provides methods for immunotherapy of a patient afflicted with an advanced, metastatic and/or refractory cancer, which method comprises administering to the patient a therapeutically effective amount of an Ab or an antigen-binding portion thereof that disrupts the interaction of PD-1 with PD-L1 and/or PD-L2. In certain embodiments of any of the therapeutic methods disclosed herein, the subject has been pre-treated for the cancer; for example, the subject had undergone at least one, two, or three prior lines of therapy for cancer.

Second, the present therapeutic methods have been shown to be applicable to a broad genus of different cancers. Based on the surprising discovery that even a "non-immunogenic" cancer such as NSCLC (Holt *et al.*, 2011) and hard-to-treat cancers such as ovarian and gastric cancers (as well as other cancers tested, including MEL, RCC, and CRC) are amendable to treatment with anti-PD-1 and/or anti-PD-L1 (*see* Examples 7 and 14), this disclosure generally provides methods for immunotherapy of a patient afflicted with practically any of a very wide range of cancers.

Third, treatment with an anti-PD-1 or anti-PD-L1 Ab has been shown to produce strikingly durable clinical activity in cancer patients. Accordingly, this disclosure provides immunotherapeutic methods of inducing a durable clinical response in a cancer patient comprising administering to the patient a therapeutically effective amount of an Ab or an antigen-binding portion thereof that disrupts the interaction of PD-1 with PD-L1 and/or PD-L2. In preferred embodiments of any of the therapeutic methods described herein, the clinical response is a durable response.

As used herein, a "durable" response is a therapeutic or clinical response that exceeds the anticipated median OS rate in a patient population. The anticipated median OS rate varies with different cancers and different patient populations. In certain embodiments, a durable response exceeds the anticipated median OS rate in the relevant patient population by at least 10%, preferably by at least 20%, more preferably by at least 30%, and even more preferably by at least 50%. A major benefit of immunotherapeutic approaches based on PD-1 pathway blockade may be the functional restoration of exhausted T cells with long-term generation of memory T cells that may maintain antitumor immune surveillance and inhibit tumor growth for prolonged periods extending to many years, even in the absence of continued therapy (Kim and Ahmed, 2010). Indeed, long-term follow-up studies on patients following cessation of nivolumab therapy have

confirmed that a patient with CRC experienced a complete response which was ongoing after 3 years; a patient with RCC experienced a partial response lasting 3 years off therapy, which converted to a complete response that was ongoing at 12 months; and a patient with melanoma achieved a partial response that was stable for 16 months off therapy, and recurrent disease was successfully treated with reinduction anti-PD-1 therapy (Lipson *et al.*, 2013).

Immune-related clinical responses

It has become evident that conventional response criteria may not adequately assess the activity of immunotherapeutic agents because progressive disease (by initial radiographic evaluation) does not necessarily reflect therapeutic failure. For example, treatment with the anti-CTLA-4 Ab, ipilimumab, has been shown to produce four distinct response patterns, all of which were associated with favorable survival: (a) shrinkage in baseline lesions, without new lesions; (b) durable stable disease (in some patients followed by a slow, steady decline in total tumor burden); (c) response after an increase in total tumor burden; and (d) response in the presence of new lesions. Accordingly, to properly evaluate immunotherapeutic agents, long-term effects on the target disease must also be captured. In this regard, systematic immune-related response criteria (irRC) that make allowances for an early increase in tumor burden and/or the appearance of new lesions, and which seek to enhance the characterization of immune-related response patterns, have been proposed (Wolchok *et al.*, 2009). While the full impact of these unconventional response patterns remains to be defined in randomized trials of nivolumab with survival endpoints, the present observations are reminiscent of findings with ipilimumab in which a significant extension of OS was observed in treated patients (Hodi *et al.*, 2010; Robert *et al.*, 2011).

The overall risk/benefit profile of anti-PD-1 immunotherapy is also favorable, with a low incidence of more severe drug-related adverse events (AEs; > grade 3), the specific events observed to date being consistent with other immunotherapeutic agents. This suggests that anti-PD-1 immunotherapy can be delivered in an outpatient setting with minimal supportive care.

Broad spectrum of cancers treatable by anti-PD-1 immunotherapy

The clinical data presented herein demonstrate that immunotherapy based on PD-1 blockade is not limited to only "immunogenic" tumor types, such as MEL and RCC, but extends to tumor types not generally considered to be immune-responsive, including

NSCLC. The unexpected successes with treatment-refractory metastatic NSCLC underscore the possibility that any neoplasm can be "immunogenic" in the context of proper immune modulation, and suggest that PD-1 blockade as an immunotherapeutic approach is broadly applicable across a very diverse range of tumor types. Thus, cancers that may be treated using the anti-PD-1 Abs of the invention also include cancers typically responsive to immunotherapy as well as cancers that have traditionally been regarded as non-immunogenic. Non-limiting examples of preferred cancers for treatment include NSCLC, MEL, RCC, CRC, CRPC, HCC, squamous cell carcinoma of the head and neck, carcinomas of the esophagus, ovary, gastrointestinal tract and breast, and a hematologic malignancy. Although NSCLC is not generally considered responsive to immunotherapy, data disclosed herein unexpectedly demonstrate that both squamous and non-squamous NSCLC are responsive to treatment with an anti-PD-1 Ab. Additionally, the disclosure provides for the treatment of refractory or recurrent malignancies whose growth may be inhibited using an anti-PD-1 Ab of the invention.

Examples of cancers that may be treated using an anti-PD-1 Ab in the methods of the present invention, based on the indications of very broad applicability of anti-PD-1 immunotherapy provided herein, 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 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 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 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.

Medical uses of anti-PD-1 Abs

One aspect of this invention is the use of any anti-PD-1 Ab or antigen-binding portion thereof of the invention for the preparation of a medicament for inhibiting signaling from the PD-1/PD-L1 pathway so as to thereby potentiate an endogenous immune response in a subject afflicted with cancer. Another aspect is the use of any anti-PD-1 Ab or an antigen-binding portion thereof of the invention for the preparation of a medicament for immunotherapy of a subject afflicted with cancer comprising disrupting the interaction between PD-1 and PD-L1. These uses for the preparation of medicaments are broadly applicable to the full range of cancers disclosed herein. In preferred embodiments of these uses, the cancers include squamous NSCLC, non-squamous NSCLC, MEL, RCC, CRC, CRPC, HCC, squamous cell carcinoma of the head and neck, and carcinomas of the esophagus, ovary, gastrointestinal tract and breast, and a hematologic malignancy. This disclosure also provides medical uses of any anti-PD-1 Ab or antigen-binding portion thereof of the invention corresponding to all the embodiments of the methods of treatment employing an anti-PD-1 Ab described herein.

The disclosure also provides an anti-PD-1 Ab or an antigen-binding portion thereof of the invention for use in treating a subject afflicted with cancer comprising potentiating an endogenous immune response in the subject by inhibiting signaling from the PD-1/PD-L1 pathway. The disclosure further provides an anti-PD-1 Ab or an antigen-binding portion thereof of the invention for use in immunotherapy of a subject afflicted with cancer comprising disrupting the interaction between PD-1 and PD-L1. These Abs may be used in potentiating an endogenous immune response against, or in immunotherapy of, the full range of cancers disclosed herein. In preferred embodiments, the cancers include squamous NSCLC, non-squamous NSCLC, MEL (*e.g.*, metastatic malignant MEL), RCC, CRC, CRPC, HCC, squamous cell carcinoma of the head and neck, and carcinomas of the esophagus, ovary, gastrointestinal tract and breast, and a hematologic malignancy.

Combination Therapy Including Anti-PD-1 Antibodies

Whereas monotherapy with anti-PD-1 and anti-PD-L1 Abs has been shown herein to significantly increase the survival of patients with lung cancer, melanoma, kidney cancer, and potentially other malignancies, preclinical data indicate that synergistic treatment combinations based on PD-1 pathway blockade could have even more potent effects. Clinical evaluation of nivolumab combined with ipilimumab (anti-CTLA-4), whose mechanism of action is similar yet distinct from nivolumab's (Parry *et al.*, 2005; Mellman *et al.*, 2011; Topalian *et al.*, 2012c), is ongoing and results from a Phase 1 trial are provided herein (*see, also*, NCT01024231; NCT01844505; NCT01783938; Wolchok *et al.*, 2013a; Wolchok *et al.*, 2013b; Hodi *et al.*, 2013). Clinical studies have also been initiated involving the administration of nivolumab in combination with melanoma vaccines (NCT01176461, NCT01176474; Weber *et al.*, 2013), with lirilumab (BMS-986015), a human IgG4 anti-KIR Ab, in patients with advanced solid tumors (NCT01714739; Sanborn *et al.*, 2013), with cytokines, for example, IL-21, in patients with advanced or metastatic solid tumors (NCT01629758; Chow *et al.*, 2013), with chemotherapeutic drugs, for example, with platinum-based doublet chemotherapy in chemotherapy-naïve NSCLC patients (NCT01454102; Rizvi *et al.*, 2013), and small-molecule targeted therapies in patients with metastatic RCC (NCT01472081; Amin *et al.*, 2013).

In certain aspects, this disclosure relates to the combination of an anti-PD-1 Ab with different cancer treatments, including chemotherapeutic regimes, radiation, surgery, hormone deprivation and angiogenesis inhibitors, for the treatment of various cancers. PD-1 blockade may also be effectively combined with an immunogenic agent, for example, a preparation of cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), antigen-presenting cells such as dendritic cells bearing tumor-associated antigens, cells transfected with genes encoding immune stimulating cytokines (He *et al.*, 2004), and/or another immunotherapeutic Ab (*e.g.*, an anti-CTLA-4, anti-PD-L1 and/or anti-LAG-3 Ab). 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.

Combination of anti-PD-1 and anti-CTLA-4 Abs to treat advanced melanoma

Given that immunologic checkpoints are non-redundant and can inhibit T-cell activation, proliferation and effector function within lymph nodes and/or the tumor microenvironment, and based on preclinical data that the combination of anti-CTLA-4 and anti-PD-1 had a stronger anti-tumor effect in mouse tumor models than either Ab alone (*see* U.S. Patent No. 8,008,449), the hypothesis that combined blockade of CTLA-4 and PD-1 could produce greater anti-tumor activity than single agents was tested in a clinical trial in MEL patients (Example 15).

Although not formally compared in this study, the concurrent nivolumab/ipilimumab regimen, which comprised achieved ORRs that exceed the rates achieved with either nivolumab (Example 7) or ipilimumab alone (Hodi *et al.*, 2010). Most importantly, rapid and deep responses were achieved in a substantial portion of treated patients, a "deep" tumor response referring to a response in target lesions characterized by a reduction of 80% or more from baseline measurements by radiographic assessment. In the present study, the majority of responding patients, including some with extensive and bulky tumor burden, achieved > 80% tumor regression at the time of the initial tumor assessment. Particularly striking was the observation that 31% of response-evaluable patients treated with concurrent-regimens (which comprise (i) an induction dosing schedule with combined administration of the anti-PD-1 and anti-CTLA-4 Abs followed by administration of the anti-PD-1 Ab alone, and (ii) a maintenance dosing schedule comprising less frequent combined administration of the anti-PD-1 and anti-CTLA-4 Abs) demonstrated > 80% tumor regression by week 12. At the MTD for the concurrent regimen, all 9 responding patients demonstrated > 80% tumor regression with 3 CRs. In contrast, in clinical experience to date, < 3% of MEL patients who received nivolumab or ipilimumab at 3 mg/kg achieved a CR (Example 7; Hodi *et al.*, 2010). Thus, the overall activity of this immunotherapy combination in this preliminary phase 1 trial compares very favorably with that of other agents approved or being developed for advanced melanoma, including the targeted inhibitors of activated kinases (Chapman *et al.*, 2011). An additional advantage of this combination is the durability of response as demonstrated in the present study as well as longer-term immunotherapy trials with nivolumab (as described herein) and ipilimumab (Wolchok *et al.*, 2013d).

These initial data suggest that rapid responses of a greater magnitude may be achieved in patients treated with a nivolumab/ipilimumab combination compared with the

historical experience of either agent alone. Responses were generally durable, and were observed even in patients whose treatment was terminated early because of toxicity. Responding patients included those with elevated LDH, M1c disease and bulky multi-focal tumor burden. Similar to prior reports regarding ipilimumab or nivolumab monotherapy, conventional ORRs may not fully capture the spectrum of clinical activity and potential benefit in patients treated with the concurrent nivolumab/ipilimumab regimen in that a number of patients experienced either long-term SD or unconventional immune-related patterns of response. Indeed, even among the 7 patients in the concurrent regimen with SD $\geq$ 24 weeks or irSD $\geq$ 24 weeks as best response, 6 demonstrated meaningful tumor regression of at least 19%, and the seventh patient has declining tumor burden after prolonged SD. Prior experience with checkpoint blockade monotherapy supports the observation that some patients may survive for extended periods of time with SD as the best OR, lending credence to the hypothesis that re-establishment of the equilibrium phase of immune surveillance is a desirable outcome (Screiber *et al.*, 2011).

The observation that patients can achieve ORs when treated sequentially with nivolumab after prior ipilimumab indicates that lack of response to CTLA-4 blockade does not preclude clinical benefit from PD-1 blockade and further supports the non-redundant nature of these co-inhibitory pathways. Notably, data disclosed herein (Example 8) suggest an association between the occurrence of response and tumor PD-L1 expression in patients receiving nivolumab, and prior data reveal a correlation between OS and increases in peripheral ALC in patients treated with ipilimumab (Berman *et al.*, 2009; Ku *et al.*, 2010; Postow *et al.*, 2012; Delyon *et al.*, 2013). In the present study of the nivolumab/ipilimumab combination, clinical responses were observed in patients irrespective of lymphocyte count or baseline tumor PD-L1 expression (Example 17), suggesting that the immune response generated by combination therapy has unique features compared to either monotherapy, While the data suggest that baseline tumor PD-L1 expression and lymphocyte count may be less relevant in the setting of active combination regimens capable of inducing rapid and pronounced tumor regression, it is also notable that a different anti-PD-L1 Ab (rabbit mAb 28-8 versus mouse 5H1 mAb) was used in a different IHC assay to measure PD-L1 expression in the combination therapy study compared to the nivolumab monotherapy study. In addition to changes in the IHC assay and Ab, the different results may also reflect differences in biopsy samples and tumor heterogeneity. The utility of PD-L1 expression as a biomarker for anti-PD-1

efficacy will be further evaluated prospectively in randomized phase 3 studies (*see, e.g.*, NCT01721772, NCT01668784, and NCT01721746).

The spectrum of adverse events observed among patients treated with the concurrent regimen was qualitatively similar to experience with nivolumab or ipilimumab monotherapy, although the rate of AEs was increased in patients treated with the combination. Grade 3-4 treatment-related AEs were observed in 53% of patients treated with the concurrent nivolumab/ipilimumab regimen, compared with historical rates of 20% in patients treated with ipilimumab monotherapy (Hodi *et al.*, 2010) and 17% in patients treated with nivolumab alone (Example 5) at a dose of 3 mg/kg. In the sequenced-regimen cohorts, 18% of patients experienced grade 3-4 treatment-related AEs. AEs experienced by patients treated with the concurrent and sequenced regimens were manageable and/or generally reversible using existing treatment algorithms.

Collectively, these results suggest that nivolumab and ipilimumab can be administered concurrently with a manageable safety profile and leading to durable clinical responses. More rapid and deeper clinical tumor responses were observed in patients treated with the combination compared with the responses obtained with either single agent.

As of the February 2013 clinical cut-off date for the study described in Example 15, of the 52 subjects on the concurrent regimen evaluable for response, 21 (40%) had an OR by modified World Health Organization (mWHO) criteria (Wolchok *et al.*, 2009). In an additional 2 subjects (4%), there was an unconfirmed OR. In Cohort 1 (0.3 mg/kg nivolumab plus 3 mg/kg ipilimumab), 3 out of 14 evaluable subjects had an OR by mWHO (ORR: 21%, including 1 CR and 2 PRs). In Cohort 2 (1 mg/kg nivolumab plus 3 mg/kg ipilimumab), 9 out of 17 evaluable subjects had an OR by mWHO (ORR: 53%; including 3 CRs and 6 PRs). In Cohort 2a (3 mg/kg nivolumab plus 1 mg/kg ipilimumab), 6 out of 15 response evaluable subjects had an OR by mWHO (ORR: 40%; including 1 CR and 5 PRs). In Cohort 3 (3 mg/kg nivolumab plus 3 mg/kg ipilimumab), 3 out of 6 evaluable subjects had an objective response by mWHO (ORR: 50%, including 3 PRs). Based on these data, the invention disclosed herein includes a method for treating a subject afflicted with a cancer comprising administering to the subject: (a) an Ab or an antigen-binding portion thereof that specifically binds to and inhibits PD-1; and (b) an Ab or an antigen-binding portion thereof that specifically binds to and inhibits CTLA-4; each Ab being administered at a dosage ranging from 0.1 to 20.0 mg/kg body weight in a

concurrent regimen comprising: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 Abs 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 10 doses, followed by administration of the anti-PD-1 Ab 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 Abs at a dosing frequency of at least once every 8, 12 or 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 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 Abs. In other embodiments, the concurrent regimen comprises: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 Abs at a dosing frequency of once every 2, 3 or 4 weeks, or once a month, for 2, 4, 6 or 8 doses, followed by administration of the anti-PD-1 Ab alone at a dosing frequency of once every 2, 3 or 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 Abs at a dosing frequency of once every 8, 12 or 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. In certain other embodiments, each of the anti-PD-1 and anti-CTLA-4 Abs is individually administered at a dosage of 0.1, 0.3, 0.5, 1, 3, 5, 10 or 20 mg/kg. In further embodiments, the dosage of each of the anti-PD-1 and anti-CTLA-4 Abs is kept constant during the induction dosing schedule and the maintenance dosing schedule. In yet other embodiments, the anti-PD-1 and anti-CTLA-4 Abs are administered at the following dosages: (a) 0.1 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab; (b) 0.3 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab; (c) 1 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab; (d) 3 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab; (e) 5 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab; (f) 10 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab; (g) 0.1 mg/kg anti-PD-1 Ab and 1 mg/kg of anti-CTLA-4 Ab; (h) 0.3 mg/kg anti-PD-1 Ab and 1 mg/kg of anti-CTLA-4 Ab; (i) 1 mg/kg anti-PD-1 Ab and 1 mg/kg of anti-CTLA-4 Ab; (j) 3 mg/kg anti-PD-1 Ab and 1 mg/kg of anti-CTLA-4 Ab; (k) 5 mg/kg anti-PD-1 Ab and

1 mg/kg of anti-CTLA-4 Ab; or (l) 10 mg/kg anti-PD-1 Ab and 1 mg/kg of anti-CTLA-4 Ab.

In the protocol described in Example 15, the 3 mg/kg nivolumab plus 3 mg/kg ipilimumab dosing regimen exceeded the MTD (though combinations with ipilimumab and other anti-PD-1 Abs may have a higher or lower MTD), whereas both Cohort 2 (1 mg/kg nivolumab plus 3 mg/kg ipilimumab) and Cohort 2a (3 mg/kg nivolumab plus 1 mg/kg ipilimumab) had similar clinical activity. In addition, the majority of responses to the combination of nivolumab and ipilimumab occurred in the first 12 weeks. Given the uncertainty of whether the ipilimumab administered past week 12 contributes to the clinical benefit and the fact that the U.S. Food and Drug Administration (FDA)- and European Medicines Agency (EMA)-approved schedule for ipilimumab is every 3 weeks for a total of four doses, in a preferred embodiment the anti-CTLA-4 Ab is administered during the induction dosing schedule once every 3 weeks for a total of 4 doses. Monotherapy treatment with nivolumab at 3 mg/kg every two weeks until progression has been shown to be associated with durable responses (Examples 4-7), and a maintenance dosing schedule comprising administration of nivolumab every 12 weeks has been shown to be efficacious (Example 15). Thus, starting at week 12, which is after the completion of the four doses of combined nivolumab and ipilimumab, nivolumab at 3 mg/kg may be administered every 2 to at least 12 weeks until progression. Accordingly, in preferred embodiments of the concurrent regimen method, the anti-PD-1 and anti-CTLA-4 Abs are administered at dosages of: (a) 1 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab; or (b) 3 mg/kg anti-PD-1 Ab and 1 mg/kg of anti-CTLA-4 Ab, and the concurrent regimen further comprises: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 Abs at a dosing frequency of once every 3 weeks for 4 doses, followed by administration of the anti-PD-1 alone at a dosing frequency of once every 3 weeks for 4 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 2 to 12 or more weeks for up to 8 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs.

Exposure-response analysis of nivolumab monotherapy across dose ranges of 1 mg/kg to 10 mg/kg reveals similar clinical activity (Example 7) while exposure-response analysis of 0.3 mg/kg, 3 mg/kg, and 10 mg/kg of ipilimumab monotherapy has

demonstrated increasing activity with increase in dose in a phase 2 trial (Wolchok *et al.*, 2010). Therefore, a dose of 3 mg/kg of ipilimumab (Cohort 2) may be more clinically impactful than selection of 3 mg/kg of nivolumab (Cohort 2a). Thus, in more preferred embodiments of the concurrent regimen method, the anti-PD-1 and anti-CTLA-4 Abs are administered at dosages of 1 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab.

In certain embodiments of the present methods, the anti-PD-1 and anti-CTLA-4 Abs are formulated for intravenous administration. In certain other embodiments, when the anti-PD-1 and anti-CTLA-4 Abs are administered in combination, they are administered within 30 minutes of each other. Either Ab may be administered first, that is, in certain embodiments, the anti-PD-1 Ab is administered before the anti-CTLA-4 Ab, whereas in other embodiments, the anti-CTLA-4 Ab is administered before the anti-PD-1 Ab. Typically, each Ab is administered intravenously over a period of 60 minutes. In further embodiments, the anti-PD-1 and anti-CTLA-4 Abs are administered concurrently, either admixed as a single composition in a pharmaceutically acceptable formulation for concurrent administration, or concurrently as separate compositions with each Ab in a pharmaceutically acceptable formulation.

Data disclosed in Example 7 demonstrate that immunotherapy with nivolumab produced significant clinical activity in MEL patients who were unresponsive to prior ipilimumab therapy. Accordingly, this disclosure provides a sequenced regimen method for treating a subject afflicted with a cancer, the subject having previously been treated with an anti-CTLA-4 Ab, which method comprises administering to the subject an Ab or an antigen-binding portion thereof that specifically binds to and inhibits PD-1 at a dosage ranging from 0.1 to 20.0 mg/kg body weight and at a dosing frequency of at least once every week, at least once every 2, 3 or 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 certain embodiments of this method, administration of the anti-PD-1 Ab to the subject is initiated within 1-24 weeks after last treatment with the anti-CTLA-4 Ab. In other embodiments, administration of the anti-PD-1 Ab to the subject is initiated within 1, 2, 4, 8, 12, 16, 20 or 24 weeks after last treatment with the anti-CTLA-4 Ab. In preferred embodiments, administration of the anti-PD-1 Ab is initiated within 4, 8 or 12 weeks after last treatment of the subject with the anti-CTLA-4 Ab. Certain embodiments of the method comprise administering the anti-PD-1 Ab at a dosage of 0.1-20 mg/kg, *e.g.*, 0.1, 0.3, 0.5, 1, 3, 5, 10 or 20 mg/kg. In preferred embodiments, the

anti-PD-1 Ab is administered at a dosage of 1 or 3 mg/kg. In certain embodiments, the sequenced regimen comprises administering the anti-PD-1 Ab to the subject at a dosing frequency of once every week, once every 2, 3 or 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 preferred embodiments, the anti-PD-1 is administered at a dosage of 1 or 3 mg/kg at a dosing frequency of once every 2 weeks for up to 48 doses. In other preferred embodiments, the anti-PD-1 Ab is formulated for intravenous administration.

In certain aspects of any of the present concurrent or sequenced regimen methods, the treatment produces at least one therapeutic effect chosen from a reduction in size and/or growth of a tumor, elimination of the tumor, reduction in number of metastatic lesions over time, complete response, partial response, and stable disease. Based on the broad spectrum of cancers in which nivolumab has shown clinical response, the present combination therapy methods are also applicable to diverse cancers. Examples of cancers that may be treated by these methods 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 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 childhood, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the 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 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, lymphocytic lymphoma, 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, refractory or recurring cancers. In preferred embodiments, the cancer to be treated is chosen from MEL, RCC, squamous NSCLC, non-squamous NSCLC, CRC, CRPC, OV, GC, HCC, PC, squamous cell carcinoma of the head and neck, carcinomas of the esophagus, gastrointestinal tract and breast, and a hematological malignancy. In more preferred embodiments, the cancer is MEL.

In certain embodiments, the subject has been pre-treated for the cancer. For example, the patient may have been treated with 1 or 2 or more prior systemic regimens of the type described herein as standard-of-care therapeutics. In certain other embodiments, the cancer is an advanced, recurring, metastatic and/or refractory cancer. In preferred embodiments, the concurrent or sequenced regimen treatment induces a durable clinical response in the subject. In preferred embodiments, the subject is a human, the anti-PD-1 Ab inhibits human PD-1, and the anti-CTLA-4 Ab inhibits human CTLA-4.

The anti-PD-1 Ab used in the present methods may be any therapeutic anti-PD-1 Ab of the invention. In preferred embodiments, the anti-PD-1 Ab is a mAb, which may be a chimeric, humanized or human Ab. In certain embodiments, the anti-PD-1 Ab comprises the CDR1, CDR2 and CDR3 domains in the heavy chain variable region and the CDR1, CDR2 and CDR3 domains in the light chain variable region of 17D8, 2D3, 4Hl, 5C4 (nivolumab), 4A11, 7D3 or 5F4, respectively, as described and characterized in U.S. Patent No. 8,008,449. In further embodiments, the anti-PD-1 Ab comprises the heavy and light chain variable regions of 17D8, 2D3, 4H1, 5C4 (nivolumab), 4A11, 7D3 or 5F4, respectively. In additional embodiments, the anti-PD-1 Ab is 17D8, 2D3, 4H1, 5C4 (nivolumab), 4A11, 7D3 or 5F4. In preferred embodiments, the anti-PD-1 Ab is nivolumab.

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. Anti-CTLA-4 Abs are described in, for example, U.S. Patent Nos. 6,051,227, 7,034,121 in PCT Application Publication Nos. WO 00/37504 and WO 01/14424. An exemplary clinical anti-CTLA-4 Ab is the human mAb 10D1 (now known as ipilimumab

and marketed as YERVOY®) as disclosed in U.S. Patent No. 6,984,720. In certain aspects of any of the present methods, the anti-CTLA-4 Ab is a mAb. In certain other embodiments, the anti-CTLA-4 antibody is a chimeric, humanized or human antibody. In preferred embodiments, the anti-CTLA-4 antibody is ipilimumab.

This disclosure also provides the use of an anti-PD-1 Ab or an antigen-binding portion thereof in combination with an anti-CTLA-4 Ab or an antigen-binding portion thereof for the preparation of a co-administered medicament for treating a subject afflicted with a cancer in a concurrent regimen, wherein the anti-PD-1 and anti-CTLA-4 Abs are each administered at a dosage ranging from 0.1 to 20.0 mg/kg body weight, and further wherein the concurrent regimen comprises: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 Abs 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 administration of the anti-PD-1 Ab 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 10 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 Abs at a dosing frequency of at least once every 8, 12 or 16 weeks, or at least once a quarter, for up to 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. The disclosure provides uses of the combination of anti-PD-1 and anti-CTLA-4 Abs for the preparation of co-administered medicaments corresponding to all the embodiments of the methods of treatment employing these Abs described herein, and are broadly applicable to the full range of cancers disclosed herein.

The disclosure also provides an anti-PD-1 Ab or an antigen-binding portion thereof for use in combination with an anti-CTLA-4 Ab or an antigen-binding portion thereof for treating a subject afflicted with a cancer in a concurrent regimen, wherein the anti-PD-1 and anti-CTLA-4 Abs are each administered at a dosage ranging from 0.1 to 20.0 mg/kg body weight, and further wherein 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 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 administration of the anti-PD-1 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 10 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 every 8, 12 or 16 weeks, or at least once a quarter, for up to 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.

Methods for screening a patient population and selecting a patient as suitable for immunotherapy with the combination of anti-PD-1 and anti-CTLA-4 using the concurrent or sequenced regimens, and methods of predicting efficacy of the Ab combination, based on a PD-L1 biomarker assay are performed as described for anti-PD-1 monotherapy, to the extent this biomarker is applicable to combination therapy with anti-PD-1 and anti-CTLA-4 Abs.

The disclosure additionally provides a kit for treating a subject afflicted with a cancer, the kit comprising: (a) a dosage ranging from 0.1 to 20.0 mg/kg body weight of an Ab or an antigen-binding portion thereof that specifically binds to and inhibits PD-1; (b) a dosage ranging from 0.1 to 20.0 mg/kg of an Ab or an antigen-binding portion thereof that specifically binds to and inhibits CTLA-4; and (c) instructions for using the combination of the anti-PD-1 and anti-CTLA-4 antibodies in any of the concurrent regimen methods. In certain embodiments, some of the dosages of the anti-PD-1 and anti-CTLA-4 Abs are admixed within a single pharmaceutical formulation for concurrent administration. In other embodiments, the dosages of the anti-PD-1 and anti-CTLA-4 Abs are formulated as separate compositions with each Ab in a pharmaceutically acceptable formulation.

The disclosure further provides a kit for treating a subject afflicted with a cancer, the kit comprising: (a) a dosage ranging from 0.1 to 20.0 mg/kg body weight of an Ab or an antigen-binding portion thereof that specifically binds to and inhibits PD-1; and (b) instructions for using the anti-PD-1 Ab in any of the sequenced regimen methods.

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, for example, further combination with dacarbazine or IL-2, for the treatment of MEL. In these instances, it may be possible to reduce the dose of the chemotherapeutic reagent. 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.

Immunotherapy of Cancer Patients Using an Anti-PD-L1 Antibody

PD-L1 is the primary PD-1 ligand up-regulated within solid tumors, where it can inhibit cytokine production and the cytolytic activity of PD-1-positive, tumor-infiltrating $CD4^+$ and $CD8^+$ T-cells, respectively (Dong *et al.*, 2002; Hino *et al.*, 2010; Taube *et al.*, 2012). These properties make PD-L1 a promising target for cancer immunotherapy. The clinical trials of anti-PD-L1 immunotherapy described in the Examples demonstrate for the first time that mAb blockade of the immune inhibitory ligand, PD-L1, produces both durable tumor regression and prolonged ($\geq$ 24 weeks) disease stabilization in patients with metastatic NSCLC, MEL, RCC and OV, including those with extensive prior therapy. The human anti-PD-L1 HuMAb, BMS-936559, had a favorable safety profile overall at doses up to and including 10 mg/kg, as is evident from the low (9%) incidence of grade 3-4 drug-related AEs. These findings are consistent with the mild autoimmune phenotype seen in PD-$L1^{-/-}$ mice (Dong *et al.*, 2004) and the more severe hyperproliferation seen in CTLA-$4^{-/-}$ mice relative to PD-$1^{-/-}$ mice (Phan *et al.*, 2003; Tivol *et al.*, 1995; Nishimura *et al.*, 1999). Most of the toxicities associated with anti-PD-L1 administration in patients were immune-related, suggesting on-target effects. The spectrum and frequency of adverse events of special interest (AEOSIs) is somewhat different between anti-PD-L1 and anti-CTLA-4, emphasizing the distinct biology of these pathways (Ribas *et al.*, 2005). Infusion reactions were observed with BMS-936559, although they were mild in most patients. Severe colitis, a drug-related AE observed in ipilimumab-treated patients (Beck *et al.*, 2006), was infrequently noted with anti-PD-L1.

As noted above for anti-PD-1 immunotherapy, another important feature of anti-PD-L1 therapy is the durability of responses across multiple tumor types. This is particularly notable considering the advanced disease and prior treatment of patients on the current study. Although not compared directly, this durability appears greater than that observed with most chemotherapies and kinase inhibitors used to treat these cancers.

Because peripheral blood T-cells express PD-L1, it is possible to assess *in vivo* RO by BMS-963559 as a pharmacodynamic measure. Median RO was 65.8%, 66.2%,

and 72.4% for the doses tested. Whereas these studies provide a direct assessment and evidence of target engagement in patients treated with BMS-936559, relationships between RO in peripheral blood and the tumor microenvironment remain poorly understood.

Based on the clinical data disclosed herein, this disclosure provides a method for immunotherapy of a subject afflicted with cancer, which method comprises administering to the subject a composition comprising a therapeutically effective amount of an anti-PD-L1 Ab of the invention or an antigen-binding portion thereof. The disclosure also provides a method of inhibiting growth of tumor cells in a subject, comprising administering to the subject an anti-PD-L1 Ab of the invention or an antigen-binding portion thereof. In preferred embodiments, the subject is a human. In certain embodiments, the Ab or antigen-binding portion thereof is of an IgG1 or IgG4 isotype. In other embodiments, the Ab or antigen-binding portion thereof is a mAb or an antigen-binding portion thereof. In further embodiments, the Ab or antigen-binding portion thereof is a chimeric, humanized or human Ab or an antigen-binding portion thereof. In preferred embodiments for treating a human patient, the Ab or antigen-binding portion thereof is a human Ab or an antigen-binding portion thereof.

Clinical trials described in the Examples employed the anti-PD-L1 HuMAb BMS-936559 to treat cancer. While BMS-936559 (designated HuMAb 12A4 in U.S. Patent No. 7,943,743) was selected as the lead anti-PD-L1 Ab for entering the clinic, it is notable that several anti-PD-L1 Abs of the invention share with 12A4 functional properties that are important to the therapeutic activity of 12A4, including high affinity binding specifically to human PD-L1, increasing T-cell proliferation, IL-2 secretion and interferon-γ production in an MLR assay, inhibiting the binding of PD-L1 to PD-1, and reversing the suppressive effect of T regulatory cells on T cell effector cells and/or dendritic cells. Moreover, certain of the anti-PD-L1 Abs of the invention, namely 1B12, 7H1 and 12B7 are structurally related to 12A4 in comprising V_H and V_κ regions that have sequences derived from V_H 1-69 and V_κ L6 germline sequences, respectively. In addition, at least 12B7, 3G10, 1B12 and 13G4 cross-compete with 12A4 for binding to the same epitope region of hPD-L1, whereas 5F8 and 10A5 may bind to the same or an overlapping epitope region as 12A4 (Examples 2 and 3). Thus, the preclinical characterization of 12A4 and other anti-PD-L1 HuMabs indicate that the methods of treating cancer provided herein may be performed using any of the broad genus of anti-

PD-L1 Abs of the invention.

Accordingly, this disclosure provides immunotherapy methods comprising administering to a patient an anti-PD-L1 Ab or antigen-binding portion thereof comprising (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-18 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6 germline sequence; (b) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-69 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L6 germline sequence; (c) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-3 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L15 germline sequence; (d) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 1-69 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ A27 germline sequence; (e) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-9 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L15germline sequence; or (f) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_H 3-9 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human V_κ L18 germline sequence.

In certain embodiments, the anti-PD-L1 Ab or antigen-binding portion thereof administered to the patient cross-competes for binding to PD-L1 with a reference Ab or a reference antigen-binding portion thereof comprising: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 15 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 25; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26; (c) a

human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 17 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 27; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 18 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 28; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 19 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 29; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 20 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 30; (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 21 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 31; (h) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 22 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 32; (i) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 23 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 33; or (j) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 24 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 34. In preferred embodiments, the Ab or antigen-binding portion thereof cross-competes for binding to PD-1 with a reference Ab or reference antigen-binding portion thereof comprising a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26.

In certain preferred embodiments of the immunotherapy methods disclosed herein, the anti-PD-L1 Ab or antigen-binding portion thereof administered to the subject

comprises: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 15 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 25; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 17 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 27; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 18 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 28; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 19 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 29; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 20 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 30; (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 21 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 31; (h) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 22 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 32; (i) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 23 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 33; or (j) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 24 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 34. In more preferred embodiments, the anti-PD-L1 Ab or antigen-binding portion comprises a human heavy chain variable

region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26.

Broad spectrum of cancers treatable by anti-PD-L1 immunotherapy

The clinical activity of anti-PD-L1 in patients with advanced NSCLC, similar to the activity of anti-PD-1 in these patients, was surprising and unexpected since NSCLC has been considered to be poorly responsive to immune-based therapies (Holt and Disis, 2008; Holt *et al.*, 2011). The present clinical data obtained with BMS-936559, an anti-PD-L1 Ab of the invention, substantiate and extend the evidence obtained using the anti-PD-1 Ab that immunotherapy based on PD-1 blockade is not applicable only to "immunogenic" tumor types, such as MEL and RCC, but is also effective with a broad range of cancers, including treatment-refractory metastatic NSCLC, that are generally not considered to be immune-responsive. Preferred cancers that may be treated using the anti-PD-L1 Abs of the invention include MEL (*e.g.*, metastatic malignant melanoma), RCC, squamous NSCLC, non-squamous NSCLC, CRC, ovarian cancer (OV), gastric cancer (GC), breast cancer (BC), pancreatic carcinoma (PC) and carcinoma of the esophagus. Additionally, the invention includes refractory or recurrent malignancies whose growth may be inhibited using the anti-PD-L1 Abs of the invention.

Accordingly, examples of cancers that may be treated using an anti-PD-L1 Ab in the methods of the invention, based on the indications of very broad applicability of anti-PD-L1 immunotherapy provided herein, include bone cancer, skin cancer, cancer of the head or neck, breast cancer, lung cancer, cutaneous or intraocular malignant melanoma, renal cancer, uterine cancer, castration-resistant prostate cancer, colon 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, carcinomas of the ovary, gastrointestinal tract and breast, 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, multiple myeloma, environmentally induced cancers including those induced by asbestos, metastatic cancers, and any combinations of said cancers. The present invention is also applicable to treatment of metastatic cancers.

Combination therapy with anti-PD-L1 Abs

Optionally, Abs to PD-L1 can be combined with an immunogenic agent, for example a preparation of cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), antigen-presenting cells such as dendritic cells bearing tumor-associated antigens, and cells transfected with genes encoding immune stimulating cytokines (He *et al.*, 2004). 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. PD-L1 blockade may also be effectively combined with standard cancer treatments, including chemotherapeutic regimes, radiation, surgery, hormone deprivation and angiogenesis inhibitors, as well as another immunotherapeutic Ab (*e.g.*, an anti-PD-1, anti-CTLA-4 or anti-LAG-3 Ab).

Uses of anti-PD-L1 Abs

This disclosure provides the use of any anti-PD-L1 Ab or antigen-binding portion thereof of the invention for the preparation of a medicament for inhibiting signaling from the PD-1/PD-L1 pathway so as to thereby potentiate an endogenous immune response in a subject afflicted with cancer. This disclosure also provides the use of any anti-PD-L1 Ab or antigen-binding portion thereof of the invention for the preparation of a medicament for immunotherapy of a subject afflicted with cancer comprising disrupting the interaction between PD-1 and PD-L1. The disclosure provides medical uses of any anti-PD-L1 Ab or antigen-binding portion thereof of the invention corresponding to all the embodiments of the methods of treatment employing an anti-PD-L1 Ab described herein.

This disclosure also provides an anti-PD-L1 Ab or an antigen-binding portion thereof of the invention for use in treating a subject afflicted with cancer comprising potentiating an endogenous immune response in a subject afflicted with cancer by inhibiting signaling from the PD-1/PD-L1 pathway. The disclosure further provides an anti-PD-L1 Ab or an antigen-binding portion thereof of the invention for use in

immunotherapy of a subject afflicted with cancer comprising disrupting the interaction between PD-1 and PD-L1. These Abs may be used in potentiating an endogenous immune response against, or in immunotherapy of, the full range of cancers disclosed herein. In preferred embodiments, the cancers include MEL (*e.g.*, metastatic malignant MEL), RCC, squamous NSCLC, non-squamous NSCLC, CRC, ovarian cancer (OV), gastric cancer (GC), breast cancer (BC), pancreatic carcinoma (PC) and carcinoma of the esophagus.

Validation of Cancer Immunotherapy by Immune Checkpoint Blockade

A major implication of the clinical activity of immune checkpoint blockade is that significant endogenous immune responses to tumor antigens are generated and these responses may be harnessed therapeutically to mediate clinical tumor regression upon checkpoint inhibition. In fact, there is evidence that inhibitory ligands such as PD-L1 are induced in response to immune attack, a mechanism termed adaptive resistance (Gajewski *et al.*, 2010; Taube *et al.*, 2012). This potential mechanism of immune resistance by tumors suggests that PD-1/PD-L1-directed therapy might synergize with other treatments that enhance endogenous antitumor immunity. Follow-up studies have verified that patients continue to demonstrate tumor control after cessation of PD-1/PD-L1 pathway blockade (Lipson *et al.*, 2013). Such tumor control may reflect a persistent antitumor immune response and the generation of effective immunologic memory to enable sustained control of tumor growth.

The data disclosed herein on the clinical testing of Abs that block the immunoregulatory receptor, PD-1, and also of Abs that block one of its cognate ligands, PD-L1, are unprecedented. These data constitute the largest clinical experience to date with PD-1 pathway-directed cancer immunotherapy, and the first report specifically describing the safety, tolerability, and initial clinical activity of an anti-PD-L1-directed agent. These findings show that both anti-PD-1 and anti-PD-L1 have favorable overall safety profiles and provide clear evidence of clinical activity across diverse cancers, including NSCLC, a tumor not historically considered responsive to immunotherapy, as well as tumors known to respond to immunotherapy, including MEL, RCC and OV. Thus, these data strongly validate the PD-1/PD-L1 pathway as an important target for therapeutic intervention in cancer.

The remarkable similarities observed between the patterns of clinical activity obtained with the anti-PD-1 and anti-PD-L1 mAbs, and among the tumor types analyzed

to date, validate the general importance of the PD-1/PD-L1 signaling pathway in tumor immune resistance and as a target for therapeutic intervention. Although the molecular interactions blocked by these two Abs are not identical, it has been clearly demonstrated herein that, irrespective of mechanistic details, both anti-PD-1 and anti-PD-L1 Abs of the invention are effective in treating patients afflicted with a wide variety of cancers.

Infectious Diseases

Other methods of the invention are used to treat patients that have been exposed to particular toxins or pathogens. For example, another aspect of this disclosure provides a method of treating an infectious disease in a subject comprising administering to the subject an anti-PD1 or an anti-PD-L1 Ab, or antigen-binding portion thereof, of the invention such that the subject is treated for the infectious disease. Preferably, the Ab is a human anti-human PD-1 or PD-L1 Ab (such as any of the human Abs described herein). Alternatively, the Ab is a chimeric or humanized Ab.

Similar to its application to tumors as discussed above, Ab-mediated PD-1 or PD-L1 blockade can be used alone, or as an adjuvant, in combination with vaccines, to potentiate an immune response to pathogens, toxins, and/or 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, and C), Influenza, Herpes, Giardia, Malaria, Leishmania, Staphylococcus aureus, Pseudomonas aeruginosa. PD-1 and/or PD-L1 blockade is particularly useful against established infections by agents such as HIV that present altered antigens over the course of an infection. Novel epitopes on these antigens are recognized as foreign at the time of anti-human PD-1 or PD-L1 administration, thus provoking a strong T cell response that is not dampened by negative signals through the PD-1/PD-L1 pathway.

In the above methods, PD-1 or PD-L1 blockade can be combined with other forms of immunotherapy such as cytokine treatment (*e.g.*, administration of interferons, GM-CSF, G-CSF or IL-2).

Kits

Also within the scope of the present invention are kits, including pharmaceutical kits, comprising an anti-PD-1 and/or an anti-PD-L1 Ab of the invention, or a combination of an anti-PD-1 and an anti-CTLA-4 Ab, for therapeutic uses, and diagnostic kits

comprising an anti-PD-L1 Ab of the invention for assaying membranous PD-L1 expression as a biomarker for screening patients for immunotherapy or for predicting the efficacy of an immunotherapeutic agent. 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 and/or anti-PD-L1 Abs may be co-packaged with other therapeutic agents in unit dosage form. In certain embodiments of a diagnostic kit, the anti-PD-L1 Ab may be co-packaged with other reagents for performing an assay to detect and/or quantify PD-L1 expression.

In certain preferred embodiments, the pharmaceutical kit comprises the anti-human PD-1 HuMAb, nivolumab. In other preferred embodiments, the pharmaceutical kit comprises the anti-human PD-L1 HuMAb, BMS-936559. In yet other preferred embodiments, the pharmaceutical kit comprises the anti-human CTLA-4 HuMAb, ipilimumab. In certain preferred embodiments, the diagnostic kit comprises the rabbit anti-human PD-L1 mAb, 28-8, comprising the V_H and V_κ regions whose amino acid sequences are set forth in SEQ ID NOs. 35 and 36, respectively. In other preferred embodiments, the diagnostic kit comprises the murine anti-human PD-L1 mAb, 5H1 (Dong *et al.*, 2002).

PD-L1 Biomarker for Predicting anti-PD-1 Efficacy

A particular challenge in cancer immunotherapy has been the identification of mechanism-based predictive biomarkers to enable patient selection and guide on-treatment management. Data disclosed in the Examples below indicate that cell surface PD-L1 expression in tumors is a useful molecular marker for predicting the efficacy of, and selecting patients for, immunotherapy with anti-PD-1 and potentially other immune checkpoint inhibitors.

There are conflicting reports in the literature about the clinical implications of PD-L1 being expressed in tumors. Several studies have concluded that PD-L1 expression in tumors correlates with a poor prognosis for the patient. *See, e.g.*, Hino *et al.* (2010) (MEL); Hamanishi *et al.* (2007) (OV); Thompson *et al.* (2006) (RCC). These findings may be rationalized on the basis that the interaction of PD-L1 on tumor cells and PD-1 on T cells helps abrogate immune responses directed against the tumor, resulting in immune evasion from tumor-specific T cells (Blank *et al.*, 2005). However, in contrast to the foregoing studies, Gadiot *et al.* (2011) and Taube *et al.* (2012) have recently reported that

PD-L1 expression in melanoma tumors correlates with a trend toward better survival. These seemingly contradictory data may reflect the relatively small numbers of patients analyzed, different histologic subtypes studied, or different methodologies used, *e.g.*, the use of different Abs to stain PD-L1, the use of frozen versus paraffin-embedded material for IHC, and the detection of membranous and/or cytoplasmic staining of PD-L1. Taube *et al.* (2012) note that PD-L1 is a type I transmembrane molecule, and hypothesize that while the cytoplasmic presence of PD-L1 may represent intracellular stores of this polypeptide that may be deployed to the cell surface upon appropriate stimulation, it is cell surface PD-L1 expression of that is biologically relevant as a potential biomarker for predicting clinical response to PD-1 blockade. *See, also*, Brahmer *et al.* (2010), which describes preliminary evidence, obtained on a small sample size of only 9 patients, of a correlation between membranous PD-L1 expression and anti-PD-1 efficacy. The data described in the Examples below on the use of membranous PD-L1 expression as a biomarker for anti-PD-1 efficacy, which was obtained from analysis of a much larger sample, substantiate the hypothesis that PD-L1 expression may be used as a biomarker for predicting anti-PD-1 clinical response and for screening patients to identify suitable candidates for immunotherapy with an anti-PD-1 Ab. Though the utility of this biomarker was demonstrated for screening patients for, or predicting the clinical response to, anti-PD-1 immunotherapy, PD-L1 expression may also potentially be applicable more broadly as a companion biomarker for other types of inhibitors of inhibitory immunoregulators.

Specifically, membranous PD-L1 expression was assayed using an automated IHC protocol and a rabbit anti-hPD-L1 Ab. Strikingly, in the initial set of data analyzed (*see* Example 8), no patients with cell surface PD-L1-negative tumors (MEL, NSCLC, CRC, RCC and CRPC) experienced an OR following treatment with the anti-PD-1 Ab, nivolumab. In contrast, cell surface expression of PD-L1 on tumor cells in pretreatment biopsies may be associated with an increased rate of OR among patients treated with nivolumab. While tumor cell expression of PD-L1 may be driven by constitutive oncogenic pathways, it may also reflect "adaptive immune resistance" in response to an endogenous antitumor immune response, part of a host inflammatory response, which may remain in check unless unleashed by blockade of the PD-1/PD-L1 pathway (Taube *et al.*, 2012). This emerging concept of adaptive immune resistance in cancer immunology suggests that inhibitory ligands such as PD-L1 are induced in response to immune attack (Gajewski *et al.*, 2010; Taube *et al.*, 2012). A major implication of the clinical activity of

immune checkpoint blockade as described herein is that significant endogenous immune responses to tumor antigens are generated and these responses may be harnessed therapeutically to mediate clinical tumor regression upon checkpoint inhibition. This potential mechanism of immune resistance by tumors suggests that PD-1/PD-L1-directed therapy might synergize with other treatments that enhance endogenous antitumor immunity.

Assaying Cell-Surface PD-L1 Expression by Automated IHC

As described in the Examples, an automated IHC method was developed for assaying 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 Ab or portion thereof and human PD-L1. Preferably, 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.

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 110ºC 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 post-primary blocking agent; incubating with NovoLink Polymer; adding a chromogen substrate and developing; and counterstaining with hematoxylin.

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%.

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.

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.

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)]

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).

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.*, 2012).

Cancer Immunotherapy with Anti-PD-1 Comprising a Patient Selection Step

This disclosure also provides a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is a suitable candidate for immunotherapy, *e.g.*, administration of an anti-PD-1 Ab, the selecting comprising (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells, (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface, and (iii) selecting the subject as a suitable candidate based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and (b) administering to the selected subject a composition comprising a therapeutically effective amount of an agent that inhibits signaling from an inhibitory immunoregulator *e.g.*, an anti-PD-1 Ab.

There is evidence that membranous PD-L1 expression is a surrogate for an endogenous antitumor immune response that is part of a host inflammatory response (Gajewski *et al.*, 2010; Taube *et al.*, 2012). Accordingly, cell surface expression of PD-L1 in tumors and/or inflammatory cells in the tumor microenvironment may be a marker not just for selecting cancer patients who would benefit from treatment with an anti-PD-1 Ab, but also for treatment with an anti-PD-L1 Ab as well as treatments targeting inhibitory immunoregulatory pathways other than the PD-1/PD-L1 pathway. For example, cell surface expression of PD-L1 in tumors and/or tumor-infiltrating inflammatory cells may be used as a marker for identifying or selecting suitable cancer patients who would benefit from immunotherapy with agents, including Abs, that target, and disrupt or inhibit signaling from, immune checkpoints such as PD-L1, Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4), B and T Lymphocyte Attenuator (BTLA), T cell Immunoglobulin and Mucin domain-3 (TIM-3), Lymphocyte Activation Gene-3 (LAG-3), Killer Immunoglobulin-like Receptor (KIR), Killer cell Lectin-like Receptor G1 (KLRG-1), Natural Killer Cell Receptor 2B4 (CD244), and CD160 (Pardoll, 2012; Baitsch et al., 2012). In certain preferred embodiments, the inhibitory immunoregulator is a component of the PD-1/PD-L1 signaling pathway. In other preferred embodiments, the inhibitory immunoregulator is an anti-PD-1 Ab of the invention. In yet other preferred embodiments, the inhibitory immunoregulator is an anti-PD-L1 Ab of the invention.

Where any immunotherapy methods comprising assaying PD-L1 expression, *i.e.*, employing a PD-L1 expression biomarker, are described below as comprising the selection of a patient who is, or is not, suitable for anti-PD-1 immunotherapy, or as comprising the administration of an anti-PD-1 Ab for immunotherapeutic purposes, it should be understood that these methods apply more broadly to the selection of a patient who is, or is not, suitable for immunotherapy with, or to the administration of an inhibitor of, an inhibitory immunoregulator (*e.g.*, CTLA-4, BTLA, TIM3, LAG3 or KIR) or a component or ligand thereof. Further, in any the methods comprising the measurement of PD-L1 expression in a test tissue sample, 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 preferred embodiments the "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 medical practitioner for use in selecting a suitable candidate for immunotherapy and/or administering an immunotherapeutic agent to the patient. In certain embodiments, the steps that provide the intermediate result may be 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.

The disclosure also provides a method for treatment of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is not suitable for treatment with an agent that inhibits an inhibitory immunoregulator, *e.g.*, anti-PD-1 Ab immunotherapy, the selecting comprising (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the surface of the cells; and (iii) selecting the

subject as not suitable for immunotherapy with an inhibitor of an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is less than a predetermined threshold level; and (b) administering a standard-of-care therapeutic other than an inhibitor of an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, to the selected subject.

Measurement of PD-L1 expression

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 preferred embodiments, PD-L1 expression is assayed by IHC. Flow cytometry may be particularly suitable for assaying PD-L1 expression in cells of hematologic tumors. In preferred embodiments of all of these methods, cell surface expression of PD-L1 is assayed using, *e.g.*, IHC or *in vivo* imaging.

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, 2010). Ab 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, 2010; Olafsen *et al.*, 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 certain preferred 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 further embodiments, the IHC assay is performed using an anti-PD-L1 mAb to bind to the PD-L1 polypeptide.

Abs that bind specifically to cell-surface-expressed PD-L1 in FFPE tissues

An Ab may bind to an antigen in fresh tissues but completely fail to recognize the antigen in an FFPE tissue sample. This phenomenon, well known in the art, is thought to be due primarily to intra- and inter-molecular cross-linking of polypeptides induced by formalin fixation, which alters the epitope recognized by the Ab (Sompuram *et al.*, 2006). In addition, several factors known to influence staining in FFPE tissue, including variable time to fixation, inadequate fixation period, differences in fixative used, tissue processing, Ab clone and dilution, antigen retrieval, detection system, and interpretation of results using different threshold points are important variables that can affect tissue antigenicity and IHC measurements (Bordeaux *et al.*, 2010). In particular, a lack of anti-human PD-L1 Abs that stain PD-L1 in FFPE specimens has been noted in the art (Hamanishi *et al.*, 2007). Taube *et al.* (2012) and Gadiot *et al.* (2011) have also reported difficulties in identifying anti-PD-L1 Abs that bind specifically to PD-L1 in FFPE tissues, and inconsistent test results on the same Abs. Our own analysis of five commercially available anti-hPD-L1 Abs shows that these Abs failed to distinguish FFPE cells expressing PD-L1 from cells that did not express PD-L1 (see Example 9, Table 7). Thus, the contradictory results reported by different groups on the implications of PD-L1 expression for prognosis of a tumor may, in part, reflect the differential abilities of anti-PD-L1 Abs used to detect PD-L1 polypeptide in FFPE tissue samples. Accordingly, in order to detect hPD-L1 on the surface of cells using an IHC assay on FFPE tissues, there is a need for anti-hPD-L1 Abs that bind specifically to cell surface-expressed PD-L1 in FFPE tissue samples.

This disclosure provides a mAb or an antigen-binding portion thereof that binds specifically to a cell surface-expressed PD-L1 antigen in a FFPE tissue sample. In preferred embodiments, the mAb or antigen-binding portion thereof does not bind to a

cytoplasmic PD-L1 polypeptide in the FFPE tissue sample or exhibits a very low level of background binding. In certain other embodiments, the presence or absence of binding specifically to a cell surface-expressed or a cytoplasmic PD-L1 polypeptide is detected by immunohistochemical staining. In certain preferred aspects of the invention, the mAb or antigen-binding portion is a rabbit Ab or a portion thereof. In other preferred embodiments, the mAb is the rabbit mAb designated 28-8, 28-1, 28-12, 29-8 or 20-12. In more preferred embodiments, the mAb is the rabbit mAb designated 28-8 or an antigen-binding portion thereof. In further embodiments, the mAb is an Ab comprising a heavy chain variable region (V_H) comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 35 and a light chain variable region (V_κ) comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 36. In other embodiments, the mAb comprises the CDR1, CDR2 and CDR3 domains in a V_H having the sequence set forth in SEQ ID NO: 35, and the CDR1, CDR2 and CDR3 domains in a V_κ having the sequence set forth in SEQ ID NO: 36.

It is known in the art that rabbit Abs have certain advantages over murine Abs. For example, rabbit Abs generally exhibit more diverse epitope recognition, improved immune response to small-size epitopes, and higher specificity and affinity compared to murine Abs (*see, e.g.*, Fischer *et al.*, 2008; Cheang *et al.*, 2006; Rossi *et al.*, 2005). For example, the rabbit's lower immune dominance and larger B-cell repertoire results in greater epitope recognition compared to murine Abs. Further, the high specificity and novel epitope recognition of rabbit antibodies translates to success with recognition of post-translational modifications (Epitomics, 2013). In addition, many protein targets relevant to signal transduction and disease are highly conserved between mice, rats and humans, and can therefore be recognized as self-antigens by a mouse or rat host, making them less immunogenic. This problem is avoided by generating Abs in rabbits. Moreover, in applications in which two antigen-specific Abs are required, it is more convenient to have the Abs come from two different species. Thus, for example, it is easier to multiplex a rabbit Ab such as 28-8 with other Abs (likely to be murine Abs since the best immune marking Abs are murine Abs) that can mark immune cells that also express PD-L1 (*e.g.*, macrophages and lymphocytes). Accordingly, rabbit anti-hPD-L1 mAbs, *e.g.*, 28-8, are particularly suited to IHC assays for detecting surface-expressed PD-L1 in FFPE tissue samples and potentially have distinct advantages over murine Abs, such as 5H1.

As described in Example 9, a large number (185) of Ab multiclones from both

rabbit and mouse immunizations were screened, and only ten rabbit Ab, but no mouse Ab, multiclones specifically detected the membranous form of hPD-L1. After further extensive screening by multiple rounds of IHC, 15 purified rabbit subclones were selected based on their specificity and intensity of staining (*see* Table 5). Following further characterization of the antibodies to determine their binding affinity and cross-competition by surface plasmon resonance, as well as screening by IHC on FFPE tissues, mAb 28-8 was selected as the Ab with the best combination of binding to membranous PDF-L1 with high affinity and specificity, and low background staining.

In certain aspects of this invention, the mAb or antigen-binding portion cross-competes with mouse mAb 5H1 for binding to PD-L1, which indicates that these antibodies bind to the same epitope region of PD-L1. In certain other aspects, the mAb or antigen-binding portion thereof does not cross-compete with mouse mAb 5H1 for binding to PD-L1, indicating that they do not bind to the same epitope region of PD-L1.

The disclosure also provides nucleic acids encoding all of the rabbit anti-hPD-L1 Abs or portions thereof disclosed herein.

Immunotherapeutic methods comprising measurement of cell surface PD-L1 expression

The availability of rabbit Abs that bind with high affinity specifically to membranous PD-L1 in FFPE tissue specimens facilitates methods comprising a step of detecting PD-L1 polypeptide on the surface of cells in FFPE tissue samples. Accordingly, this disclosure also provides a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is a suitable candidate for immunotherapy, the selecting comprising: (i) optionally providing a FFPE test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface by IHC using a rabbit anti-human PD-L1 Ab, *e.g.*, mAb 28-8, to bind to the PD-L1; and (iii) selecting the subject as a suitable candidate based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and (b) administering a composition comprising a therapeutically effective amount of an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, to the selected subject.

In certain embodiments of methods employing IHC to assay PD-L1 expression in FFPE tissues, an automated IHC assay is used. The automated IHC process is performed on an autostainer and comprises: (a) de-paraffinizing the FFPE sample with xylene and rehydrating the sample; (b) retrieving the antigen using a decloaking chamber; (c) blocking nonspecifc protein binding sites by incubation with a Protein Block; (d) incubating the sample with a primary anti-PD-L1 Ab; (e) adding a polymeric horseradish peroxidase (HRP)-conjugated secondary Ab; (f) detecting the bound secondary Ab comprising staining with a 3,3'-diaminobenzidine (DAB) chromogen; and/or (g) counterstaining with hematoxylin. This automated IHC process has been optimized by minimization of the number of steps, optimization of incubation times, and selection of primary Abs, blocking and detection reagents that produce strong specific staining with a low level of background staining. In preferred embodiments of this automated IHC assay, the primary anti-PD-L1 Ab is rabbit mAb 28-8 or murine mAb 5H1. In certain embodiments of this invention, this IHC assay, and any other IHC assay described herein to measure PD-L1 expression, may be used as part of a method of immunotherapy. In other embodiments, any of the IHC methods described herein is used independently of any therapeutic process requiring the administration of a therapeutic, *i.e.*, solely as a diagnostic method to assay PD-L1 expression.

In certain embodiments any of the immunotherapy methods described herein, the Ab administered to the selected subject is any anti-PD-1 or anti-PD-L1 Ab or antigen-binding portion thereof of the invention. In certain preferred embodiments, the subject is a human. In other preferred embodiments, the Ab is a human Ab or antigen-binding portion thereof. In more preferred embodiments, the anti-PD-1 Ab is nivolumab and the anti-PD-L1 Ab is BMS-936559. In certain other embodiments, the anti-PD-1 Ab is an Ab or antigen-binding portion thereof that cross-competes with nivolumab for binding to PD-1, and the anti-PD-L1 Ab is an Ab or antigen-binding portion thereof that cross-competes with BMS-936559 for binding to PD-L1. In certain preferred embodiments, the cancer to be treated is selected from the group consisting MEL, RCC, squamous NSCLC, non-squamous NSCLC, CRC, castration-resistant prostate cancer CRPC, HCC, squamous cell carcinoma of the head and neck, carcinomas of the esophagus, ovary, gastrointestinal tract and breast, and a hematological malignancy.

In certain embodiments of the disclosed methods, the predetermined threshold is based on a proportion of (a) tumor cells, (b) tumor-infiltrating inflammatory cells, (c)

particular tumor-infiltrating inflammatory cells, *e.g.*, TILs or macrophages, or (d) a combination of tumor cells and tumor-infiltrating inflammatory cells, in a test tissue sample that expresses PD-L1 on the cell surface. In certain embodiments, the predetermined threshold is at least 0.001% of tumor cells expressing membranous PD-L1 as determined by IHC. In other embodiments, the predetermined threshold is at least 0.01%, preferably at least 0.1%, more preferably at least 1% of tumor cells expressing membranous PD-L1, as determined by IHC. In certain embodiments, the predetermined threshold is at least 5% of tumor cells expressing membranous PD-L1 as determined by IHC. In certain embodiments, the predetermined threshold is at least 0.01%, at least 0.1%, at least 1%, or at least 5% of tumor cells expressing membranous PD-L1 as determined by IHC, and/or a single tumor-infiltrating inflammatory cell expressing membranous PD-L1 as determined by IHC. In certain other embodiments, the predetermined threshold is at least 0.01%, at least 0.1%, at least 1%, or at least 5% of a tumor-infiltrating inflammatory cell expressing membranous PD-L1 as determined by IHC. In certain other embodiments, the predetermined threshold is at least 0.01%, at least 0.1%, at least 1%, or at least 5% of a tumor-infiltrating lymphocyte expressing membranous PD-L1 as determined by IHC. In certain other embodiments, the predetermined threshold is at least 0.01%, at least 0.1%, at least 1%, or at least 5% of a tumor-infiltrating macrophage expressing membranous PD-L1 as determined by IHC. In yet other embodiments, the predetermined threshold is at least a single tumor cell or a single tumor-infiltrating inflammatory cell expressing membranous PD-L1 as determined by IHC. Preferably, PD-L1 expression is assayed by automated IHC using mAb 28-8 or 5H1 as the primary Ab.

This disclosure also provides a method for treatment of a subject afflicted with cancer, which method comprises: (a) screening a plurality of subjects to identify a subject that is not a suitable candidate for immunotherapy comprising the administration of an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, to the subject, the screening comprising: (i) optionally providing test tissue samples from the plurality of subjects, the test tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue samples that express PD-L1 on the surface of the cells; and (iii) selecting the subject as a candidate that is not suitable for immunotherapy based on an assessment that the proportion of cells that express PD-L1 on the surface of cells in the subject's test tissue sample is below a predetermined threshold level; and (b) administering a standard-of-care therapeutic other

than an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, to the selected subject.

This disclosure further provides a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) screening a plurality of subjects to identify a subject that is a suitable candidate for immunotherapy, the screening comprising: (i) optionally providing test tissue samples from the plurality of subjects, the test tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue samples that express PD-L1 on the surface of the cells; and (iii) selecting the subject as a candidate that is suitable for immunotherapy with an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and (b) administering a composition comprising a therapeutically effective amount of said agent to the selected subject.

This disclosure additionally provides a method for treatment of a subject afflicted with cancer, which method comprises: (a) screening a plurality of subjects to identify a subject that is a suitable candidate for the treatment, the screening comprising: (i) optionally providing test tissue samples from the plurality of subjects, the test tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue samples that express PD-L1 on the surface of the cells, wherein the subject is identified as a suitable candidate for anti-PD-1 Ab immunotherapy if the proportion of cells in the tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level, and the subject is identified as a candidate that is not a suitable candidate for anti-PD-1 Ab immunotherapy if the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is below a predetermined threshold level; and (b) administering a composition comprising a therapeutically effective amount of an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, to the subject identified as a suitable candidate for anti-PD-1 Ab immunotherapy, or (c) administering a standard-of-care therapeutic other than said agent to the subject identified as not a suitable candidate for anti-PD-1 Ab immunotherapy.

One aspect of this invention is a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor

cells and tumor-infiltrating inflammatory cells; (b) determining that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is above a predetermined threshold level; and (c) based on that determination administering a composition comprising a therapeutically effective amount of an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, to the subject. Another aspect of the invention is a method for treatment of a subject afflicted with cancer, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) determining that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is below a predetermined threshold level; and (c) based on that determination administering a standard-of-care therapeutic other than an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, to the subject.

Yet another aspect of the invention is a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) determining the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; (c) selecting an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, as a treatment for the subject based on the recognition that an anti-PD-1 Ab is effective in patients whose test tissue sample contains a proportion of cells above a predetermined threshold level that express PD-L1 on the cell surface; and (d) administering a composition comprising a therapeutically effective amount of said agent to the subject. In another embodiment, this disclosure provides a method for treatment of a subject afflicted with cancer, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) determining the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; (c) selecting a standard-of-care therapeutic other than an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, as a treatment for the subject based on the recognition that said agent is ineffective in patients in whom the proportion of cells that express PD-L1 on the cell surface in the test tissue sample is below a predetermined threshold level; and (d) administering the standard-of-care therapeutic to the subject.

This disclosure also provides a method of selecting an immunotherapy for a subject afflicted with cancer, which method comprises: (a) assaying cells of a test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells to assess the proportion of cells in the test tissue sample that express PD-L1; and (b) based on an assessment that the proportion of cells that express membranous PD-L1 is above a predetermined threshold level, selecting an immunotherapy comprising a therapeutically effective amount of an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, for the subject. The disclosure further provides a method of selecting a treatment for a subject afflicted with cancer, which method comprises: (a) assaying cells of a test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells to assess the proportion of cells in the test tissue sample that express PD-L1; and (b) based on an assessment that the proportion of cells that express membranous PD-L1 is below a predetermined threshold level, selecting a standard-of-care treatment other than an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, for the subject.

In addition, this disclosure provides a method for treatment of a subject afflicted with cancer, which method comprises administering to the subject a composition comprising a therapeutically effective amount of an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, the subject having been selected on the basis that the proportion of cells in a test tissue sample from the subject that express PD-L1 is determined to exceed a predetermined threshold level, wherein the test tissue sample comprises tumor cells and tumor-infiltrating inflammatory cells. This disclosure also provides a method for treatment of a subject afflicted with cancer, which method comprises administering to the subject a standard-of-care treatment other than an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, the subject having been selected on the basis that the proportion of cells in a test tissue sample from the subject that express PD-L1 is determined to be below a predetermined threshold level, wherein the test tissue sample comprises tumor cells and tumor-infiltrating inflammatory cells.

This disclosure further provides a method for selecting a cancer patient for immunotherapy with an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the

proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold proportion; and (d) selecting the patient for immunotherapy based on an assessment that the proportion of cells in the test tissue sample that express surface PD-L1 is above the predetermined threshold level.

In any of the methods described herein comprising a step for assessing PD-L1 expression, the test tissue sample may be a FFPE tissue sample and PD-L1 polypeptide on the cell surface is detected by IHC using an anti-PD-L1 Ab, *e.g.*, mAb 28-8 or 5H1.

In addition, in any method where an immunotherapy is selected or administered based on an assessment that the proportion of cells in a test tissue sample from the subject expresses PD-L1 at a level above a predetermined threshold level, it follows that a complementary method of treatment may be performed wherein a standard-of-care treatment other than the immunotherapy is selected or administered based on an assessment that the proportion of cells in a test tissue sample from the subject expresses PD-L1 at a level below the predetermined threshold level.

This disclosure further provides a method for predicting the therapeutic effectiveness of an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, for treating a cancer patient, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold value; and (d) predicting the therapeutic effectiveness of said agent, wherein if the proportion of cells that express PD-L1 on the cell surface exceeds the threshold proportion the agent is predicted to be effective in treating the patient, and wherein if the proportion of cells that express PD-L1 on the cell surface is below the threshold proportion the agent is predicted to not be effective in treating the patient.

This disclosure also provides a method for determining an immunotherapeutic regimen comprising an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, for treating a cancer patient, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the

cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold proportion; and (d) determining an immunotherapeutic regimen comprising said agent based on the determination that the proportion of cells that express PD-L1 on the cell surface exceeds the predetermined threshold proportion.

Standard-of-Care Therapeutics

Several of the methods of treatment described herein comprise the administration of a standard-of-care therapeutic to a patient. As used herein, a "standard-of-care therapeutic" is a treatment process, including a drug or combination of drugs, radiation therapy (RT), surgery or other medical intervention that is recognized by medical practitioners as appropriate, accepted, and/or widely used for a certain type of patient, disease or clinical circumstance. Standard-of-care therapies for different types of cancer are well known by persons of skill in the art. For example, the National Comprehensive Cancer Network (NCCN), an alliance of 21 major cancer centers in the USA, publishes the NCCN Clinical Practice Guidelines in Oncology (NCCN GUIDELINES®) that provide detailed up-to-date information on the standard-of-care treatments for a wide variety of cancers (*see* NCCN GUIDELINES®, 2013). By way of example, standard-of-care treatments for MEL, RCC and NSCLC are summarized below.

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.*, 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.* (2013).

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 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.

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).

There is no consensus on the best treatments for metastatic MEL, though a variety of treatments including excision to clear margins, intralesional injections, laser ablation, radiation and biochemotherapy (combination of chemotherapy and biological agents such as interferon-alpha and IL-2) are being investigated. The therapeutic landscape for metastatic MEL has recently seen dramatic improvements with the development of novel drugs such as vemurafenib and ipilimumab. Vemurafenib specifically inhibits signaling by a mutated intracellular kinase, BRAF, that is present in about 50% of patients with metastatic MEL. In clinical trials, vemurafenib delivered an estimated progression-free survival (PFS) of 5.3 months in BRAF mutation-positive patients versus merely 1.6 months for dacarbazine, and a median OS of 15.9 months for vemurafenib versus 5.6-7.8 months for dacarbazine (Chapman *et al.*, 2011; Sosman *et al.*, 2012). Ipilimumab is a HuMAb that inhibits the immune checkpoint receptor, CTLA-4, and thereby stimulates a T cell immune response. Ipilimumab, with or without a glycoprotein 100 (gp100) peptide vaccine, improved median OS in patients with previously treated metastatic MEL to 10.0-10.1 months as compared with 6.4 months among patients receiving gp100 alone (Hodi *et al.*, 2010). Besides these two agents, no other agent has demonstrated an OS benefit in a Phase 3 randomized study. Dacarbazine is approved by the FDA and the EMA for treatment of metastatic MEL with a reported objective response rate of 5-20% and a median OS of about 6.4 months, but these responses are short-lived. Other drugs such as temozolomide and fotemustine have not resulted in significant improvement in survival when compared to dacarbazine. IL-2 has also been approved by the FDA for the

treatment of metastatic MEL as it is associated with a 15-20% response rate including 4-6% complete responses which can be durable, but it is associated with significant toxicities including hypotension, cardiac arrhythmias, and pulmonary edema. Further details on standard-of-care treatments for melanoma are provided by Garbe *et al.* (2012) and the NCCN GUIDELINES®, 2013 – Melanoma. Despite the recent approval of ipilimumab and vemurafenib for advanced MEL, there is still a large unmet need for patients who have progressed on anti-CTLA-4 therapy and a BRAF inhibitor (depending on BRAF status) or patients with previously untreated, unresectable or metastatic BRAF wild-type MEL. The 5-year survival rate for late-stage MEL is currently only 15%.

Renal Cell Carcinoma

RCC is the most common type of kidney cancer in adults, responsible for approximately 90% of renal tumors, and 80-90% of these are clear cell tumors (NCCN GUIDELINES®, 2013 – Kidney Cancer). It is also the most lethal of all the genitourinary tumors. An estimated 65,150 patients will be diagnosed with renal cancer and 13,680 will die of the disease in the United States in 2013 (Siegel *et al.* (2013).

For clinically localized RCC (Stage IA and IB), surgical resection, including radical nephrectomy and nephron-sparing surgery, is an effective therapy. Partial nephrectomy is generally not suitable for patients with locally advanced tumors (Stage II and III), in which case radical nephrectomy is preferred. Where the tumor is confined to the renal parenchyma, the 5-year survival rate is 60-70%, but this is lowered considerably in Stage IV disease where metastases have spread. Stage IV RCC is relatively resistant to RT and chemotherapy, although patients may benefit from surgery, and cytoreductive nephrectomy before systemic therapy is recommended for patients with a potentially surgically resectable primary and multiple resectable metastases.

Until recently, the cytokines IL-2 and IFNα were the only active systemic treatments for advanced or metastatic RCC, both providing reported ORRs of 5-27%. However, due to each of these agent's limited clinical benefit and substantial toxicity profile, newer targeted agents have largely replaced cytokines in the treatment of advanced or metastatic renal cell carcinoma. The recognition of the importance of hypoxia inducible factor alpha (HIFα) signaling in the pathogenesis of clear-cell RCC has led to widespread study of two classes of targeted therapies, anti-angiogenic tyrosine kinase inhibitors (TKIs) and mammalian target of rapamycin (mTOR) inhibitors, in 1L and 2L treatments (Mulders, 2009). Targeting of angiogenesis is rational because

constitutive HIFα activation leads to the upregulation or activation of several proteins including vascular endothelial growth factor (VEGF), which can subsequently lead to tumor proliferation and neovasculature formation. Targeting of the mTOR pathway is important because activation of the upstream PI3K/Akt/mTOR signaling pathway is one method by which constitutive HIFα activation or upregulation occurs (Mulders, 2009). Agents that target angiogenesis include VEGF-receptor (VEGFr) TKIs (*e.g.*, sorafenib, sunitinib, pazopanib, axitinib, and tivozanib) and VEGF-binding mAbs (*e.g.*, bevacizumab), while agents that target the mTOR pathway include the mTOR inhibitors (*e.g.*, everolimus and temsirolimus) (Mulders, 2009; NCCN GUIDELINES®, 2013 – Kidney Cancer). However, most patients develop resistance, and OS improvement has only been shown in one phase 3 trial in poor-risk patients: temsirolimus showed a statistically significant benefit for OS in patients with advanced RCC compared to IFNα (10.9 months versus 7.3 months) (Hudes *et al.*, 2007). Everolimus has also demonstrated a 2.1-month improvement in median PFS versus placebo, but with no OS improvement (Motzer et al., 2008). Among the five approved anti-angiogenic agents (sorafenib, sunitinib, bevacizumab, pazopanib, and axitinib) and two approved mTOR inhibitors (temsirolimus, everolimus), only everolimus is approved specifically for use after the failure of treatment with anti-angiogenic therapy. In the U.S., everolimus is indicated for the treatment of advanced RCC after failure of treatment with sunitinib or sorafenib, whereas in the EU, everolimus is more broadly indicated for patients with advanced RCC, whose disease has progressed on or after treatment with VEGF-targeted therapy.

Non-Small Cell Lung Cancer

NSCLC is the leading cause of cancer death in the U.S. and worldwide, exceeding breast, colon and prostate cancer combined. In the U.S., an estimated 228,190 new cases of lung and bronchial will be diagnosed in the U.S., and some 159,480 deaths will occur because of the disease (Siegel *et al.*, 2013). The majority of patients (approximately 78%) are diagnosed with advanced/recurrent or metastatic disease. Metastases to the adrenal gland from lung cancer are a common occurrence, with about 33% of patients having such metastases. NSCLC therapies have incrementally improved OS, but benefit has reached a plateau (median OS for late stage patients is just 1 year). Progression after 1L therapy occurred in nearly all of these subjects and the 5-year survival rate is only 3.6% in the refractory setting. From 2005 to 2009, the overall 5-year relative survival rate for

lung cancer in the U.S. was 15.9% (NCCN GUIDELINES®, 2013 – Non-Small Cell Lung Cancer).

Surgery, RT and chemotherapy are the three modalities commonly used to treat NSCLC patients. As a class, NSCLCs are relatively insensitive to chemotherapy and RT, compared to small cell carcinoma. In general, for patients with Stage I or II disease, surgical resection provides the best chance for cure, with chemotherapy increasingly being used both pre-operatively and post-operatively. RT can also be used as adjuvant therapy for patients with resectable NSCLC, the primary local treatment, or as palliative therapy for patients with incurable NSCLC.

Patients with Stage IV disease who have a good performance status (PS) benefit from chemotherapy. Many drugs, including platinum agents (*e.g.*, cisplatin, carboplatin), taxanes agents (*e.g.*, paclitaxel, albumin-bound paclitaxel, docetaxel), vinorelbine, vinblastine, etoposide, pemetrexed and gemcitabine are useful for Stage IV NSCLC. Combinations using many of these drugs produce 1-year survival rates of 30% to 40% and are superior to single agents. Specific targeted therapies have also been developed for the treatment of advanced lung cancer. For example, bevacizumab (AVASTIN®) is a mAb that blocks vascular endothelial growth factor A (VEGF-A). Erlotinib (TARCEVA®) is a small-molecule TKI of epidermal growth factor receptor (EGFR). Crizotinib (XALKORI®) is a small-molecule TKI that targets ALK and MET, and is used to treat NSCLC in patients carrying the mutated *ALK* fusion gene. Cetuximab (ERBITUX®) is a mAb that targets EGFR.

There is a particular unmet need among patients who have squamous cell NSCLC (representing up to 25% of all NSCLC) as there are few treatment options after 1L therapy. Single-agent chemotherapy is standard of care following progression with platinum-based doublet chemotherapy (Pt-doublet), resulting in median OS of approximately 7 months. Docetaxel remains the benchmark treatment in this line of therapy although erlotinib may also be used with less frequency. Pemetrexed has also been shown to produce clinically equivalent efficacy outcomes but with significantly fewer side effects compared with docetaxel in the 2L treatment of patients with advanced NSCLC (Hanna *et al.*, 2004). No therapy is currently approved for use in lung cancer beyond the 3L setting. Pemetrexed and bevacizumab are not approved in squamous NSCLC, and molecularly targeted therapies have limited application. The unmet need in advanced lung cancer has been compounded by the recent failure of Oncothyreon and

Merck KgaA's STIMUVAX® to improve OS in a phase 3 trial, inability of ArQule's and Daiichi Sankyo's c-Met kinase inhibitor, tivantinib, to meet survival endpoints, failure of Eli Lilly's ALIMTA® in combination with Roche's AVASTIN® to improve OS in a late-stage study, and Amgen's and Takeda Pharmaceutical's failure to meet clinical endpoints with the small-molecule VEGF-R antagonist, motesanib, in late-stage trials.

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.

EXAMPLE 1

Cross-Competition between Anti-PD-1 HuMAbs for Binding to CHO Cells Expressing Human PD-1

Chinese Hamster Ovary (CHO) cells transfected to express human PD-1 (CHO/PD-1 cells) were incubated with 10 μg/ml of Fab fragment of the anti-PD-1 HuMAb 5C4 or human IgG1 (hIgG1) isotype control Ab for 30 minutes at 4°C before addition of anti-PD-1 HuMAbs 2D3, 7D3 or 4H1 at a concentration of 0.2 μg/ml. Binding of 4H1, 2D3 or 7D3 to CHO/PD-1 cells were detected by fluorescein isothiocyanate (FITC)-conjugated goat anti-hIgG, Fc-gamma specific Ab. In the case of cross-competition assay with 5C4 and 17D8, CHO/PD-1 cells were incubated with the whole molecule of 5C4 before addition of FITC-labeled 17D8. Binding of 2D3, 7D3, 4H1 or 17D8 to the CHO/PD-1 cells was measured by flow cytometric analysis using a FACScalibur flow cytometer (Becton Dickinson, San Jose, CA).

The results are depicted in Figure 1. The data show that the 5C4 Fab fragment substantially blocked the binding of mAbs 5C4 itself, as well as the binding of 2D3, 7D3 (Figure 1A) and 4H1 (Figure 1B), while the 5C4 whole mAb substantially blocked the binding of 17D8 (Figure 1C) to CHO/PD-1 cells as measured by mean fluorescence intensity (MFI) of staining.

EXAMPLE 2

Cross-Competition between Anti-PD-L1 HuMAbs for Binding to CHO Cells Expressing Human PD-L1

CHO cells transfected to express hPD-L1 (CHO/PD-L1 cells) were incubated with 10 μg/ml of each of ten unconjugated human anti-PD-L1 mAbs (5F8, 7H1, 10H10, 1B12, 3G10, 10A5, 11E6, 12A4, 12B7, and 13G4) or human IgG1 (hIgG1) isotype control Ab

for 20 min at 4°C. FITC-conjugated 10H10 (A), 3G10 (B), 10A5 (C), 11E6 (D), 12A4 (E) or 13G4 (F) was added to the cells to a final concentration of 0.09 μg/ml (B, D), 0.27 μg/ml (A, C), 0.91 μg/ml (F), or 2.73 μg/ml (E) for an additional 20 min at 4°C without prior washout of unbound, unconjugated Ab. Different quantities of the various FITC-conjugated HuMAbs were used due to differences in binding efficiency following labeling, and the optimal amounts of these FITC-conjugated HuMAbs were previously determined by dose-titration analysis of binding to CHO/PD-L1 cells. Binding of FITC-conjugated 10H10, 3G10, 10A5, 11E6, 12A4 or 13G4 to the CHO/PD-L1 cells was measured by flow cytometry.

The results are depicted in Figure 2. Binding of labeled 10H10 was partially blocked by 10A5, 11E6 and 13G4, but was substantially blocked only by itself (Figure 2A). Conversely, 10H10 substantially blocked the binding only of itself to CHO/PD-L1 cells. Each of anti-PD-L1 HuMAbs 5F8, 7H1, 1B12, 3G10, 10A5, 11E6, 12A4, 12B7 and 13G4 substantially blocked binding of labeled mAbs 3G10 (Figure 2B), 10A5 (Figure 2C), 11E6 (Figure 2D), 12A4 (Figure 2E) and 13G4 (Figure 2F) to CHO/PD-L1 cells as measured by MFI, though mAbs 5F8 and 13G4 generally blocked binding of the labeled mAbs to a slightly lesser extent.

EXAMPLE 3

Cross-Competition between Anti-PD-L1 mAbs for Binding to Ovarian Carcinoma Cells Expressing Human PD-L1

Anti-PD-L1 HuMAbs 5F8, 12B7, 3G10, 1B12, 13G4, 10H10, 10A5 and 12A4, and a human IgG1 (huIgG1) isotype control Ab were serially diluted from 10 μg/ml and incubated with hPD-L1-expressing ES-2 ovarian carcinoma cells for 20 minutes at 4°C. Without washing, biotinylated-12A4 Ab was added to a final concentration of 0.4 μg/ml for an additional 20 minutes at 4°C. After washing, bound biotin-12A4 was detected using fluorescent streptavidin-PE secondary reagent and measured by flow cytometry. Figure 3 shows the fluorescence of bound biotin-12A4 plotted against the concentration of unlabeled hPD-L1 HuMAbs. Binding of biotin-12A4 to ES-2 cells was substantially blocked by 12A4 itself and by 1B12 and 12B7, and was moderately to significantly blocked by mAbs 5F8, 10A5, 13G4 and 3G10, but was not blocked by mAb 10H10.

EXAMPLE 4

Design of Phase 1 Clinical Study of Anti-PD-1 Ab

A Phase 1 study was conducted to assess the safety, antitumor activity, and pharmacokinetics of an anti-PD-1 in patients with selected advanced solid tumors. The human anti-PD-1 mAb, BMS-936558 (also referred to herein as nivolumab, and in U.S. Patent No. 8,008,449 as 5C4), was administered as an intravenous infusion every 2 weeks of each 8-week treatment cycle. Tumor status was re-assessed following each cycle. Patients continued treatment for up to 2 years (12 cycles), until they experienced complete remission, unacceptable toxicity, disease progression, or withdrew consent. In patients who were otherwise clinically stable, study treatment was continued beyond apparent initial disease progression until further progression was noted as recommended by proposed immune response criteria (Wolchok *et al.*, 2009). Patients with stable disease (SD) or an ongoing objective response (OR: complete response [CR] or partial response [PR]) at the end of treatment were followed for 1 year and were offered retreatment for 1 additional year in the event of progression.

Dose escalation

Patients with advanced melanoma (MEL), non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), castration-resistant prostate cancer (CRPC) and colorectal cancer (CRC) were eligible to enroll. Cohorts of 3-6 patients per dose level were enrolled sequentially at 1.0, 3.0, and 10.0 mg/kg. Dose escalation proceeded when a minimum of 3 patients had completed the safety evaluation period (56 days) at the given dose level, with dose-limiting toxicity in less than one-third of patients. Intra-patient dose escalation was not permitted.

Cohort expansion

A maximum tolerated dose (MTD) was not reached. Initially, 5 expansion cohorts of approximately 16 patients each were enrolled at 10 mg/kg for MEL, NSCLC, RCC, CRPC and CRC. Based on initial signals of activity, and following a 6.5-month hiatus for protocol amendment, additional expansion cohorts of approximately 16 patients each were enrolled for MEL (at 1.0 and 3.0 mg/kg, followed by cohorts randomized to 0.1, 0.3, or 1.0 mg/kg), NSCLC (squamous or nonsquamous histology cohorts randomized to 1, 3, or 10 mg/kg), and RCC (at 1.0 mg/kg). Intra-patient dose escalation to 1 mg/kg was permitted for MEL patients with progressive disease after receiving 0.1 or 0.3 mg/kg.

Patients

Eligible patients had documented advanced solid tumors; age > 18 years; life expectancy >12 weeks; Eastern Cooperative Oncology Group performance status of ≤ 2; measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST), v1.0 with modification (*see* Topalian *et al.*, 2012b); adequate hematologic, hepatic, and renal function; and received 1-5 prior systemic treatment regimens. Patients with stable treated brain metastases were enrolled. Exclusion criteria included a history of chronic autoimmune disease, prior therapy with T-cell modulating Abs (*e.g.*, anti-CTLA-4, anti-PD-1, anti-PD-L1), conditions requiring immunosuppressive medications, and chronic infections (*e.g.*, HIV, hepatitis B or C).

A total of 296 patients with advanced solid tumors including MEL (n=104), NSCLC (n=122), RCC (n=34), CRPC (n=17), and CRC (n=19) were treated with BMS-936558 for 40 months up to February 2012. By March 2013, 306 patients including patients with non-small cell lung cancer (n=129), melanoma (n=107), RCC (n=34), CRPC (n=17), and CRC (n=19) had been treated with BMS-936558 from October 2008 through January 2012, all with a minimum of one year of observation. Two patients did not receive a full cycle of treatment and were not considered response-evaluable. Median age was 63 years (range, 29-85). ECOG performance score was 0 or 1 in 98% of patients. The majority of patients were heavily pretreated, with 47% having received at least 3 prior regimens. Notable prior therapies included immunotherapy (64%) and B-RAF inhibitor (8%) in MEL patients; platinum-based chemotherapy (94%) and tyrosine kinase inhibitors (TKIs, 34%) in NSCLC patients; and nephrectomy (94%), immunotherapy (59%), and anti-angiogenic therapy (74%) in RCC patients. Baseline characteristics of the total treated population (N=306) were similar to those of the efficacy population (response evaluable patients, N=270). Details on the patient pre-treatments are provided in Topalian *et al.* (2012b).

Statistical analysis

Baseline characteristics and adverse events for all treated patients (N=306), and efficacy results for 270 patients with lung cancer, melanoma, and kidney cancer as of March 2013 are reported. Pharmacokinetic and molecular-marker populations consisted of treated patients with available data as of the February 2012 date of preliminary analysis. The efficacy population consisted of response-evaluable patients commencing treatment at least 8 months before the date of analysis. Tumor measurements were

collected after each treatment cycle (4 doses) by investigators. Individual best objective responses based on the tumor measurements were assessed by the sponsor per modified RECIST v1.0. Objective response was confirmed by at least one sequential tumor assessment. Objective response and stable disease rates were estimated with confidence intervals using the Clopper-Pearson method. Time-to-event endpoints including PFS, OS, survival rates, and duration of response, were estimated using the Kaplan-Meier method. AEs were coded using Medical Dictionary for Regulatory Activities (MedDRA), version 15.1. Select adverse events with potential immunologic etiologies, also referred to as "immune-related adverse events" of "AEs of special interest" (AEOSIs), defined as adverse events that require more frequent monitoring and/or unique intervention, were identified using a pre-defined list of MeDRA terms. Individual best ORs were derived from investigator-reported data per modified RECIST v1.0. OR was confirmed by at least one sequential tumor assessment and OR rate (ORR={[CR + PR] ÷ n} × 100) was calculated.

EXAMPLE 5

Safety Evaluations on Patients Treated with Anti-PD-1 Antibody

Safety evaluations, including clinical examination and laboratory assessments, were conducted in all treated patients at baseline and regular intervals up to 100 days following last administration of drug. The severity of AEs was graded based on the NCI Common Terminology Criteria for Adverse Events (NCI CTCAE), v3.0. Computed tomography (CT) or magnetic resonance imaging was performed for tumor assessment at baseline and following each treatment cycle.

A MTD was not defined across the doses of BMS-936558 tested on this study, up to the highest planned dose of 10 mg/kg. A relative BMS-936558 dose intensity of 90% or higher was achieved in 87% of patients (*see* Topalian *et al.*, 2012b; Topalian *et al.*, 2013, for details). AEs were coded using Medical Dictionary for Regulatory Activities (MedDRA), version 14.1. AEOSIs were identified using a pre-defined list of MeDRA terms. Fifteen of 296 (5%) patients discontinued treatment due to BMS-936558-related AEs. As of the February 2012 date of preliminary analysis, 62 (21%) patients had died, and by March 2013, 195 patients (64%) had died, with disease progression being the most common cause of death (Topalian *et al.*, 2012b; Topalian *et al.*, 2013).

The most common adverse events, regardless of causality included fatigue, decreased appetite, diarrhea, nausea, cough, dyspnea, constipation, vomiting, rash,

pyrexia and pruritus (Topalian *et al.*, 2012b; Topalian *et al.*, 2013). Common BMS-936558-related AEs included fatigue, rash, diarrhea, decreased appetite, and nausea. The majority of the events were low grade, with grade 3-4 drug-related AEs observed in 41 of 296 (14%) patients. Treatment-related AEs (any grade) were observed in 230 of 306 patients (75%), the most common being fatigue, rash, diarrhea and pruritus (Topalian *et al.*, 2013). The spectrum, frequency, and severity of BMS-936558-related AEs were generally similar across the dose levels tested, with no clear relationship to treatment duration. Fifty-two of 306 patients (17%) experienced Grade 3-4 treatment-related adverse events, with fatigue (2%), pneumonitis, lymphopenia, diarrhea, abdominal pain, and hypophosphatemia (1% each) being the most common. Treatment-related serious adverse events occurred in 42 of 306 patients (14%) (Topalian *et al.*, 2013).

Drug-related AEOSIs, with potential immune-related etiologies, were categorized by organ involvement to provide a more accurate estimate of frequency and included pneumonitis, vitiligo, colitis, hepatitis, hypophysitis, and thyroiditis among others. Treatment-related select adverse events of any grade were observed in 140 of 306 patients (46%), the most common being rash (15%), diarrhea (13%), and pruritus (11%) (Table 2). Grade 3-4 treatment-related select events were seen in 19 patients (6%).

Fifty-two of 230 patients (23%) with drug-related adverse events required management with systemic glucocorticoids and/or other immunosuppressive agents. Hepatic or gastrointestinal AEOSIs were managed with treatment interruption and, as necessary, with administration of corticosteroids. Among patients treated to-date, these AEs were reversible in all cases. Endocrine AEOSIs were managed with replacement therapy. Thirty-two of 306 patients (11%) discontinued therapy due to treatment-related adverse events. At the discretion of the treating physician, patients successfully reinitiated treatment with BMS-936558. Twenty-one (40%) were able to resume nivolumab therapy after toxicity resolved.

Drug-related pneumonitis (any grade) occurred in 12 of 306 (4%) patients (Topalian *et al.*, 2013). Clinical presentations ranged from radiographic abnormalities in asymptomatic patients, to progressive, diffuse pulmonary infiltrates associated with symptoms (cough, fever, dyspnea). Grade 3-4 pneumonitis developed in 4 patients (1%), of which 3 cases were fatal (2 NSCLC patients, 1 CRC). No clear relationship between the occurrence of pneumonitis and tumor type, dose level, or the number of doses received was noted. In 9 of 12 patients, pneumonitis was reversible with treatment

discontinuation and/or immunosuppression (glucocorticoids, infliximab, mycophenolate). Following the most recent pneumonitis-associated death in November 2011, 79 patients continued to receive nivolumab (median 29 weeks, range 2-69 weeks) with no further mortalities from this or other treatment-related causes.

EXAMPLE 6

Pharmacokinetics/Pharmacodynamics Analyses on Anti-PD-1 Antibody

For pharmacokinetic (PK) analyses, serial blood samples were collected and serum concentrations of BMS-936558 were quantified using by ELISA. For pharmacodynamic (PD) analysis, peripheral blood mononuclear cells were isolated from patients at baseline and following cycle 1 to estimate PD-1 receptor occupancy (RO) by BMS-936558 on circulating CD3+ T-cells via flow cytometry (Brahmer *et al.*, 2010).

The maximum concentration of BMS-936558 was observed at a median T_{max} of 1-4 hours after the start of infusion. The PK of BMS-936558 was linear with a dose proportional increase in C_{max} and $AUC_{(0\text{-}14\ d)}$ in the dose range of 0.1-10 mg/kg (n=35). BMS-936558 PD was assessed by PD-1 RO on circulating T-cells. PBMCs from 65 MEL patients treated with one cycle of BMS-936558 at 0.1-10 mg/kg biweekly demonstrated median occupancy of PD-1 molecules on circulating $CD3^+$ T-cells by BMS-936558 ranging from 64%-70% (*see* Topalian *et al.*, 2012b, for details).

EXAMPLE 7

Antitumor Efficacy Exhibited by Anti-PD-1 Antibody

Data analyzed as of February 2012

Clinical antitumor activity was observed at all BMS-936558 doses tested. ORs (confirmed CR or PR) were observed in a substantial portion of patients with NSCLC, MEL, and RCC (Tables 1 and 2; Figure 4), and in various sites of metastatic disease including liver, lung, lymph nodes, and bone (Figures 5-7 and not shown). Tumor regressions followed conventional as well as "immune-related" patterns of response, such as prolonged reduction in tumor burden in the presence of new lesions. Individual best overall responses were derived from investigator-reported data according to modified RECIST v1.0. OR was confirmed by at least one sequential tumor assessment. At the time of data analysis, 2 patients with NSCLC who were treated with 10 mg/kg had unconfirmed responses, and 8 additional patients (with MEL, NSCLC, or RCC) had a persistent reduction in baseline target lesions in the presence of new lesions, (*i.e.*, an

"immune-related" response pattern). None of these patients was categorized as a responder for the purpose of calculating OR rates. Antitumor responses and/or prolonged disease stabilization were observed in patients irrespective of prior therapies received (*see* summary of progression free interval for patients with OR and SD in Supplementary Appendix 4 of Topalian *et al.*, 2012b).

In NSCLC patients, 14 ORs were observed at BMS-936558 doses of 1, 3, or 10 mg/kg with response rates of 6%, 32%, and 18%, respectively. ORs were observed across NSCLC histologies: 6 responders of 18 squamous (33%), 7 responders of 56 nonsquamous (13%), and 1 of 2 unknown. All 14 patients with ORs started treatment ≥ 24 weeks before data analysis, and of these, 8 had response duration ≥ 24 weeks (Table 1). Stable disease (SD) lasting ≥ 24 weeks was observed in 5 (7%) NSCLC patients, all with nonsquamous histology. Among MEL patients, 26 ORs were observed at doses ranging from 0.1-10 mg/kg, with response rates ranging from 19%-41% per dose level. At the 3 mg/kg dose level, ORs were noted in 7 of 17 (41%) patients. Of 26 MEL patients who achieved an OR, 17 started treatment ≥ 1 year before data analysis, and of these, 13 patients had an OR duration ≥ 1 yr. The remaining 8 patients with OR were on study < 1 year and 6 had responses ranging from 1.9-5.6 months. SD lasting ≥ 24 weeks was observed in 6 (6%) patients. In RCC patients, ORs occurred in 4 of 17 (24%) patients treated with a BMS-936558 dose of 1 mg/kg and 5 of 16 (31%) patients treated with 10 mg/kg. Among 8 RCC patients with OR who started treatment ≥ 1 year prior to data analysis, 5 (63%) had OR duration ≥ 1 yr. SD lasting ≥ 24 weeks was observed in an additional 9 (27%) patients.

Table 1. Clinical Activity of BMS-936558 in the Efficacy Population* (N=236)[†] as Assessed to February 2012

Tumor Type	Dose (mg/kg)	n	ORR[‡] No. Patients (%) [95% CI][†]	SD ≥ 24 wk No. Patients (%) [95% CI]	PFSR[§] at 24 wk (%) [95% CI]
MEL	0.1	14	4 (29) [8-58]	1 (7) [0.2-34]	40 [13-66]
	1.0	27	8 (30) [14-50]	3 (11) [2-29]	45 [26-65]
	3.0	17	7 (41) [18-67]	1 (6) [0.1-29]	55 [30-80]
	10.0	20	4 (20) [6-44]	0	30 [9-51]
ALL MEL		94	26 (28) [19-38]	6 (6) [2-13]	41 [30-51]

Tumor Type	Dose (mg/kg)	n	ORR‡ No. Patients (%) [95% CI]†	SD ≥ 24 wk No. Patients (%) [95% CI]	PFSR§ at 24 wk (%) [95% CI]
NSCLC**					
All	1	18	1 (6) [0.1-27]	1 (6) [0.1-27]	16 [0-34]
Squamous	1	5	0	0	0
Nonsquamous	1	12	0	1 (8) [0.2-39]	14 [0-37]
Unknown	1	1	1(100) [3-100]	0	1
All	3	19	6 (32) [13-57]	2 (11) [1-33]	41 [18-64]
Squamous	3	6	3 (50) [12-88]	0	50 [10-90]
Nonsquamous	3	13	3 (23) [5-54]	2 (15) [2-45]	37 [10-64]
All	10	39	7 (18) [8-34]	2 (5) [0.6-17]	24 [11-38]
Squamous	10	7	3 (43) [10-82]	0	43 [6-80]
Nonsquamous	10	31	4 (13) [4-30]	2 (7) [0.8-21]	21 [6-36]
Unknown	10	1	0	0	0
ALL NSCLC		76	14 (18) [11-29]	5 (7) [2-15]	26 [16-36]
All Squamous		18	6 (33) [13-59]	0	33 [12-55]
All Nonsquamous		56	7 (13) [5-24]	5 (9) [3-20]	22 [11-34]
RCC	1	17	4 (24) [7-50]	4 (24) [7-50]	47 [23-71]
	10	16	5 (31) [11-59]‡	5 (31) [11-59]¶	67 [43-91]
ALL RCC		33	9 (27) [13-46]	9 (27) [13-46]	56 [39-73

* The efficacy population consists of response-evaluable patients whose treatment was initiated at least 8 months before data analysis in February 2012, and had measurable disease at baseline and one of the following: at least 1 on-treatment scan or clinical evidence of disease progression or death.

† CR denotes complete response, MEL melanoma, NSCLC non-small cell lung cancer, ORR objective response rate, PFSR progression-free survival rate, PR partial response, RCC renal cell cancer, SD stable disease, n number of patients.

‡ Objective response rates ({[CR + PR] ÷ n} × 100) have been calculated based on confirmed responses with confidence intervals calculated using the Clopper-Pearson method. Individual patient responses were adjudicated per RECIST v1.0 with

modification (*see* Topalian *et al.*, 2012b).

§ Progression-free survival rate was the proportion of patients who did not progress and were alive at 24 weeks calculated by the Kaplan-Meier methodology with confidence intervals using the Greenwood method.

¶ One CR.

**One NSCLC patient who was treated at the 3 mg/kg dose level had an initial evaluation of progressive disease, subsequently had a PR, and was classified as a responder.

Table 2. Duration of Objective Responses to BMS-936558* as Assessed to February 2012

Tumor Type	Dose (mg/kg)	No. of Patients with OR	Duration of Response (months)†
MEL	0.1	4	7.5+, 5.6+, 5.6, 5.6
	0.3	3	3.8+, 2.1+, 1.9+
	1	8	24.9+, 22.9, 20.3+, 19.3+, 18.4+, 7.6+, 5.6+, 5.3+
	3	7	22.4+, 18.3+, 15.2+, 12.9, 11.1, 9.3, 9.2+
	10	4	24.6+, 23.9+, 18.0+, 17.0
NSCLC§	1	1	9.2+
	3	6	30.8+, 7.6+, 5.5+, 3.7+, 1.9+, NA‡
	10	7	14.8+, 7.6+, 7.3+, 6.7, 4.2, 3.7+, 3.7
RCC	1	4	17.5+, 9.2+, 9.2, 5.6+
	10	5	22.3+. 21.7+, 12.9, 12.0, 8.4

* MEL denotes melanoma, NA not applicable, NSCLC non-small cell lung cancer, RCC renal cell cancer.

† Time from first response to time of documented progression, death, or for censored data, time to last tumor assessment.

‡ One patient was treated beyond an initial evaluation of progressive disease and subsequently had a PR; this patient was classified as a responder for the purposes of calculating response rates by RECIST v1.0 but was not eligible for calculation of duration of response.

Data analyzed as of March 2013

Objective responses were observed in patients with NSCLC (17%), MEL (31%), and RCC (29%), but not with CRC or CRPC. Responses were observed across all nivolumab doses tested; response rates in NSCLC patients receiving 1 mg/kg, versus 3 or 10 mg/kg, appeared to be decreased (3%, versus 24% and 20%, respectively) (Tables 3 and 4). Thirteen of 270 patients (4.8%) with responding histologies had unconventional response patterns that did not meet RECIST criteria (*e.g.*, persistent reduction in target lesions in the presence of new lesions or regression following initial progression) (Wolchok *et al*, 2009). Additional patients manifested SD for 24 weeks or longer (10% NSCLC, 7% MEL, 27% RCC). Sustained survival, reflected by 1- and 2-year landmark OS rates, was noted in each of the responding populations as follows: NSCLC, 42% and 14%; MEL, 62% and 43%; and RCC, 70% and 50% (Table 3). Median OS of 9.6 months for lung cancer (9.2 and 10.1 months for squamous and non-squamous NSCLC histologies, respectively), 16.8 months for MEL, and more than 22 months for RCC, were observed; median PFS was 2.3 months in NSCLC, 3.7 months in MEL, and 7.3 months in RCC; and the median duration of response was 74, 104, and 56 weeks, respectively (Table 4). Among 16 responders who discontinued therapy for reasons other than disease progression and were followed for at least 24 weeks, 13 (81%) remained in response at the time of analysis (Figure 8).

Table 3. Clinical Activity of Nivolumab in the Efficacy Population (N = 306)* as Assessed to March 2013

Tumor Type	ORRs[†] No. of patients/total no. of patients (%) [95% CI]	Median Duration of Response[‡], wk (range)	Stable Disease No. of patients/total no. of patients (%) [95% CI]		Median Progression-free Survival, mo (95% CI)	Median Overall Survival, mo (95% CI)	Overall Survival Rate, % (95% CI); patients at risk, n	
			≥ 24 wk	≥ 48 wk			1 yr	2 yr
Non-small cell lung cancer**	22/129 (17.1) [11.0, 24.7]	74.0 (6.1+, 133.9+)	13/129 (10.1) [5.5, 16.6]	6/129 (4.7) [1.7, 9.8]	2.3 (1.9, 3.7)	9.6 (7.8, 12.4)	42 (33, 51); 43	14 (4, 24); 5
Squamous	9/54 (16.7) [7.9, 29.3]	NR[§] (16.1, 133.9+)	8/54 (14.8) [6.6, 27.1]	3/54 (5.6) [1.2, 15.4]	3.7 (1.8, 7.2)	9.2 (7.3, 12.5)	39 (25, 53):16	¶
Non-squamous	13/74 (17.6) [9.7, 28.2]	63.9 (6.1+, 74.0+)	5/74 (6.8) [2.2, 15.1]	3/74 (4.1) [0.8, 11.4]	2.0 (1.8, 3.6)	10.1 (7.2, 13.7)	43 (31, 54): 26	¶
Melanoma	33/107 (30.8) [22.3, 40.5]	104 (18.4, 117.0+)	7/107 (6.5) [2.7, 13.0]	4/107 (3.7) [1.0, 9.3]	3.7 (1.9, 9.1)	16.8 (12.5, 31.6)	62 (53, 72); 55	43 (32, 53); 26
Kidney cancer	10/34 (29.4) [15.1, 47.5]	56.1 (36.6, 126.7+)	9/34 (26.5) [12.9, 44.4]	2/34 (5.9) [0.7, 19.7]	7.3 (3.7, 12.9)	>22[#] (13.6, NE^)	70 (55, 86); 23	50 (31, 70); 8

* No objective responses were seen in 19 patients with colorectal cancer or 17 patients with castration-resistant prostate cancer.

† Objective response rates ({[CR + PR] ÷ n} × 100) have been calculated based on confirmed responses with confidence intervals calculated using the Clopper-Pearson method. Individual patient responses were adjudicated per RECIST v1.0 with modification (see Methods S1 and study protocol, NEJM.org).

‡ Time from first response to time of documented progression, death, or for censored data (denoted by "+"), time to last tumor assessment.

** Among 129 patients with non-small-cell lung cancer, 1 had an unknown histology and did not show an objective response. Others had squamous or non-squamous histologies, as indicated.

§ NR, not reached; the time point at which the probability that responders progress drops below 50% has not been reached due to insufficient number of events and/or follow-up.

¶ Insufficient period of follow-up.

‖ 1 CR was noted in melanoma, and 1 CR in kidney cancer.

The median overall survival was not reached at 22 months, the longest time to death so far in patients with kidney cancer in this trial.

^ NE, not estimable.

Table 4. Clinical Activity of Nivolumab by Dose Level as Assessed to March 2013

Tumor Type	Dose, mg/kg	ORRs* No. of patients/total no. of patients (%) [95% CI]	Median Duration of Response wk (range)†	Stable Disease No. of patients/total no. of patients (%) [95% CI]		Median Progression-free Survival, mo (95% CI)	Median Overall Survival, mo (95% CI)
				≥ 24 wk	≥ 48 wk		
Non-small cell lung cancer	All doses	22/129 (17.1) [11.0, 24.7]	74.0 (6.1+, 133.9+)	13/129 (10.1) [5.5, 16.6]	6/129 (4.7) [1.7, 9.8]	2.3 (1.9, 3.7)	9.6 (7.8, 12.4)
	1	1/33 (3.0) [0.1, 15.8]	63.9 (63.9, 63.9)	5/33 (15.2) [5.1, 31.9]	2/33 (6.1) [0.7, 20.2]	1.9 (1.8, 3.6)	9.2 (5.6, 11.1)
	3	9/37 (24.3) [11.8, 41.2]	NR‡ (16.1+, 133.9+)	3/37 (8.1) [1.7, 21.9]	2/37 (5.4) [0.7, 18.2]	1.9 (1.7, 7.3)	14.9 (9.5, NE^)
	10	12/59 (20.3) [11.0, 32.8]	83.1 (6.1+, 117.1+)	5/59 (8.5) [2.8, 18.7]	2/59 (3.4) [0.4, 11.7]	3.6 (1.9, 3.8)	9.2 (5.2, 12.4)
Squamous	All doses	9/54 (16.7) [7.9, 29.3]	NR (16.1, 133.9+)	8/54 (14.8) [6.6, 27.1]	3/54 (5.6) [1.2, 15.4]	3.7 (1.8, 7.2)	9.2 (7.3, 12.5)
	1	0	0	4/15 (26.7) [7.8, 55.1]	1/15 (6.7) [0.2, 31.9]	1.9 (1.8, 7.5)	8.0 (2.6, 13.3)
	3	4/18 (22.2) [6.4, 47.6]	NR (16.1, 133.9+)	1/18 (5.6) [0.1, 27.3]	1/18 (5.6) [0.1, 27.3]	3.8 (1.7, 14.9)	9.5, (6.7, NE)

Tumor Type	Dose, mg/kg	ORRs* No. of patients/total no. of patients (%) [95% CI]	Median Duration of Response wk (range)†	Stable Disease No. of patients/total no. of patients (%) [95% CI]		Median Progression-free Survival, mo (95% CI)	Median Overall Survival, mo (95% CI)
				≥ 24 wk	≥ 48 wk		
	10	5/21 (23.8) [8.2, 47.2]	83.1 (16.1, 117+)	3/21 (14.3) [3.0, 36.3]	1/21 (4.8) [0.1, 23.8]	4.1 (1.8, 7.6)	10.5 (7.8, 12.5)
Non-squamous	All doses	13/74 (17.6) [9.7, 28.2]	63.9 (6.1+, 74.0+)	5/74 (6.8) [2.2, 15.1]	3/74 (4.1) [0.8, 11.4]	2.0 (1.8, 3.6)	10.1 (7.2, 13.7)
	1	1/18 (5.6) [0.1, 27.3]	63.9 (63.9, 63.9)	1/18 (5.6) [0.1, 27.3]	1/18 (5.6) [0.1, 27.3]	1.8 (1.7, 3.6)	9.9 (5.6, 22.7)
	3	5/19 (26.3) [9.1, 51.2]	74.0 (24.3, 74.0+)	2/19 (10.5) [1.3, 33.1]	1/19 (5.3) [0.1, 26.0]	1.8 (1.7, 12.5)	18.2 (10.3, 18.2)
	10	7/37 (18.9) [8.0, 35.2]	NR (6.1+, 65.7+)	2/37 (5.4) [0.7, 18.2]	1/37 (2.7) [0.1, 14.2]	2.3 (1.9, 3.8)	7.4 (4.6, 12.4)
Melanoma§	All doses	33/107 (30.8) [22.3, 40.5]	104 (18.4, 117.0+)	7 (6.5) [2.7, 13.0]	4 (3.7) [1.0, 9.3]	3.7 (1.9, 9.1)	16.8 (12.5, 31.6)
	0.1¶	6/17 (35.3) [14.2, 61.7]	NR (24.1, 48.7+)	0	0	3.6 (1.7, 9.1)	16.2 (8.6, NE)
	0.3¶	5/18 (27.8) [9.7, 53.5]	NR (18.4, 66.3+)	1 (5.6) [0.1, 27.3]	0	1.9 (1.8, 9.3)	12.5 (7.7, NE)

Tumor Type	Dose, mg/kg	ORRs* No. of patients/total no. of patients (%) [95% CI]	Median Duration of Response wk (range)†	Stable Disease No. of patients/total no. of patients (%) [95% CI]		Median Progression-free Survival, mo (95% CI)	Median Overall Survival, mo (95% CI)
				≥ 24 wk	≥ 48 wk		
	1	11/35 (31.4) [16.9, 49.3]	104 (32.4, 108.1+)	5/35 (14.3) [4.8, 30.3]	4/35 (11.4) [3.2, 26.7]	9.1 (1.8, 24.7)	25.3 (14.6, NE)
	3	7/17 (41.2) [16.9, 67.1]	75.9 (40.1+, 115.4+)	1/17 (5.9) [0.1, 28.7]	0	9.7 (1.9, 16.4)	20.3 (8.2, NE)
	10	4/20 (20.0) [5.7, 43.7]	112 (73.9, 117+)	0	0	3.7 (1.7, 20.50)	11.7 (7.2, 37.8)
Kidney cancer§	All doses	10/34 (29.4) [15.1, 47.5]	56.1 (36.6, 126.7+)	9/34 (26.5) [12.9, 44.4]	2/34 (5.9) [0.7, 19.7]	7.3 (3.7, 12.9)	>22# (13.6,NE)
	1	5/18 (27.8) [9.7, 53.5]	56.1 (40.1, 76.1+)	4/18 (22.2) [6.4, 47.6]	1/18 (5.6) [0.1, 27.3]	4.7 (1.9, 10.9)	NR (17.9, NE)
	10	5/16 (31.3) [11.0, 58.7]	56.1 (36.6, 126.7+)	5/16 (31.3) [11.0, 58.7]	1/16 (6.3) [0.2, 30.2]	8.0 (3.7, 14.0)	18.8 (11.6, NE)

* Objective response rates ({[CR + PR] ÷ n} × 100) have been calculated based on confirmed responses with confidence intervals calculated using the Clopper-Pearson method. Individual patient responses were adjudicated per RECIST v1.0 with modification (see Methods S1 and study protocol, NEJM.org).

† Time from first response to time of documented progression, death, or for censored data (denoted by "+"), time to last tumor assessment.

‡ NR, not reached; the time point at which the probability that responders progress drops below 50% has not been reached due to insufficient number of events and/or follow-up.

§ 1 CR was noted in melanoma, and 1 CR in kidney cancer.

¶ Five patients with tumor progression were dose-escalated from 0.1 to 1.0 mg/kg, and six from 0.3 to 1.0 mg/kg. None of these patients responded to therapy.

The median overall survival was not reached at 22 months, the longest time to death so far in patients with kidney cancer in this trial.

^ NE, not estimable.

EXAMPLE 8

Correlation between Membranous PD-L1 Expression and Anti-PD-1 Response

IHC staining of PD-L1 was performed on pretreatment formalin-fixed paraffin-embedded (FFPE) tumor specimens using the murine anti-human PD-L1 mAb 5H1 (Dong *et al.*, 2002) in a standard IHC protocol (Taube *et al.*, 2012; Supp. Materials). Briefly, 5μm-FFPE sections mounted on glass slides were deparaffinized in xylene and antigen retrieval was performed using a Tris-EDTA buffer, pH 9.0 at 120°C for 10 min in a Decloaking Chamber (Biocare Medical). Endogenous peroxidase, biotin and proteins were blocked (CAS system K1500, Dako; Avidin/biotin Blocking Kit, SP-2001, Vector Laboratories; Serotec Block ACE), and the primary 5H1 Ab was added at a concentration of 2 μg/ml and allowed to incubate at 4°C for 20 h. Secondary Ab (biotinylated anti-mouse IgG1, 553441 BD) was applied at a concentration of 1 μg/ml for 30 min at room temperature (RT). The signal was then developed with amplification according to the manufacturer's protocol (CAS system K1500, Dako). Sections were counterstained with hematoxylin, dehydrated in ethanol and cleared in xylene, and a coverslip was applied.

The percentage of tumor cells exhibiting cell surface staining for PD-L1 was scored by two independent pathologists who were blinded to treatment outcomes. PD-L1 positivity was defined per specimen by a 5% expression threshold (Taube *et al.*, 2012; Thompson *et al.*, 2006), and in cases with multiple specimens, if any specimen met this criterion. A Fisher's exact test was applied to assess the association between PD-L1 expression and OR, noting, however, that this analysis was based in part on optional biopsies from a non-random subset of the population and testing of a statistical hypothesis was not pre-specified.

Sixty-one pretreatment tumor specimens from 42 patients (18 MEL, 10 NSCLC, 7 CRC, 5 RCC, and 2 CRPC) were analyzed for tumor cell surface PD-L1 expression (Figure 8). Biopsy specimens from 25 of 42 patients were positive for PD-L1 expression by IHC. A Fisher's exact test was applied to assess the association between PD-L1 expression and OR in a post-hoc analysis. Among the 42 surface-PD-L1^{+} patients, 9 (36%) achieved an OR , whereas among 17 patients with PD-L1^{-} tumors, none achieved an OR (Figure 8A). Thus, in a subset of patients cell surface expression of PD-L1 on tumor cells in pretreatment biopsies is associated with an increased rate of OR among patients treated with BMS-936558, while no patients with documented PD-L1-negative tumors experienced an OR. These data indicate that tumor PD-L1 expression is a

molecular marker that can enable patient selection for anti-PD-1 immunotherapy.

EXAMPLE 9

Isolation of Rabbit mAbs that Detect Membranous hPD-L1 Antigen in FFPE Tissues

Rabbit Abs against human PD-L1 polypeptide were prepared by Epitomics, Inc. (Burlingame, CA) by immunization of rabbits using a recombinant human PD-L1 fusion protein. Antiserum titers were evaluated using standard direct ELISA with the hPD-L1 antigen, and cell ELISA using transfected cells overexpressing hPD-L1. These Abs were also screened for their ability to bind to PD-L1 by IHC assay of FFPE tissue sections. The rabbit with the highest Ab titer was selected for splenectomy. Lymphocytes isolated from the spleen were fused to myeloma cells in 40 x 96-well plates, and screened by ELISA against the immunizing PD-L1 antigen and by cell ELISA against cells overexpressing hPD-L1. Positive clones were expanded into 24-well plates, and confirmatory screens were conducted by direct ELISA and cell ELISA. The supernatants (sups) of clones that were specific to the screening antigen were re-screened by IHC.

A set of mouse anti-hPD-L1 mAbs were also produced by immunization of mice using a protocol similar to that described above for the rabbit mAbs.

Out of a total of 185 multiclones from both rabbit and mouse immunizations screened, only ten rabbit multiclone Abs specifically detected the membranous form of hPD-L1. None of the purified mouse subclones were found to specifically detect cell surface hPD-L1. Sixty subclones from the top five rabbit multiclones (designated Nos. 13, 20, 28, 29 and 49, each comprising 12 subclones) were screened initially by IHC on FFPE low density tissue microarrays (TMAs), followed by confirmation and specificity verification in narrowed 25 subclones. Rabbit IgG was used as a negative isotype control, and mAb 5H1 (Dong *et al.*, 2002) was used as the positive control. Specificity was further verified by antigen preabsorption assay. Through two rounds of IHC, the following 15 purified subclones were selected as the most promising Abs in terms of specificity and intensity of staining: 13-1, 13-3, 13-7, 13-8; 20-5, 20-7, 20-12, 20-6; 28-1, 28-8, 28-12; 29-8; 49-5, 49-7 and 49-9. Immunoreactivity data on these selected Abs are summarized in Table 5.

Table 5. Immunoreactivity of Rabbit Anti-hPD-L1 mAbs

	Specific Staining		Non-Specific staining
Antibody Name	Pos. vs Neg. Staining on PD-L1 Cells*	Intensity Range on Tissues† (Very High, High, Medium, Low)	Background Staining on Tissues (High, Medium, Low)
13-1	Pos	Low to Very High	None
13-3	Pos	Low to High	None
13-7	Pos	Low to High	None
13-8	Pos	Low to High	None
20-5	Pos	Low to Very High	High
20-6	Pos	Low to High	Medium
20-7	Pos	Low to High	Medium
20-12	Pos	Low to High	Medium
28-1	Pos	Low to Very High	None
28-8	Pos	Medium to Very High	None
28-10	Pos	Medium to Very High	Low
28-12	Pos	Low to Very High	None
49-5	Pos	Low to High	None
49-7	Pos	Low to High	Low
49-9	Pos	Low to High	None
5H1	Pos	High to Very High	None
Rb IgG	Neg	Neg	None

* PD-L1 stably transfected CHO cells vs. CHO-S control;

† PD-L1 positive tissues included placenta and one non-small cell lung cancer; Detection up to "very high" expression suggests better sensitivity at detecting membranous PD-L1.

Additional assays were performed to further characterize the purified Ab clones, including determining binding affinity and cross-competition among the Abs (to identify overlapping versus different epitope regions) by surface plasmon resonance. All the Abs exhibited high binding affinity ($K_D < 10^{-9}$ M). These 15 purified clones were also re-screened by IHC on FFPE low density TMA or regular sections against various cell and

tissue types known to be positive or negative for cell surface expression of PD-L1. Rabbit IgG was used as the isotype control, and mAb 5H1 was used as the positive control. At high concentration (10 μg/ml), clones 28-x and 49-x displayed low to moderate levels of background staining in tissues, while clones 13-x exhibited no background staining, which suggests that the 13-x clones have a wider dynamic range. The 20-x clones displayed various degree of background staining which was primarily cytoplasmic and diffuse. The clone with most robust detection specifically of membranous PD-L1, rabbit clone 28-8 (K_D = 100 p M, as determined by SPR), was selected as the lead Ab for subsequent IHC assays. MAbs 28-1, 28-12, 20-12 and 29-8 had K_D values of 130 pM, 94 pM, 160 pM and 1200 pM, respectively. The sequences of the heavy chain variable (V_H) and light (kappa) chain variable (V_κ) regions of mAb 28-8 are set forth in SEQ ID NOs. 35 and 36, respectively. The 28-8 Ab was shown to recognize a different epitope from mouse mAb 5H1, based on SPR analysis. Clones 28-1, 28-12, 29-8 and 20-12 were the next best Abs in terms of robust detection of membranous PD-L1 in FFPE tissues. Although mAb 13-1 had the best specificity in terms of detection of membranous PD-L1, the maximal detection level was lower than that of the other lead Abs. Western blotting was also performed with plus/minus antigen competition to verify the specificity of the top selected Abs for PD-L1.

The binding of mAbs 5H1 and 28-8 to membranous PD-L1 in FFPE test tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells from different types of tumors was compared. Membranous PD-L1 expression was evaluated using the histoscore method performed by 2 independent pathologists. Four NSCLC, 2 MEL, and 2 RCC tumors were stained with 28-8 at 2 mg/ml and 5H1 at 5 mg/ml. The data are tabulated in Table 6, and shown graphically in Figure 9. The rabbit mAb 28-8 showed better detection (higher histoscores) for 7 out of 10 samples using 2.5-fold less Ab, and in only one sample was the histoscore for 5H1 slightly higher than for mAb 28-8.

Table 6. Comparison of mAbs 28-8 and 5H1 by histoscore analysis

Tissue	Sample I.D.	Histoscore Average (5H1)	Histoscore Average (28-8)
NSCLC	1080754B	245	261
NSCLC	1080766B	103	130
NSCLC	1080790	40	37

Tissue	Sample I.D.	Histoscore Average (5H1)	Histoscore Average (28-8)
NSCLC	1080993B	12	16
Mel	T030668	98	113
Mel	T980744	98	123
Mel	1168657B	0	0
Mel	T980747	1	9
RCC	1164619B	4	4
RCC	1167809B	108	125

Taube *et al.* (2012) demonstrated by flow cytometry on cultured cells that mAb 5H1 bound to the cell surface, and the specificity of binding to PD-L1 was confirmed using a PD-L1 fusion protein to competitively block binding of the 5H1 mAb to tissue sections. These authors also compared 5H1 with a rabbit polyclonal anti-hPD-L1 Ab, 4059, previously described by Gadiot *et al.* (2011), and found that whereas 5H1 showed a cell surface staining pattern on FFPE samples, pAb 4059 demonstrated diffuse cytoplasmic staining. Further, when 5H1 was compared to pAb 4059 by western blot analysis, Ab 4059 bound to multiple proteins in lysates of melanoma cells in addition to a 50 kDa protein corresponding to the expected mass of glycosylated PD-L1, in contrast to 5H1 which specifically detected the 50 kDa band of glycosylated PD-L1 (Taube *et al.*, 2012). Contrary to the results of Taube *et al.* (2012) and the results disclosed herein (summarized in Table 7), Gadiot *et al.* (2011) reported that mAb 5H1 produced a high level background staining in FFPE tissue samples, whereas they found that pAb 4059 produced satisfactory specific staining of PD-L1 in FFPE samples. Thirteen other Abs tested by Gadiot *et al.* (2011) either did not stain FFPE tissues, gave a high background staining, or were not blocked by PD-L1 fusion protein competition, highlighting the difficulties in obtaining anti-PD-L1 Abs that bind specifically to PD-L1 in FFPE tissues.

In the present study, an automated IHC assay (*see* Example 10) was used to evaluate the binding of several commercially available anti-PD-L1 Abs and 5H1 (Dong *et al.*, 2002) to FFPE tissue samples containing various cells expressing PD-L1. The results, summarized in Table 7, show that none of the commercially available Abs tested specifically recognized membranous PD-L1 expression in human tissues known to express PD-L1, or to clearly distinguish CHO cells expressing PD-L1 versus the

untransfected parent CHO cells that did not express PD-L1. The inability of the polyclonal Ab (pAb) 4059 to bind specifically recognized membranous PD-L1 is consistent with the findings of Taube *et al.* (2012). The binding of 28-8 was similar to that of 5H1 in this assay, though histoscore analysis suggests that 28-8 performs better than 5H1.

Table 7. Binding of mAbs to FFPE Samples Containing PD-L1-expressing cells

Source	Antibody Types	Clone No. (mAb) /Catalog No. (pAb)	Pos. vs. Neg. Staining on PD-L1 Cells*	Human Positive Tissues†
MBL	mAb	27A2	Failed	Failed
BioLegend	mAb	29E.2A3	Failed	Failed
eBiosciences	mAb	M1H1	No Staining	No staining
Collaborator	mAb	5H1	Passed	Passed
ProSci	pAb	4059	Failed	Failed
LifeSpan BioSciences	pAb	LS-B480/0604	Failed	Failed
Bristol-Myers Squibb	mAb	28-8	Passed	Passed

*PD-L1 stably transfected CHO cells vs. parent CHO-S negative control;

†PD-L1 positive tissues included tonsil and/or thymus;

mAb, mouse monoclonal Ab; pAb, rabbit polyclonal Ab.

EXAMPLE 10

Development of Automated IHC Protocol for Assessing PD-L1 Expression

An automated IHC protocol was developed to assay PD-L1 expression in FFPE specimens. Tissue sections (4 μm) were mounted on slides, deparaffinized in an autostainer (Leica) by soaking twice for 5 min in xylene, and re-hydrated by soaking twice for 2 min each time in 100% EtOH, twice in 95% (v/v) EtOH, once in 70% (v/v) EtOH, and once in de-ionized water (dH_2O). Antigen retrieval was performed using a decloaking chamber (Biocare Medical Decloaking Chamber Plus) and Dako pH 6 buffer, heated to 110ºC (P1) for 10 min, then moved to the next step (P2 FAN ON at 98ºC; FAN OFF at 90ºC). The slides were cooled at room temperature (RT) for 15 min and rinsed

with water for about 1 min.

Reagents were set up on an autostainer (BioGenex i6000), and the tissue area was defined using a pap pen. The IHC assay, run using the autostainer in research mode, comprised the following steps: neutralizing endogenous peroxidase using the Peroxidase Block (Leica) for 10 min, followed by rinsing 3 times with IHC wash buffer (Dako); applying Protein Block (Leica) to the slides, and incubating for 10 min at RT, followed by washing 3 times with wash buffer; applying the primary Ab to the slides (2 μg/ml) and incubating for 1 h at RT, followed by washing 6 times with wash buffer; adding Post Primary Block (NovoLink Kit) to the slides and incubating for 30 min, followed by washing 6 times with wash buffer; adding NovoLink Polymer (NovoLink Kit) to the slides and incubating for 30 min, followed by washing 6 times with wash buffer; adding the DAB chromogen substrates (NovoLink Kit) and developing for 3 min, followed by rinsing 5 times with dH_2O at RT; counterstaining with hematoxylin (NovoLink Kit) for 1 min at RT, followed by washing 3 times with dH_2O for 5 times at RT. The primary Ab was selected from the rabbit anti-PD-L1 Abs shown in Table 4; mAb 28-8 was the preferred Ab. As a negative control, rabbit IgG (Dako) was used. The tissue sections were dehydrated using a Leica autostainer by washing once for 2 min in 70% EtOH, twice for 2 min in 95% EtOH, and three times for 2 min in 70% EtOH, then cleared by washing three times for 5 min in xylene. The sections were permanently mounted with permount to the slide, covered with a coverslip, and transferred to a chemical hood to dry.

EXAMPLE 11

Design of Phase 1 Clinical Study on Anti-PD-L1 Antibody

Study design

A phase 1 study was conducted to assess the safety and tolerability of BMS-936559 (also referred to herein and in U.S. Patent No. 7,943,743 as 12A4) in patients with selected advanced solid tumors. Secondary objectives included initial assessment of the antitumor activity of BMS-936559 and pharmacokinetic evaluation. Pharmacodynamic measures were included under exploratory objectives. Patients were treated in 6-week cycles of BMS-936559 administered as a 60-minute intravenous infusion every 2 weeks on days 1, 15, and 29 of each cycle. Patients continued treatment for up to 16 cycles unless they experienced unacceptable toxicity, disease progression, or withdrew consent. In some patients who were clinically stable, treatment beyond initial disease progression was permitted until further progression was confirmed.

Dose escalation

Patients with advanced NSCLC, MEL, CRC, RCC, ovarian (OV), gastric (GC), breast (BC), and pancreatic (PC) carcinoma were eligible to enroll. Using an accelerated titration design, safety was assessed at doses of 0.3, 1, 3, and 10 mg/kg. One patient was enrolled in each successive cohort until there was a ≥ grade 2 drug-related AE during cycle 1. Two additional patients were then enrolled at that dose level and the study was transitioned to a standard 3+3 design. Intra-patient dose escalation or de-escalation was not permitted. The maximum tolerated dose (MTD) was defined as the highest dose where less than one-third of patients had a dose-limiting toxicity.

Cohort expansion

Initially, 5 expansion cohorts (n=16/cohort) were enrolled at 10 mg/kg for patients with NSCLC, MEL, RCC, OV, and CRC. Based on initial signals of activity, additional expansion cohorts (up to n=16/cohort) were enrolled for MEL (at 1.0 and 3.0 mg/kg), NSCLC (squamous or nonsquamous histology cohorts randomized to 1, 3, or 10 mg/kg), and at 10 mg/kg for PC, BC, and GC.

Patients

Patients were required to have documented advanced NSCLC, MEL, RCC, OV, CRC, PC, GC, or BC, and have failed at least one prior tumor-appropriate therapy for advanced/metastatic disease (except for PC or GC patients who could be treatment-naïve). Other inclusion criteria included age ≥ 18 years, life expectancy ≥ 12 weeks, Eastern Cooperative Oncology Group performance status of ≤ 2, measurable disease as defined by RECIST v1.0, and adequate hematologic, hepatic, and renal function. Patients with treated brain metastases were allowed, if stable for at least 8 weeks. Major exclusion criteria included a history of autoimmune disease or other diseases requiring systemic steroids or immunosuppressive medication, prior therapy with T cell- modulating Abs (including anti-PD-1, anti-PD-L1 and anti-CTLA-4), history of HIV, or active hepatitis B or C.

In this ongoing study, 207 patients with NSCLC (n=75), MEL (n=55), CRC (n=18), RCC (n=17), OV (n=17), PC (n=14), GC (n=7), or BC (n=4) were treated with BMS-936559 during a 34-month period and are included in the safety data. Efficacy was characterized in 160 response-evaluable patients. The baseline demographic characteristics of the total and response-evaluable patient populations were very similar (Brahmer *et al.*, 2012). Among treated patients, 86% had received prior chemotherapy

and 28% immunologic or biological therapy. Prior therapies by tumor type included immunotherapy (56%) and B-RAF inhibitor (9%) in patients with MEL; platinum-based chemotherapy (95%) and tyrosine kinase inhibitors (TKIs; 41%) in patients with NSCLC; and nephrectomy (94%), anti-angiogenic therapy (82%), and immunotherapy (41%) in patients with RCC (*see* Brahmer *et al.*, 2012, for more details on patient pre-treatments).

Statistical analysis

All 207 patients commencing treatment as of the date of analysis were used for summaries of baseline characteristics and AEs. The efficacy population consisted of 160 response-evaluable patients who initiated treatment at least 7 months before the date of analysis. AEs were coded using MedDRA v14.1. Individual best overall responses were derived from radiographic scan measurements according to modified RECIST v1.0. ORs were confirmed by at least one sequential tumor assessment. Additional details regarding statistical methods are provided in Brahmer *et al.* (2012).

EXAMPLE 12

Safety Evaluations on Patients Treated with Anti-PD-L1 Antibody

Safety evaluations (clinical examination and laboratory assessments) were conducted on all treated patients at baseline and regular intervals (weekly during cycle 1 and biweekly thereafter). AE severity was graded based on the NCI CTCAE, v3.0. Disease assessment via computed tomography (CT) scans or magnetic resonance imaging was performed at baseline and prior to each treatment cycle.

A MTD was not reached up to the highest tested dose of 10 mg/kg of BMS-936559. The median duration of therapy was 12 weeks (range 2.0-111.1 weeks). A relative dose intensity of $\geq$ 90% was achieved in 86% of patients. Twelve of 207 patients (6%) discontinued treatment due to a BMS-936559-related AE (*see* Brahmer *et al.*, 2012, for details).

AEs regardless of causality (any grade) were reported in 188 of 207 patients. Investigator-assessed BMS-936559-related AEs were noted in 126 of 207 (61%) patients. The most common drug-related AEs were fatigue, infusion reactions, diarrhea, arthralgia, rash, nausea, pruritus, and headache. Most events were low grade with BMS-936559-related grade 3-4 events noted in 19 of 207 (9%) patients (Brahmer *et al.*, 2012). The spectrum, frequency, and severity of BMS-936559-related AEs were similar across the dose levels, with the exception of infusion reactions. Drug-related AEOSIs, with potential

immune-related etiologies, were observed in 81 of 207 (39%) of the patients and included rash, hypothyroidism, hepatitis, and single cases each of sarcoidosis, endophthalmitis, diabetes mellitus, and myasthenia gravis (Brahmer *et al.*, 2012). These AEs were predominantly grade 1-2 and generally reversible with treatment interruption or discontinuation. Notably, 9 patients were treated with corticosteroids for the management of AEs. AEs improved or resolved in all patients. Furthermore, 4 of these 9 patients maintained disease control despite treatment with corticosteroids. Endocrine AEs were managed with replacement therapy and patients reinitiated treatment with BMS-936559 at the discretion of the treating physician. Infusion reactions were observed in 21 of 207 (10%) patients, predominantly at 10 mg/kg. They were grade 1-2 with the exception of one grade 3 event at 10 mg/kg. Infusion reactions were generally rapidly reversible with antihistamines and antipyretics and, in some cases, corticosteroids. A prophylactic regimen with antihistamines and antipyretics was implemented during the study. Patients with grade 1-2 infusion reactions were able to continue treatment with BMS-936559 with prophylactic antihistamines and antipyretics and at a reduced infusion rate. BMS-936559-related serious AEs occurred in 11 of 207 (5%) patients. As of the data analysis date, 45 patients (22%) had died (Brahmer *et al.*, 2012); no drug-related deaths were observed.

EXAMPLE 13

Pharmacokinetics/Pharmacodynamics Analyses on Anti-PD-L1 Antibody

For PK analyses, serial blood samples were collected and serum concentrations of BMS-936559 were quantified by ELISA. Peripheral blood mononuclear cells were isolated from patients at baseline and following one treatment cycle to assay PD-L1 RO by BMS-936559 on circulating CD3-positive T-cells via flow cytometry (Brahmer *et al.*, 2010).

Serum concentrations of BMS-936559 increased in a dose-dependent manner from 1-10 mg/kg (n=131). Geometric mean area under the curve (0-14 days) for the 1, 3, and 10 mg/kg dose levels were 2210, 7750, and 36620 μg/mL•hr (coefficient of variation [CV] 34-59%), respectively; geometric mean peak concentrations at these dose levels were 27, 83, and 272 μg/mL (CV 30−34%), respectively, after the first dose. The half-life of BMS-936559 was estimated from population pharmacokinetic data as approximately 15 days. PD-L1 RO on CD3-positive peripheral blood lymphocytes was assessed in 29 MEL patients at the end of 1 cycle of treatment, at BMS-936559 doses from 1-10 mg/kg. Median RO exceeded 65% for all groups (Brahmer *et al.*, 2012).

EXAMPLE 14

Antitumor Efficacy Exhibited by Anti-PD-L1 Antibody

One-hundred and sixty patients out of the 207 treated were evaluable for response by February 2012 and included those with NSCLC, MEL, CRC, RCC, OV, and PC, but not patients with GC or BC. Clinical activity was observed at all doses ≥ 1 mg/kg (Brahmer *et al.*, 2012). ORs (confirmed complete [CR] or partial [PR] responses) were observed in patients with MEL, NSCLC, RCC, and OV (Table 8), as illustrated by representative spider plots and CT scans (Figures 10-13), and many ORs were also durable (Table 9). Four additional patients had a persistent reduction in target lesions in the presence of new lesions, consistent with an "immune-related" pattern of response; however, for the purpose of calculating response rates, they were not categorized as responders. Antitumor responses and/or prolonged stable disease (SD) were observed in patients with a variety of prior therapies received. ORs were observed even in patients with an extensive burden of metastatic disease.

In patients with MEL, there were 9 ORs across the 1, 3, and 10 mg/kg dose levels, with response rates of 6%, 29%, and 19%, respectively. Three MEL patients achieved a CR. All 9 MEL patients who experienced an OR started treatment ≥ 1 year prior to data analysis; of these 5 had a response duration ≥ 1 year. Additionally 14 MEL patients (27%) had SD lasting ≥ 24 weeks. In patients with NSCLC, there were 5 ORs amongst the 3 and 10 mg/kg dose levels, with response rates of 8% and 16%, respectively. There were ORs in patients with non-squamous (n=4) or squamous histology (n=1). All 5 NSCLC responders started treatment ≥ 24 weeks prior to data analysis; of these, 3 had responses lasting ≥ 24 weeks. Six additional NSCLC patients had SD lasting ≥ 24 weeks. There was 1 PR out of 17 patients with OV (6% response rate) and 3 patients (18%) with SD lasting ≥ 24 weeks, all at the 10 mg/kg dose. In patients with RCC, there were ORs in 2 of 17 (12%) patients treated at 10 mg/kg with responses lasting 4 and 18 months, respectively. Seven additional RCC patients had SD lasting ≥ 24 weeks.

Table 8. Clinical Activity of BMS-936559 in 160 Patients, Response Evaluable*

Tumor Type	Dose (mg/kg)	n	ORR[§] No. patients (%) [95% CI]	SD ≥ 24 wk No. patients (%) [95% CI]	PFSR** at 24 wk (%) [95% CI]
MEL	0.3	1	0 [0-98]	0 [0-98]	N/A
	1	18	1 (6) [0.1-27]	6 (33) [13-59]	39 [16-61]
	3	17	5 (29)[†] [10-56]	3 (18) [4-43]	47 [21-72]
	10	16	3 (19)[††] [4-46]	5 (31) [11-59]	44 [19-68]
ALL MEL		52	9 (17) (8-30)	14 (27) [16-41]	42 [28-56]
NSCLC[§]	1	11	0 [0-29]	0 [0-29]	N/A
Squamous	1	1	0 [0-98]	0 [0-98]	N/A
Non-Squamous	1	10	0 [0-31]	0 [0-31]	N/A
	3	13	1 (8) [0.2-36]	1 (8) [0.2-36]	34 [7-60]
Squamous	3	4	0 [0-60]	1 (25) [0.6-81]	50 [1-99]
Non-Squamous	3	9	1 (11) [0.3-48]	0 [0-34]	25 [0-55]
	10	25	4 (16) [5-36]	5 (20) [7-41]	46 [25-67]
Squamous	10	8	1 (13) [0.3-53]	2 (25) [3-65]	47 [10-83]
Non-Squamous	10	17	3 (18) [4-43]	3 (18) [4-43]	46 [20-72]
ALL NCSLC		49	5 (10) [3-22]	6 (12) [5-25]	31 [17-45]
ALL Squamous		13	1 (8) [0.2-36]	3 (23.1) [5-54]	43 [15-71]
ALL Non-Squamous		36	4 (11) [3-26]	3 (8) [2-23]	26 [10-42]
OV	3	1	0 [0-98]	0 [0-98]	N/A
	10	16	1 (6) [0.2-30]	3 (19) [4-46]	25 [4-46]
ALL OV		17	1 (6) [0.1-29]	3 (18) [4-43]	22 [2-43]
RCC	10	17	2 (12) [2-36]	7 (41) [18-67]	53 [29-77]

CI denotes Confidence intervals, MEL melanoma, RCC renal cell carcinoma, NSCLC non-small cell lung cancer, OV ovarian cancer, RCC renal cell carcinoma, N/A not applicable, ORR objective response rate (complete response + partial response), SD stable disease, and PFSR progression-free survival rate.

* Efficacy population consists of response-evaluable patients who initiated treatment at least 7 months prior to the date of analysis and had measurable disease at a baseline tumor assessment and at least one of the following: an on-study tumor assessment,

clinical progression, or death.

† Includes two CRs.

†† Includes one CR.

§ Objective response rates ({[CR + PR] ÷ n} × 100) are based on confirmed responses only, with confidence intervals calculated using the Clopper-Pearson method.

** Progression-free survival rate was the proportion of patients who did not progress, and were alive at 24 weeks, calculated by the Kaplan-Meier methodology, with confidence intervals using the Greenwood method.

Individual patient responses were adjudicated per RECIST v1.0 with modification (see study protocol in Brahmer *et al.* (2012) *N Engl J Med* (submitted) for additional information).

Table 9. Duration of Objective Responses to BMS-936559*

Tumor Type	Dose (mg/kg)	No. of Patients with OR	Duration of Response (months)†
MEL	1	1	6.9
	3	5	23.5+, 22.9+, 16.2+, 4.1+, 3.5
	10	4	24.6+, 23.9+, 18.0+, 17.0
NSCLC	1	0	9.2+
	3	1	2.3+
	10	4	16.6+, 12.6+, 9.8, 3.5
RCC	10	2	18, 4
OV	10	1	1.3+

* MEL denotes melanoma, NSCLC non-small cell lung cancer, RCC renal cell cancer, OV ovarian cancer.

† Time from first response to time of documented progression, death, or for censored data (denoted by +), time to last tumor assessment.

EXAMPLE 15

Design of Phase 1 Clinical Study on Anti-PD-1 plus Anti-CTLA-4 in Advanced MEL

Study Design

In this phase 1 study, successive cohorts of patients were treated with escalating

doses of intravenous nivolumab and ipilimumab administered concurrently (concurrent regimen), and separately, two cohorts of patients treated previously with ipilimumab received nivolumab alone (sequenced regimen).

For the concurrent regimen, patients received during the induction period nivolumab and ipilimumab every 3 weeks for 4 doses, followed by nivolumab alone every 3 weeks for 4 doses. Combined treatment was subsequently continued during the maintenance period every 12 weeks for up to 8 doses. When both drugs were administered together, nivolumab was administered first. Within a cohort, nivolumab and ipilimumab doses were kept constant during the induction and maintenance periods. The dose-limiting-toxicity evaluation period was through week 9. Tumor assessments were at weeks 12, 18, 24, and 36, and then every 12 weeks thereafter.

In the sequenced regimen, patients previously treated with ipilimumab prior to study entry received nivolumab every 2 weeks for up to 48 doses. Nivolumab therapy was initiated within 4-12 weeks after ipilimumab monotherapy. Tumor assessments were at week 8 and then every 8 weeks thereafter. Tumor responses were adjudicated using mWHO and immune-related mWHO criteria for both regimens.

After completion of therapy, patients without confirmed disease progression were followed for up to 2.5 years. Patients with CR, PR, or SD $\geq$ 24 weeks and subsequent disease progression could be retreated with the original regimen. Safety evaluation was performed per protocol. The severity of AEs was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0. Disease assessment, using CT and/or MRI as appropriate, was performed per protocol.

Dose escalation and cohort expansion

The study was initially planned to evaluate the concurrent regimen using a standard 3 + 3 design for the dose escalation phase, followed by cohort expansion to a total of up to 16 patients at the maximum tolerated dose or the maximum administered dose. The dose-limiting toxicity (DLT) evaluation period for dose escalation was 9 weeks. No intra-patient dose escalation was allowed and patients who experienced DLT were discontinued from therapy. Patients who withdrew from study during the DLT evaluation period for reasons other than drug-related toxicity could be replaced. The protocol was amended to permit expansion of any concurrent regimen cohort during dose escalation to N = up to 12 patients. Two sequenced-regimen cohorts (6 to 16 patients each) were later added; patients were treated with nivolumab (1 mg/kg or 3 mg/kg) after

having received prior ipilimumab.

Patients

Eligible patients were 18 years of age and had a diagnosis of measurable, unresectable stage III or IV melanoma; Eastern Cooperative Oncology Group performance status of 0-1, where 0 is asymptomatic and 1 is mildly symptomatic; adequate organ function; and life expectancy of ≥ 4 months. Patients with active, untreated central nervous system metastasis; history of autoimmune disease; prior therapy with T-cell modulating antibodies (excluding ipilimumab for sequenced-regimen cohorts); HIV; or hepatitis B or C were excluded.

In the sequenced-regimen cohorts, patients were required to have received 3 prior doses of ipilimumab, with the last dose administered within 4-12 weeks of initiation of nivolumab. Patients with CR, progression with evidence of clinical deterioration, or a history of high-grade AEs related to ipilimumab were excluded (*see* Wolchok *et al.* 2013a, for details of protocol).

Eighty-six patients were treated between December 2009 and February 2013, 53 with the concurrent regimen and 33 with the sequenced regimen. Baseline patient characteristics are detailed in Wolchok *et al.* (2013a). In the concurrent and sequenced regimens, 38% and 100% of patients, respectively, received prior systemic therapy. The majority of patients had M1c disease and >30% had elevated serum lactate dehydrogenase (LDH). Most patients enrolled to the sequenced-regimen cohorts demonstrated radiographic progression (73%) with prior ipilimumab treatment.

PD-L1 immunohistochemistry

Pre-treatment PD-L1 expression was measured by IHC in FFPE tumor specimens using the rabbit anti-PD-L1 mAb, 28-8, and an automated assay developed by Dako (Carpinteria, CA). Ab specificity was assessed by western blotting against recombinant PD-L1 protein and lysates from PD-L1 expressing and non-expressing cell lines. An IHC assay with and without antigen competition and an assessment of staining patterns in normal human tissues was performed. Analytical sensitivity, specificity, repeatability, reproducibility, and robustness of the immunohistochemistry assay were tested and met all pre-specified acceptance criteria. Two pathologists, blinded to outcome, independently read and adjudicated scores for all clinical specimens. A sample was defined as PD-L1-positive if 5% of tumor cells exhibited membrane PD-L1 staining of any intensity in a section with 100 evaluable cells.

Statistical analysis

All treated patients (N=86) as of February 2013 were used to describe baseline characteristics, safety, and absolute lymphocyte count (ALC), and analysis of PD-L1 staining. The efficacy population consisted of 82 response-evaluable patients who received at least one dose of study therapy, had measurable disease at baseline, and one of the following: > 1 on-treatment tumor evaluation, clinical progression, or death before the first on-treatment tumor evaluation. AEs were coded using MedDRA, version 15.1. Select AEs with potential immunologic etiologies were identified using a pre-defined list of MedDRA terms. Best overall responses were derived programmatically from tumor-measurements provided by the study-site radiologist and investigators per modified WHO (mWHO) or immune-related response criteria (Wolchok *et al.* 2009). Complete and partial responses were confirmed by at least one subsequent tumor assessment. An analysis also was performed to assess the magnitude of reduction in target lesions by radiographic assessment. A response was characterized as deep if a reduction of 80% from baseline measurements was noted. Unconfirmed responses as of the date of this analysis were also included in an estimate of aggregate clinical activity.

EXAMPLE 16

Safety Evaluations on MEL Patients Treated with Anti-PD-1 plus Anti-CTLA-4

For the concurrent regimen (n=53), AEs of any grade, irrespective of attribution, were observed in 98% of patients. Treatment-related AEs were observed in 93% of patients with the most common being rash (55%), pruritus (47%), fatigue (38%), and diarrhea (34%). Grade 3-4 AEs, irrespective of attribution, were observed in 72% of patients, while grade 3-4 treatment-related events were noted in 53%, with the most common being elevations of lipase (13%), aspartate aminotransferase (13%) and alanine aminotransferase (11%). Six of 28 (21%) patients had grade 3-4 dose- limiting treatment-related events. Treatment-related serious AEs were reported in 49% of patients. Common grade 3-4 treatment-related select AEs included hepatic (15%), gastrointestinal (9%), and renal (6%) events. Isolated cases of pneumonitis and uveitis were seen, consistent with historical monotherapy experiences. Eleven (21%) patients discontinued due to treatment-related AEs.

Cohort 3 (3 mg/kg nivolumab + 3 mg/kg ipilimumab) exceeded the MTD (3 of 6 patients experienced asymptomatic grade 3-4 elevated lipase that persisted for 3 weeks).

Cohort 2 (1 mg/kg nivolumab + 3 mg/kg ipilimumab) was identified as the MTD (grade 3 uveitis, grade 3 elevated AST/ALT in 1 patient each).

For the sequenced regimen (n=33), AEs of any grade, irrespective of attribution, were observed in 29 (88%) patients. Treatment-related AEs were observed in 24 (73%) patients, with the most common including pruritus (18%) and lipase elevation (12%). Grade 3-4 AEs, irrespective of attribution, were observed in 11 (33%) patients, while grade 3-4 treatment-related AEs were observed in 6 (18%) patients, with lipase elevation as the most common event (6%). Treatment-related serious AEs were reported in 7 (21%) patients. Grade 3-4 endocrine events were noted as treatment-related select AEs in 2 patients. One patient had grade-2 pneumonitis. Three (9%) patients discontinued due to treatment-related AEs.

For both the concurrent and sequenced regimens, treatment-related AEs were manageable and generally reversible with immunosuppressants and/or replacement therapy (for endocrinopathies) per previously algorithms previously established for ipilimumab (*see* YERVOY® package insert). Amongst the 86 patients treated on the study, 28 of 73 patients (38%) with drug-related adverse events required management with systemic glucocorticoids. Three patients required additional immunosuppressive therapy with infliximab (2 patients) or mycophenolate mofetil (1 patient). No treatment-related deaths were reported. Additional details of AEs and their management are provided in (Wolchok *et al.* 2013a).

EXAMPLE 17

Efficacy Exhibited by Combination of Anti-PD-1 and Anti-CTLA-4 in MEL Patients

Clinical activity was observed with both the concurrent and sequenced regimens (Tables 10 and 11). In the concurrent-regimen cohorts, confirmed objective responses (OR) by mWHO criteria were observed in 21 of 52 (40%; 95% CI: 27-55) response-evaluable patients across all doses. After noting several patients who demonstrated major responses (approaching CR), the number of patients with at least 80% tumor reduction, an empirical threshold chosen because it represents a level of tumor regression that approaches complete response, was assessed. This depth of response was uncommon in published studies of checkpoint blockade (Hodi *et al.*, 2010; Topalian *et al.*, 2012b). Sixteen patients had ≥ 80% tumor reduction at 12 weeks, including 5 CRs (Table 10, Figures 14A and 15-17). In addition to the 21 patients with OR by mWHO criteria, 4 patients experienced an objective response by immune-related response criteria and 2 patients had

unconfirmed responses. These patients were not included in the calculation of ORRs. For the concurrent regimen, overall evidence of clinical activity (conventional, unconfirmed, or immune-related response or SD ≥ 24 weeks) was observed in 65% (95% CI: 51-78; Table 10) of patients. The profound impact of the concurrent combination can best be appreciated in the waterfall plot (Figure 14B). Responses were ongoing among 19 of 21 responders, with durations ranging from 6.1+ to 72.1+ weeks at the time of data analysis (Table 12). For patients treated at the MTD (cohort 2, 1 mg/kg nivolumab + 3 mg/kg ipilimumab), OR occurred in 9 of 17 (53%; 95% CI: 28-77) patients, including 3 CRs. All 9 responders achieved ≥ 80% tumor reduction at their first scheduled on-treatment assessment (Table 10 and Figure 14A).

For patients in the sequenced-regimen cohorts, 6 of 30 patients achieved OR (20%; 95% CI: 8-39) including 1 CR. Four (13%) patients achieved 80% tumor reduction at 8 weeks (Table 11 and Figure 18). Additional patients had immune-related (n=3) or unconfirmed (n=3) responses. When objective, immune-related or unconfirmed responses or SD ≥ 24 weeks are considered, evidence of clinical activity for the sequenced regimen was observed in 43% (95% CI: 26-63). The waterfall plot reveals that patients who did not respond to prior ipilimumab can respond to subsequent nivolumab (Figure 18C).

Table 10. Clinical Activity of Patients who Received the Concurrent Regimen of Nivolumab and Ipilimumab*

Cohort	Dose	Response-Evaluable Patients† n	CR n	PR n	uPR‡ n	irPR§ n	SD ≥24 wk n	irSD§ ≥24 wk n	Objective Response Rate¶ % [95% CI]	Aggregate Clinical Activity Rate‖ % [95% CI]	80% Tumor Reduction at 12 wk n (%)
1	0.3 mg/kg nivolumab + 3 mg/kg ipilimumab	14	1	2	0	2	2 (14)	0	21 [5-51]	50 [23-77]	4 (29)
2	1 mg/kg nivolumab + 3 mg/kg ipilimumab	17	3	6	0	0	0	2 (12)	53 [28-77]	65 [38-86]	7 (41)**
2a	3 mg/kg nivolumab + 1 mg/kg ipilimumab	15	1	5	2	1	2 (13)	0	40 [16-68]	73 [45-92]	5 (33)
3	3 mg/kg nivolumab + 3 mg/kg ipilimumab	6	0	3	0	1	0	1 (17)	50 [12-88]	83 [36-100]	0
All	Concurrent treatment	52	5	16	2	4	4 (8)	3 (6)	40 [27-55]	65 [51-78]	16 (31)

* CR denotes complete response, PR partial response, uPR unconfirmed partial response, irPR immune-related partial response, SD stable disease, irSD immune-related stable disease.

† Response-evaluable patients were those who received at least one dose of study therapy, had measurable disease at baseline, and one of the following: 1) at least one on-treatment tumor evaluation, 2) clinical progression, or 3) death prior to the first on-treatment tumor evaluation.

‡ Patients who had a PR after one tumor assessment but did not have sufficient follow-up time for confirmation of the initial PR.

§ Patients who had target tumor-lesion reduction in the presence of new lesions, consistent with immune-related PR or SD.

¶ [(CR + PR) / no. response-evaluable patients] × 100. Confidence intervals were estimated by the Clopper-Pearson method.

‖ [(CR + PR + uCR + uPR + irPR + SD ≥24 wk + irSD ≥24 wk) / no. response-evaluable patients] × 100.

** Two additional patients in cohort 2 achieved ≥80% tumor reduction at their first scheduled assessment, which was conducted after week 12.

Table 11. Clinical Activity of Patients who Received the Sequenced Regimen of Nivolumab and Ipilimumab*

Cohort	Dose	Response-Evaluable Patients† n	CR n	PR n	uPR‡ n	irPR§ n	SD ≥ 24 wk n	irSD§ ≥ 24 wk n	Objective Response Rate¶ % [95% CI]	Aggregate Clinical Activity Rate‖ % [95% CI]	80% Tumor Reduction at 8 wk n (%)
6	1 mg/kg nivolumab + prior ipilimumab	16	1	5	2	2	1 (6)	0	38 [15-65]	69 [41-89]	4 (25)
7	3 mg/kg nivolumab + prior ipilimumab	14	0	0	1	1	0	0	0	14 [2-43]	0
All	Sequenced treatment	30	1	5	3	3	1 (3)	0	20 [8-39]	43 [26-63]	4 (13)

* CR denotes complete response, PR partial response, uPR unconfirmed partial response, irPR immune-related partial response, SD stable disease, irSD immune-related stable disease.

† Response-evaluable patients were those who received at least one dose of study therapy, had measurable disease at baseline, and one of the

following: 1) at least one on-treatment tumor evaluation, 2) clinical progression, or 3) death prior to the first on-treatment tumor evaluation.

‡ Patients who had a PR after one tumor assessment but did not have sufficient follow-up time for confirmation of the initial PR.

Table 12. Duration of Confirmed Objective Responses of Individual Patients

Cohort	Dose	Response	Duration of Response, weeks
1	0.3 mg/kg nivolumab + 3 mg/kg ipilimumab	CR	48.0+
		PR	72.1+, 65.4+
2	1 mg/kg nivolumab + 3 mg/kg ipilimumab	CR	36.1+, 28.9+, 24.7+
		PR	54.4+, 52.6, 23+, 18.4+, 14.0 +, 11.1+
2a	3 mg/kg nivolumab + 1 mg/kg ipilimumab	CR	7.7+
		PR	18.1+, 12.3, 6.6+, 6.4+, 6.1+
3	3 mg/kg nivolumab + 3 mg/kg ipilimumab	PR	38.7+, 31.3+, 12.1+
6	1 mg/kg nivolumab + prior ipilimumab	CR	24.1+
		PR	16.1+, 12.1+, 8.7+, 8.4+, 8.1+
7	3 mg/kg nivolumab + prior ipilimumab	CR	NA*
		PR	NA*

* Not available; as of the date of this analysis, no confirmed objective responses had been reported.

Assessment of tumor PD-L1 expression and absolute lymphocyte count

Tumor PD-L1 expression and alterations in the peripheral blood ALC have been explored as biomarkers for nivolumab and ipilimumab monotherapy, respectively (Topalian *et al.*, 2012b; Berman *et al.*, 2009; Ku *et al.*, 2010; Postow *et al.*, 2012; Delyon *et al.*, 2013). Tumor PD-L1 expression was characterized via IHC staining and pharmacodynamic changes in peripheral blood ALC was analyzed. Using a ≥ 5% cut off to define PD-L1 positivity, tumor specimens from 21 of 56 (38%) patients were PD-L1-positive. ORs were seen in patients with either PD-L1-positive (6/13) or PD-L1-negative (9/22) tumors amongst patients treated with the concurrent regimen (post-hoc P-value > 0.99; Fisher's exact test). In sequenced-regimen cohorts, a numerically higher number of overall responses were seen in patients with PD-L1-positive tumor samples (4/8) compared with patients with PD-L1-negative tumors (1/13), but the numbers are small.

In contrast to observations with ipilimumab monotherapy, a consistent rise in ALC from baseline was not detected in patients treated with the concurrent combination or in patients treated with nivolumab following ipilimumab therapy. In the concurrent-regimen cohorts, patients with a low ALC at weeks 5 to 7 (< 1000 cells/μL) (Ku *et al.*, 2010) had similar OR (43%) compared with patients with a normal ALC at weeks 5 to 7 (40%). Likewise, in the sequenced-regimen cohorts, 17% of patients with low ALC had OR and 23% of patients with normal or high ALC had OR.

Sequence Listing Summary

SEQ ID NO:	Description
1	V_H amino acid sequence of 17D8
2	V_H amino acid sequence of 2D3
3	V_H amino acid sequence of 4H1
4	V_H amino acid sequence of 5C4
5	V_H amino acid sequence of 4A11
6	V_H amino acid sequence of 7D3
7	V_H amino acid sequence of 5F4
8	V_L amino acid sequence of 17D8
9	V_L amino acid sequence of 2D3
10	V_L amino acid sequence of 4H1
11	V_L amino acid sequence of 5C4

SEQ ID NO:	Description
12	V_L amino acid sequence of 4A11
13	V_L amino acid sequence of 7D3
14	V_L amino acid sequence of 5F4
15	V_H amino acid sequence of 3G10
16	V_H amino acid sequence of 12A4
17	V_H amino acid sequence of 10A5
18	V_H amino acid sequence of 5F8
19	V_H amino acid sequence of 10H10
20	V_H amino acid sequence of 1B12
21	V_H amino acid sequence of 7H1
22	V_H amino acid sequence of 11E6
23	V_H amino acid sequence of 12B7
24	V_H amino acid sequence of 13G4
25	V_L amino acid sequence of 3G10
26	V_L amino acid sequence of 12A4
27	V_L amino acid sequence of 10A5
28	V_L amino acid sequence of 5F8
29	V_L amino acid sequence of 10H10
30	V_L amino acid sequence of 1B12
31	V_L amino acid sequence of 7H1
32	V_L amino acid sequence of 11E6
33	V_L amino acid sequence of 12B7
34	V_L amino acid sequence of 13G4
35	V_H amino acid sequence of 28-8
36	V_L amino acid sequence of 28-8
37	Heavy chain CDR1 sequence of 28-8
38	Heavy chain CDR2 sequence of 28-8
39	Heavy chain CDR3 sequence of 28-8
40	Light chain CDR1 sequence of 28-8
41	Light chain CDR2 sequence of 28-8
42	Light chain CDR1 sequence of 28-8

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CLAIMS

What is claimed is:

1. A method of treating a subject afflicted with a cancer comprising administering to the subject a therapeutically effective amount of an antibody or an antigen-binding portion thereof that disrupts the interaction between Programmed Death-1 (PD-1) and Programmed Death Ligand-1 (PD-L1, wherein the antibody or antigen-binding portion thereof binds specifically to PD-1 or to PD-L1.

2. The method of claim 1, wherein the cancer is selected from the group consisting of melanoma, renal cell carcinoma, squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, colorectal cancer, castration-resistant prostate cancer, ovarian cancer, gastric cancer, hepatocellular carcinoma, pancreatic carcinoma, squamous cell carcinoma of the head and neck, carcinomas of the esophagus, gastrointestinal tract and breast, and a hematological malignancy.

3. The method of claim 1, wherein the therapeutically effective amount of the antibody or antigen-binding portion thereof comprises a dose ranging from 0.1 to 10.0 mg/kg body weight which is administered at a dosing schedule of once per week, once every two weeks, or once a month.

4. A method for immunotherapy of a subject afflicted with cancer, which method comprises:

(a) selecting a subject that is a suitable candidate for immunotherapy, the selecting comprising:

(i) providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells;

(ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; and

(iii) selecting the subject as a suitable candidate based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and

(b) administering a composition comprising a therapeutically effective amount of an anti-PD-1 antibody to the selected subject.

5. The method of claim 4, wherein the proportion of cells 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.

6. The method of claim 5, wherein the test tissue sample is a formalin-fixed paraffin-embedded (FFPE) tissue sample.

7. The method of claim 6, wherein the presence of PD-L1 polypeptide is determined using an automated IHC assay.

8. The method of claim 12, wherein the IHC assay is performed using an anti-PD-L1 monoclonal antibody to bind to the PD-L1 polypeptide, wherein the anti-PD-L1 monoclonal antibody is selected from 28-8, 28-1, 28-12, 29-8, and 5H1.

9. The method of claim 4, wherein the predetermined threshold level is 1% of tumor cells or a single tumor-infiltrating inflammatory cell expressing cell surface PD-L1 as determined by automated IHC using mAb 28-8.

10. A method for treatment of a subject afflicted with cancer, which method comprises:

(a) selecting a subject that is not suitable for anti-PD-1 antibody immunotherapy, the selecting comprising:

(i) providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells;

(ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; and

(iii) selecting the subject as not suitable for anti-PD-1 antibody immunotherapy based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is less than a predetermined threshold level; and

(b) administering a standard-of-care therapeutic other than an anti-PD-1 antibody to the selected subject.

11. A monoclonal antibody or an antigen-binding portion thereof that binds specifically to a cell surface-expressed PD-L1 polypeptide in a formalin-fixed, paraffin-embedded (FFPE) tissue sample.

12. The monoclonal antibody or antigen-binding portion thereof of claim 16, which antibody or portion thereof comprises the CDR1, CDR2 and CDR3 regions in a heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 35, and the CDR1, CDR2 and CDR3 regions in a light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 36.

13. The monoclonal antibody or antigen-binding portion thereof of claim 16, which antibody or portion thereof comprises a heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 35, and a light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 36.

14. A method of treating a subject afflicted with a cancer comprising administering to the subject:

(a) an antibody or an antigen-binding portion thereof that specifically binds to and inhibits Programmed Death-1 (PD-1); and

(b) an antibody or an antigen-binding portion thereof that specifically binds to and inhibits Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4);

each antibody being administered at a dosage ranging from 0.1 to 20.0 mg/kg body weight in a concurrent regimen comprising:

(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 every 2, 3 or 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 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 once every 8, 12 or 16 weeks, or at least once a quarter, for at least 4, 6, 8, 10, 12 or 16 doses.

15. The method of claim 14, wherein 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.

16. A method of treating a subject afflicted with a cancer, the subject having previously been treated with an anti-CTLA-4 antibody, which method comprises administering to the subject in a sequenced regimen an antibody or an antigen-binding portion thereof that specifically binds to and inhibits PD-1 at a dosage ranging from 0.1 to 20.0 mg/kg body weight and at a dosing frequency of at least once every week, at least once every 2, 3 or 4 weeks, or at least once a month, for up to 6 to up to 72 doses.

17. The method of claim 15 or 16, wherein the anti-PD-1 antibody is nivolumab.

18. The method of 15 or 16, wherein the anti-CTLA-4 antibody is ipilimumab.

19. An anti-PD-1 antibody or an antigen-binding portion thereof for use in treating a subject afflicted with a cancer in a concurrent regimen comprising combined administration with an anti-CTLA-4 antibody or an antigen-binding portion thereof, wherein the anti-PD-1 and anti-CTLA-4 antibodies are each administered

at a dosage ranging from 0.1 to 20.0 mg/kg body weight, and further wherein 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 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 administration of the anti-PD-1 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 10 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 every 8, 12 or 16 weeks, or at least once a quarter, for up to 4, 6, 8, 10, 12 or 16 doses.

20. A kit for treating a subject afflicted with a cancer, the kit comprising:

(a) a dosage ranging from 0.1 to 20.0 mg/kg body weight of an antibody or an antigen-binding portion thereof that specifically binds to and inhibits PD-1;

(b) a dosage ranging from 0.1 to 20.0 mg/kg body weight of an antibody or an antigen-binding portion thereof that specifically binds to and inhibits CTLA-4; and

(c) instructions for using the anti-PD-1 and anti-CTLA-4 antibodies the concurrent regimen method of claim 14.

21. A kit for treating a subject afflicted with a cancer, the kit comprising:

(a) a dosage ranging from 0.1 to 20.0 mg/kg body weight of an antibody or an antigen-binding portion thereof that specifically binds to and inhibits PD-1; and

(b) instructions for using the anti-PD-1 antibody in the sequenced regimen method of claim 16.

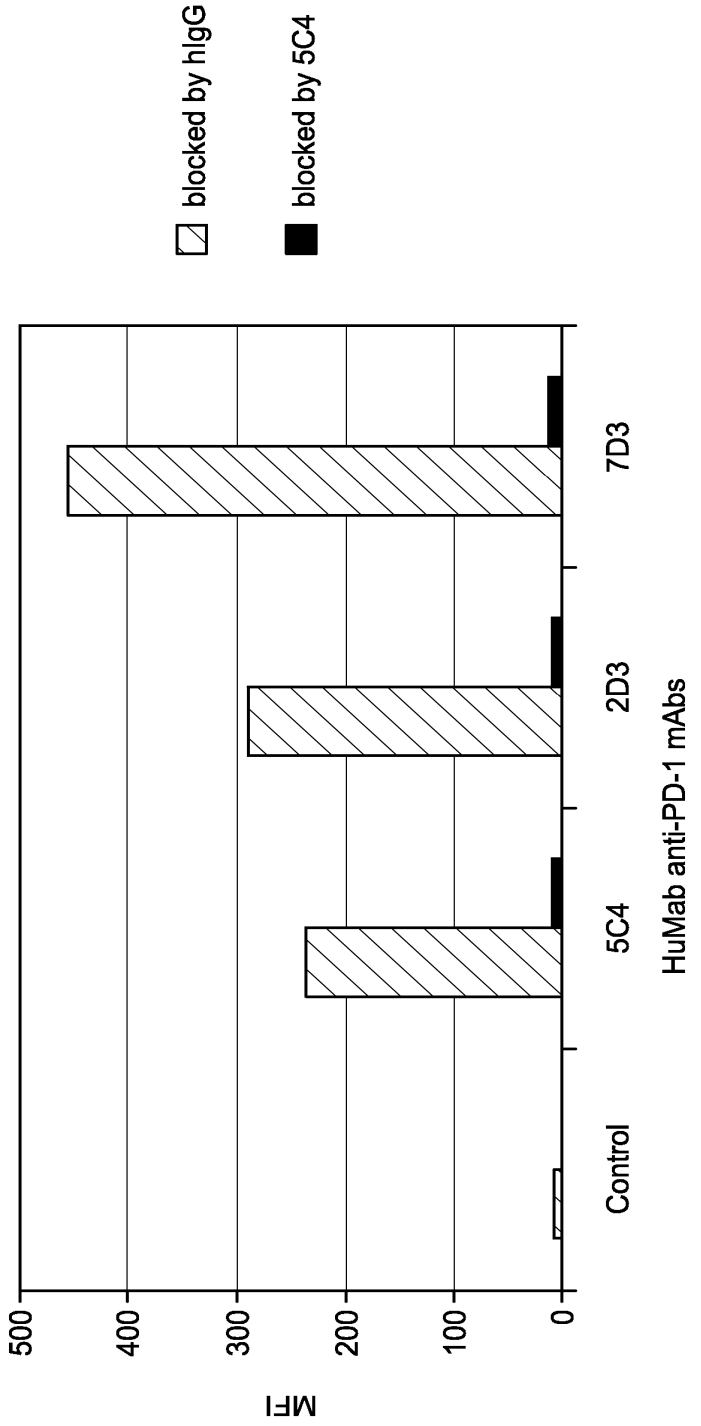

blocked by hIgG
blocked by 5C4
MFI
500
400
300
200
100
0
Control
5C4
2D3
7D3
HuMab anti-PD-1 mAbs

FIG. 1A

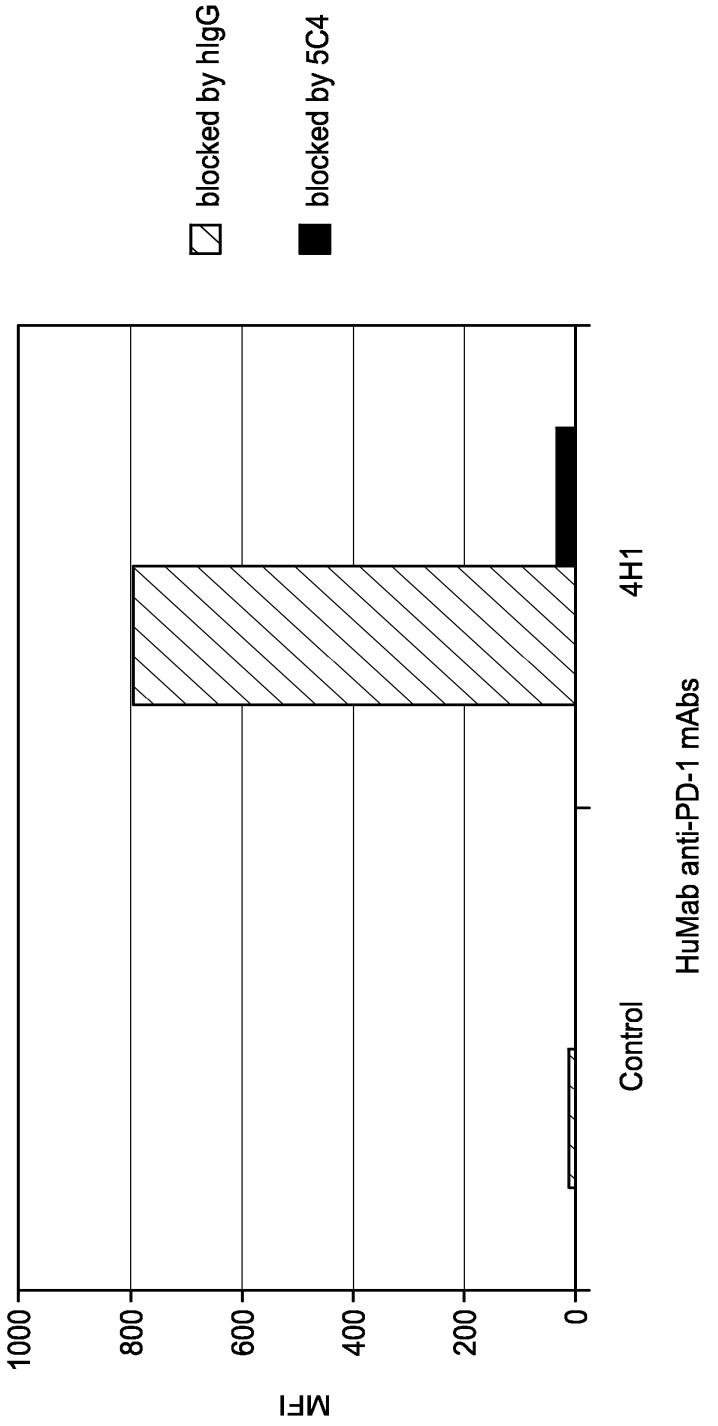

blocked by hIgG
blocked by 5C4
MFI
1000
800
600
400
200
0
Control
4H1
HuMab anti-PD-1 mAbs

FIG. 1B

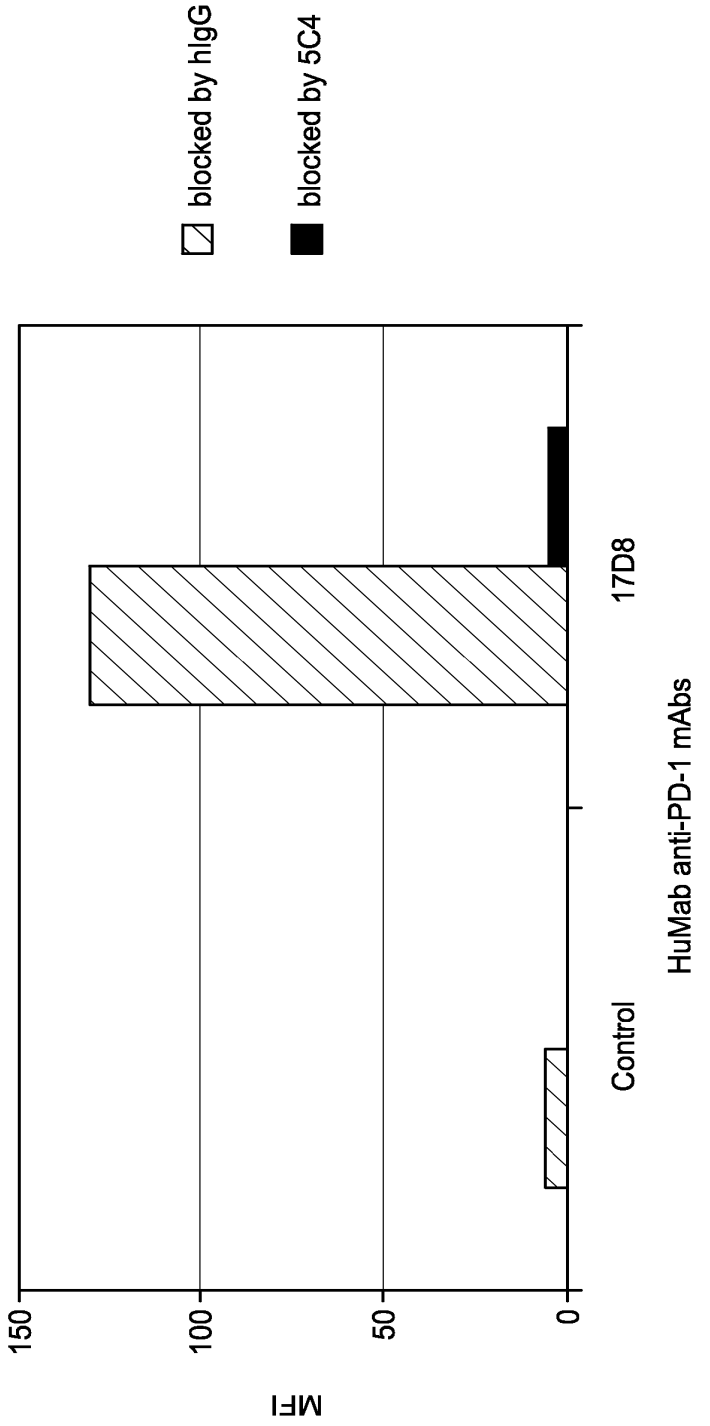

blocked by hIgG
blocked by 5C4
MFI
150
100
50
0
Control
17D8
HuMab anti-PD-1 mAbs

FIG. 1C

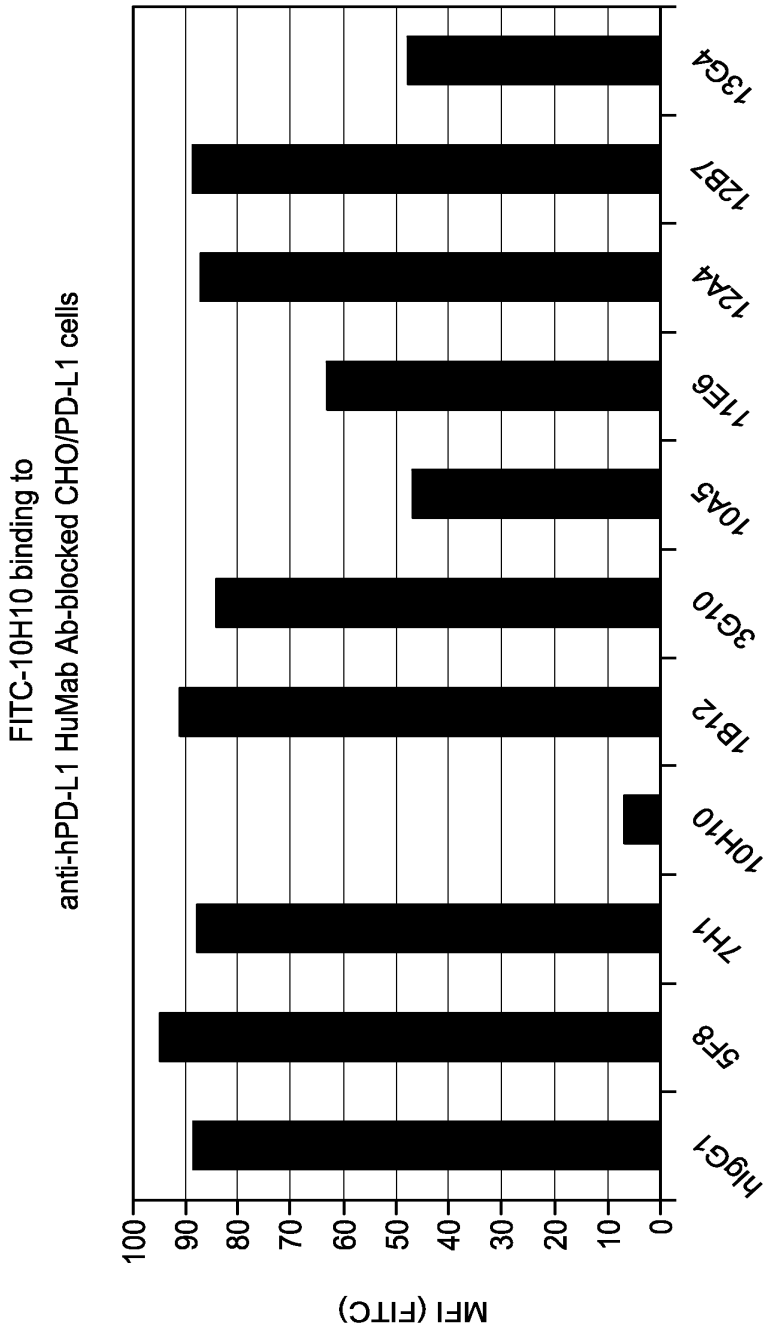

FITC-10H10 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
100
90
80
70
60
50
40
30
20
10
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2A

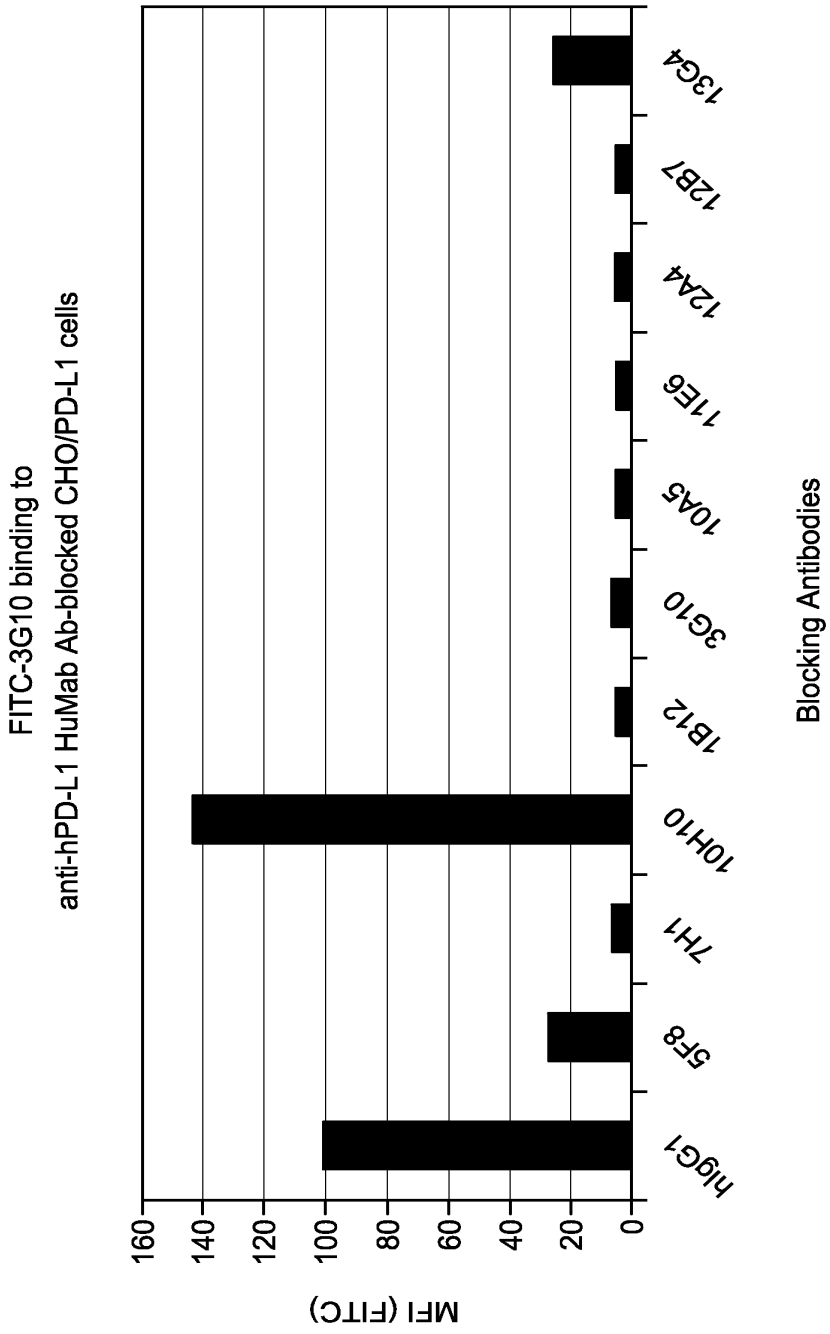

FITC-3G10 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
0
20
40
60
80
100
120
140
160
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2B

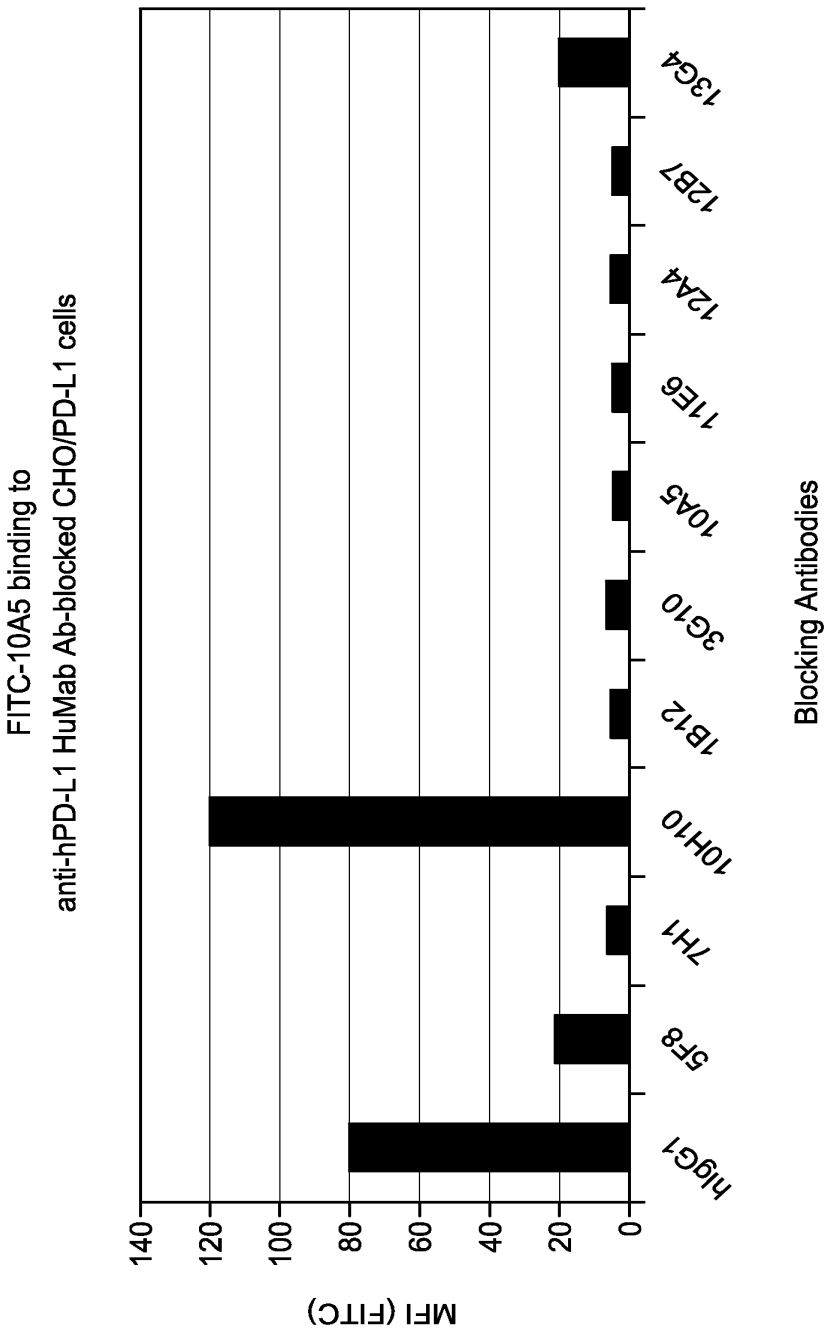

FITC-10A5 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
0
20
40
60
80
100
120
140
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2C

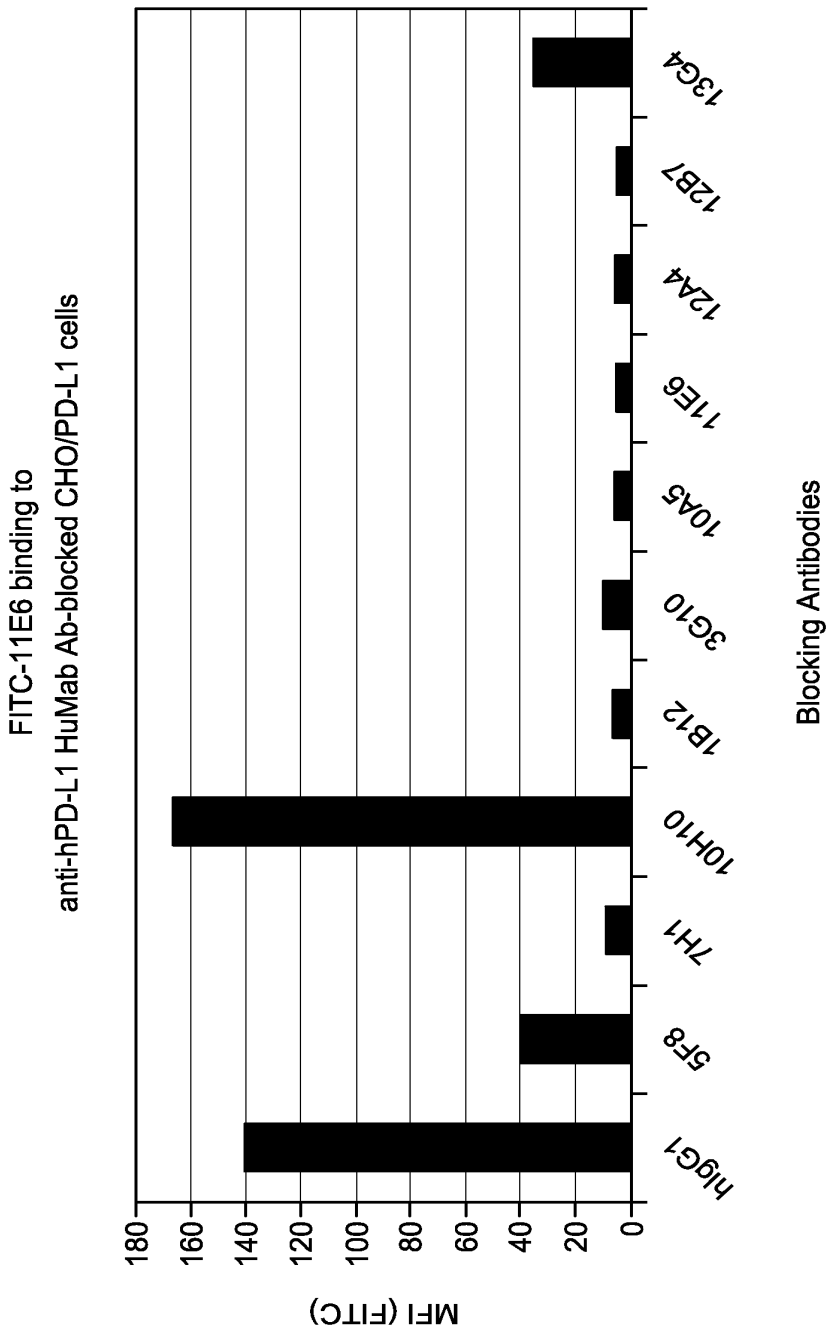

FITC-11E6 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
0
20
40
60
80
100
120
140
160
180
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2D

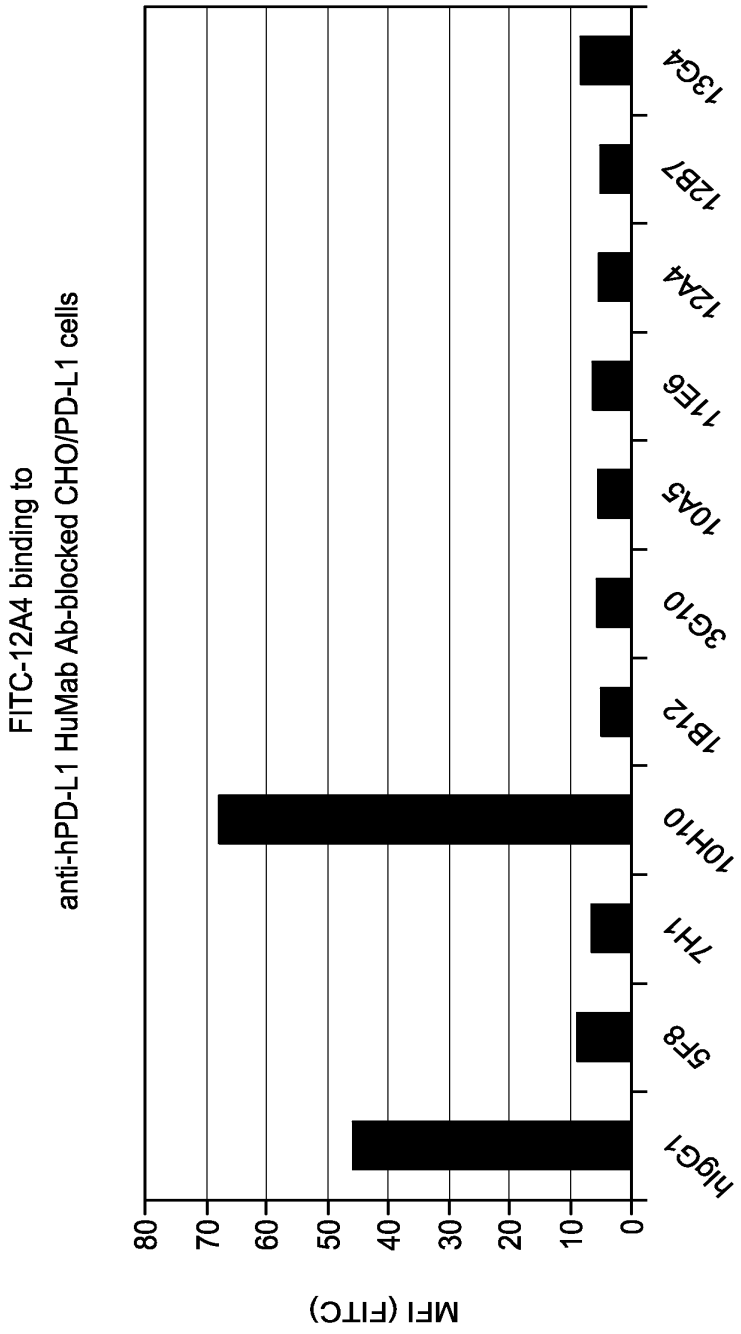

FITC-12A4 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
0
10
20
30
40
50
60
70
80
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2E

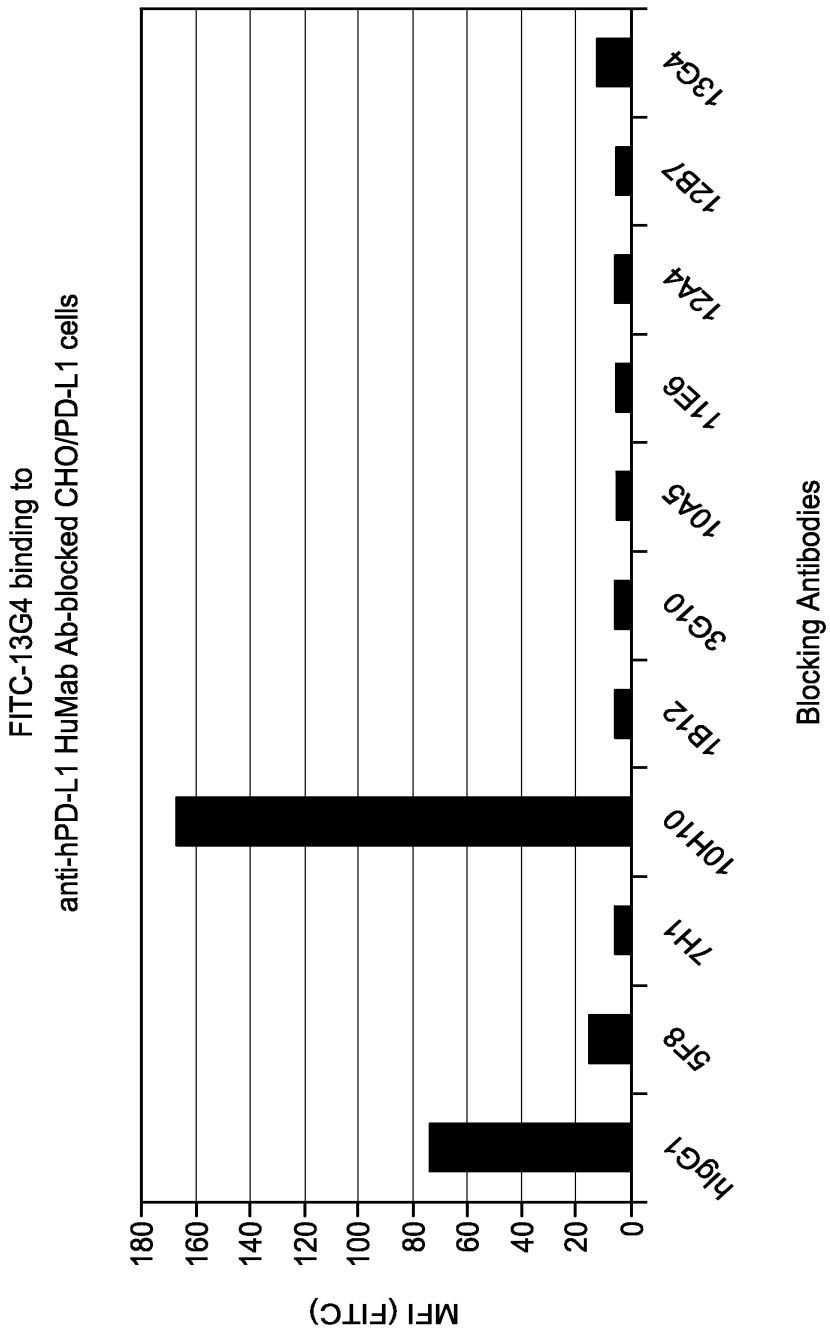

FITC-13G4 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
0
20
40
60
80
100
120
140
160
180
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2F

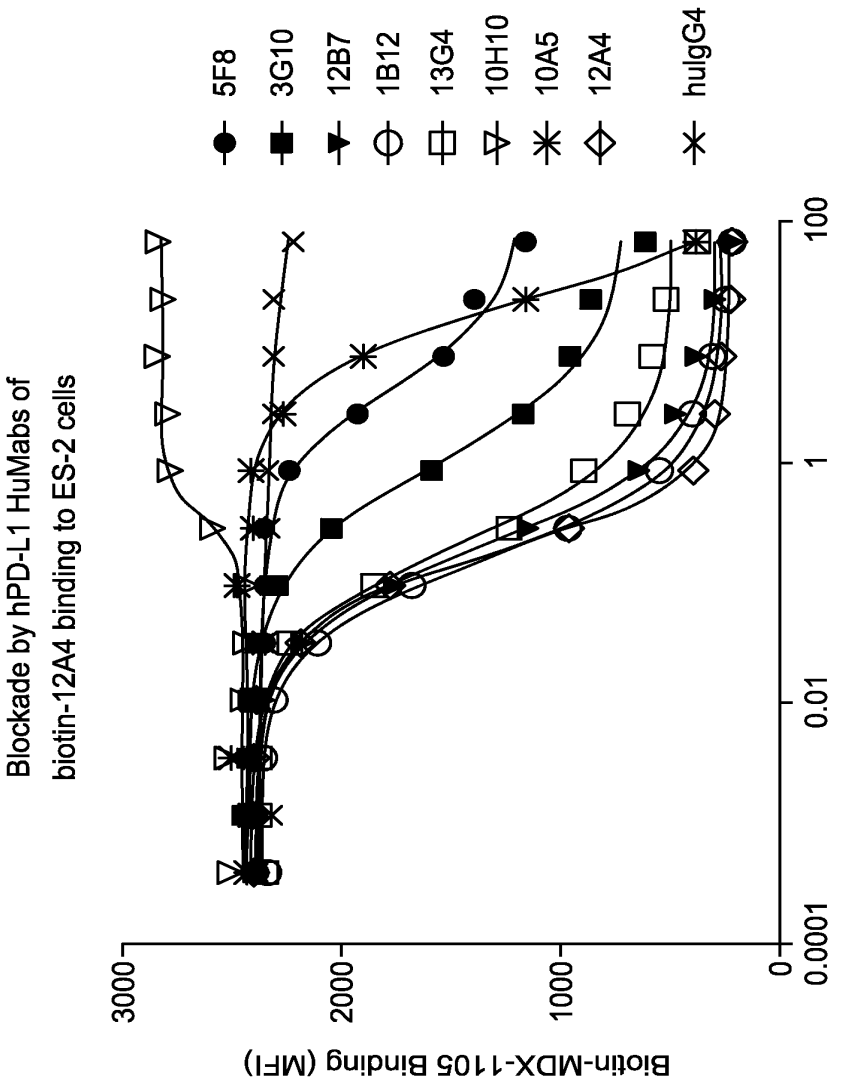

Blockade by hPD-L1 HuMabs of
biotin-12A4 binding to ES-2 cells
Biotin-MDX-1105 Binding (MFI)
3000
2000
1000
0
0.0001
0.01
1
100
Unconjugated Ab (nM)
5F8
3G10
12B7
1B12
13G4
10H10
10A5
12A4
hulgG4

FIG. 3

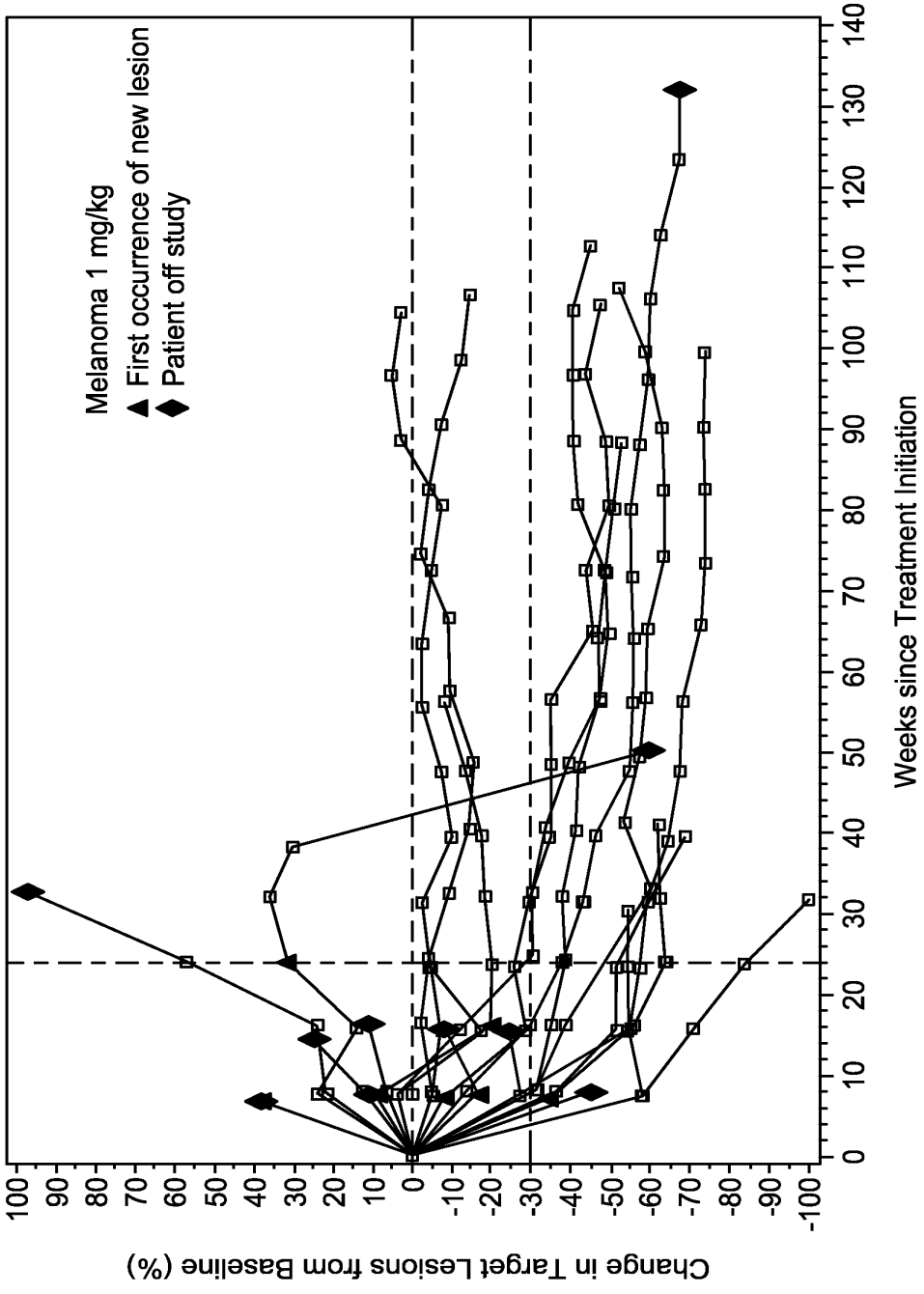

Melanoma 1 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
Weeks since Treatment Initiation
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140

FIG. 4

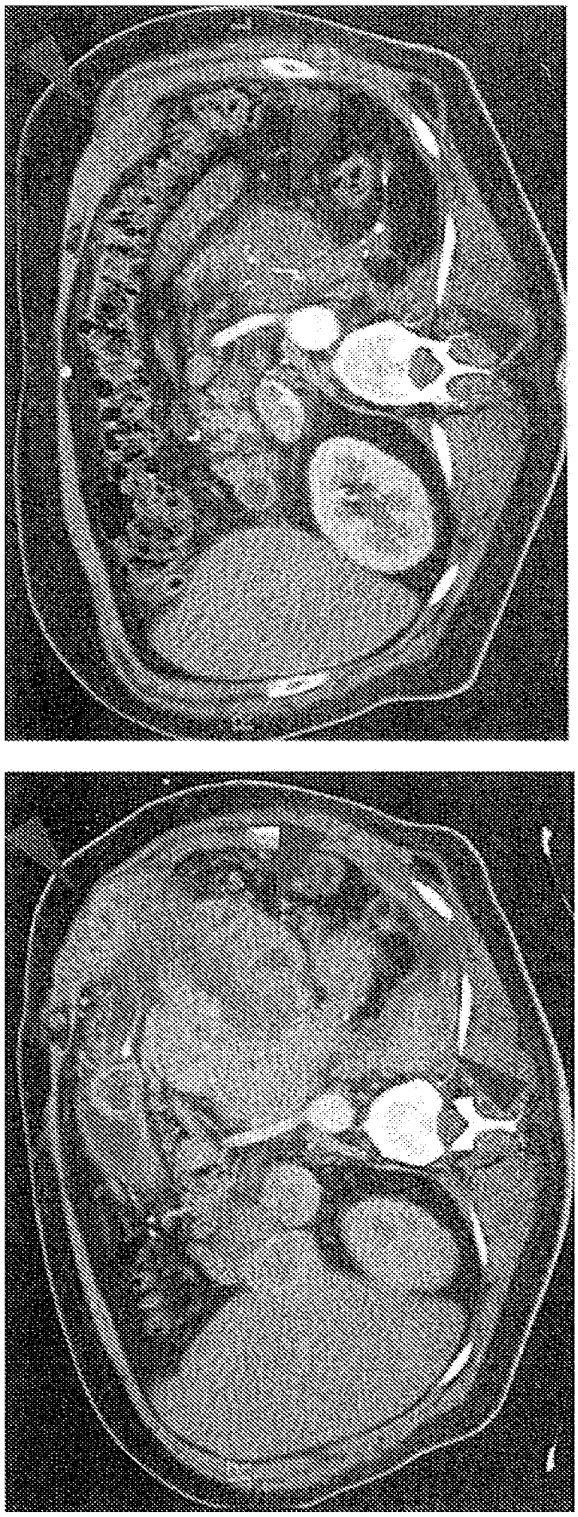


FIG. 5

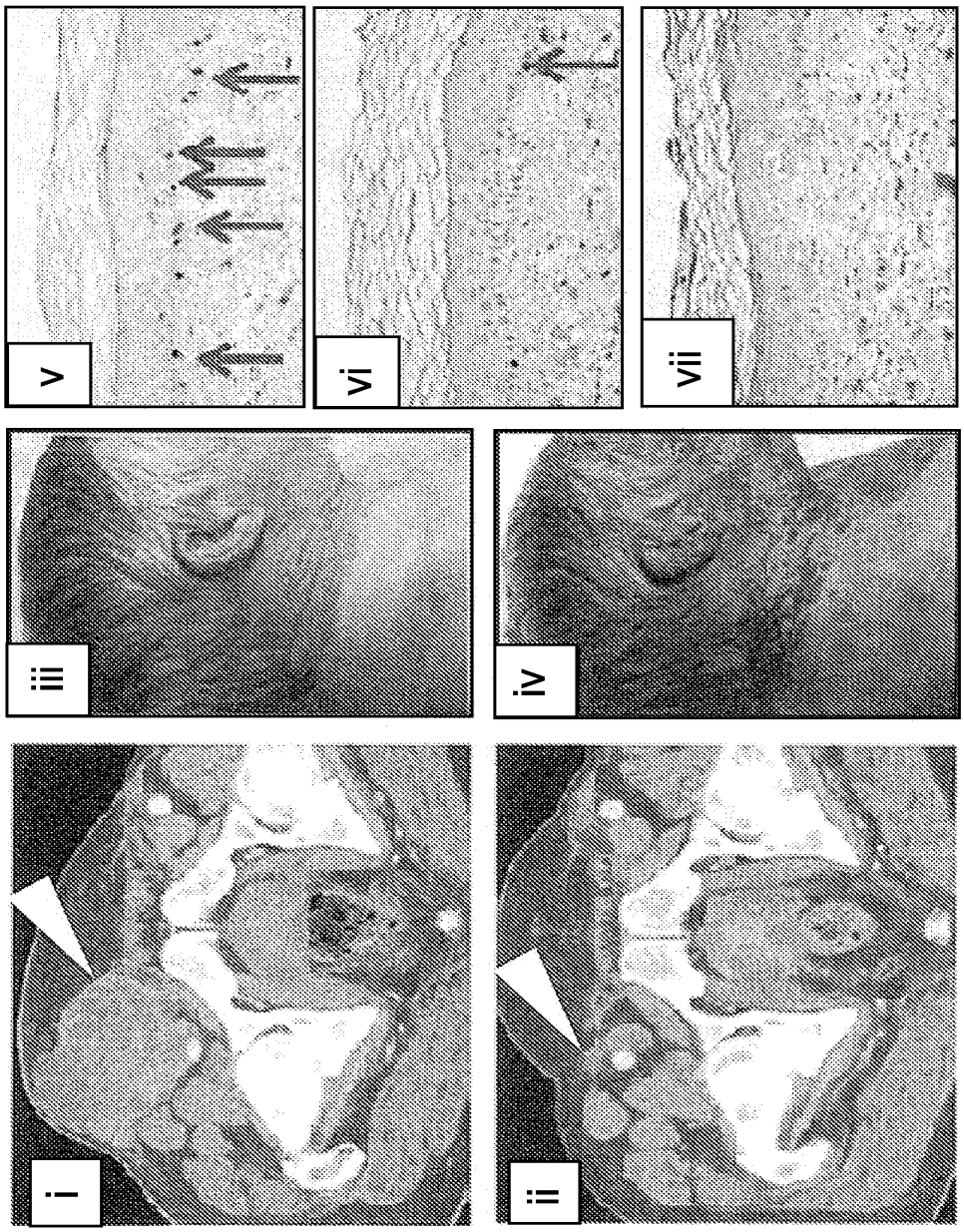


FIG. 6

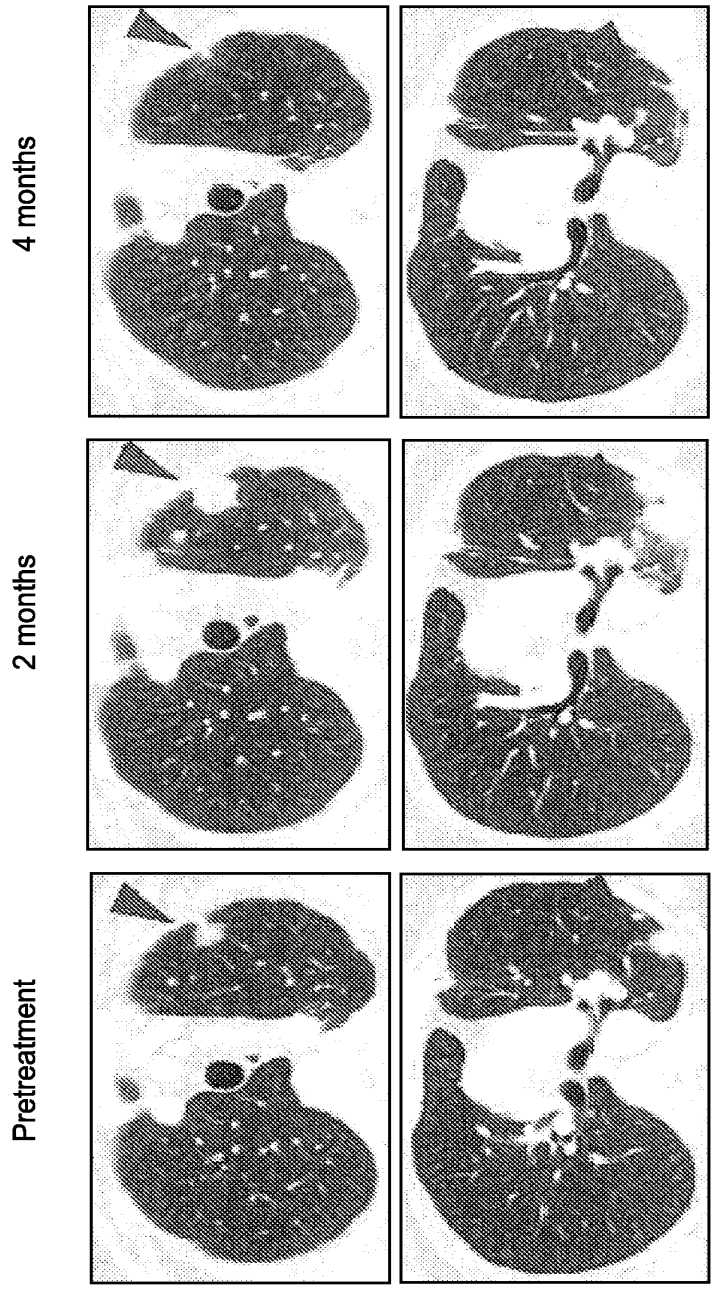
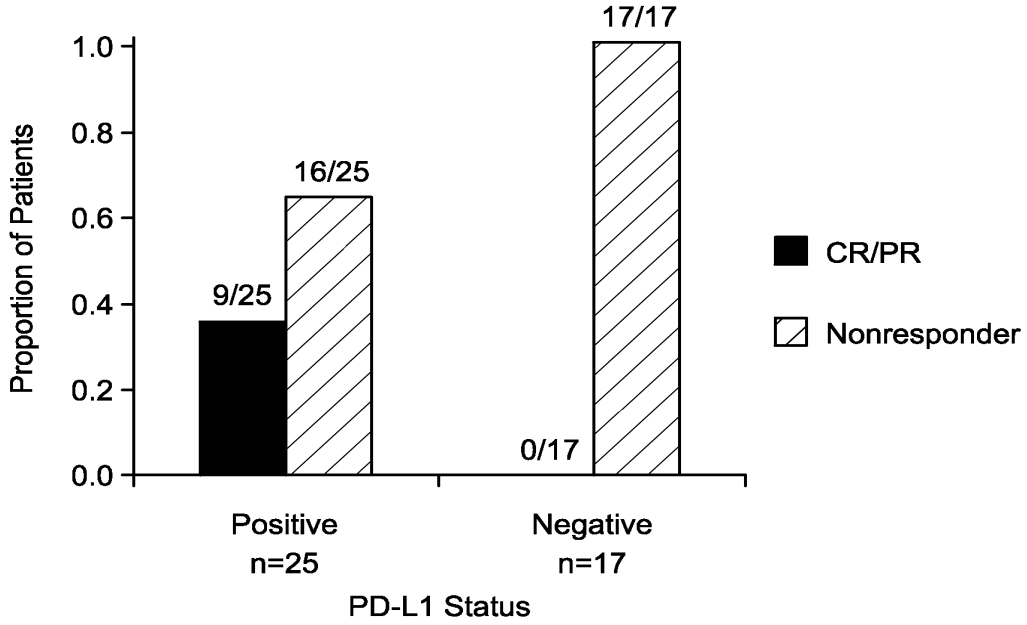


FIG. 7



Association Between Pretreatment Tumor PD-L1 Expression and Clinical Response			
Response Status	PD-L1-Positive no. (%)	PD-L1-Negative no. (%)	Total no. (%)
CR/PR	9(36)	0	9(21)
Nonresponder	16*(64)	17(100)	33(79)
All Patients	25	17	42

Fisher's exact test for association P=0.006.

*Two patients categorized as nonresponders at the time of data analysis are still under evaluation.

FIG. 8A

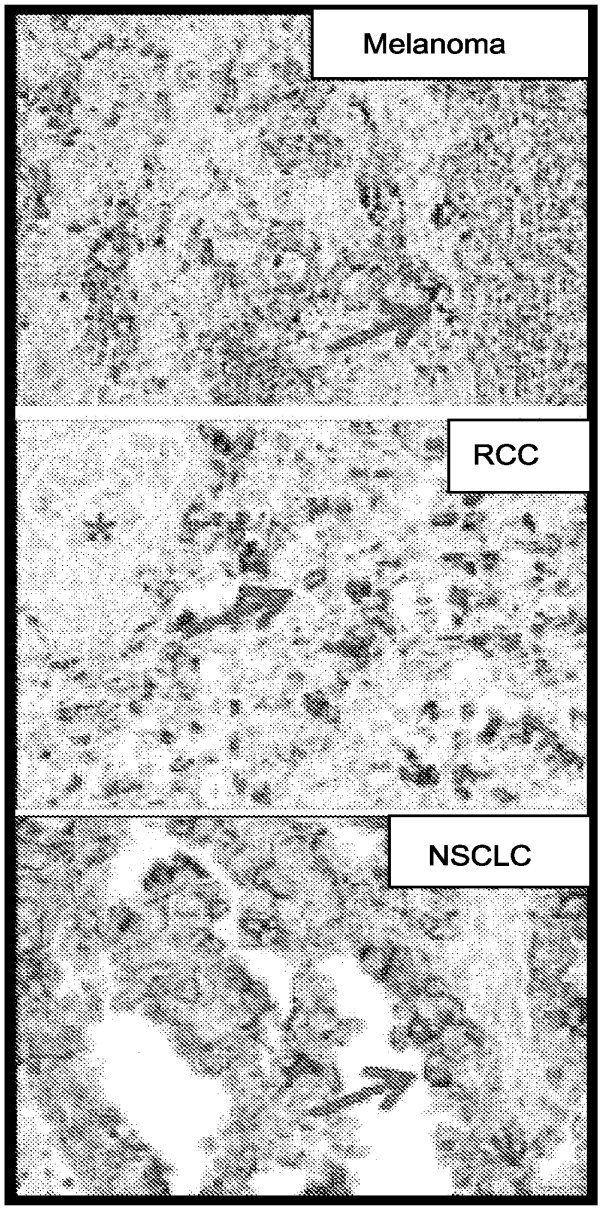


FIG. 8B

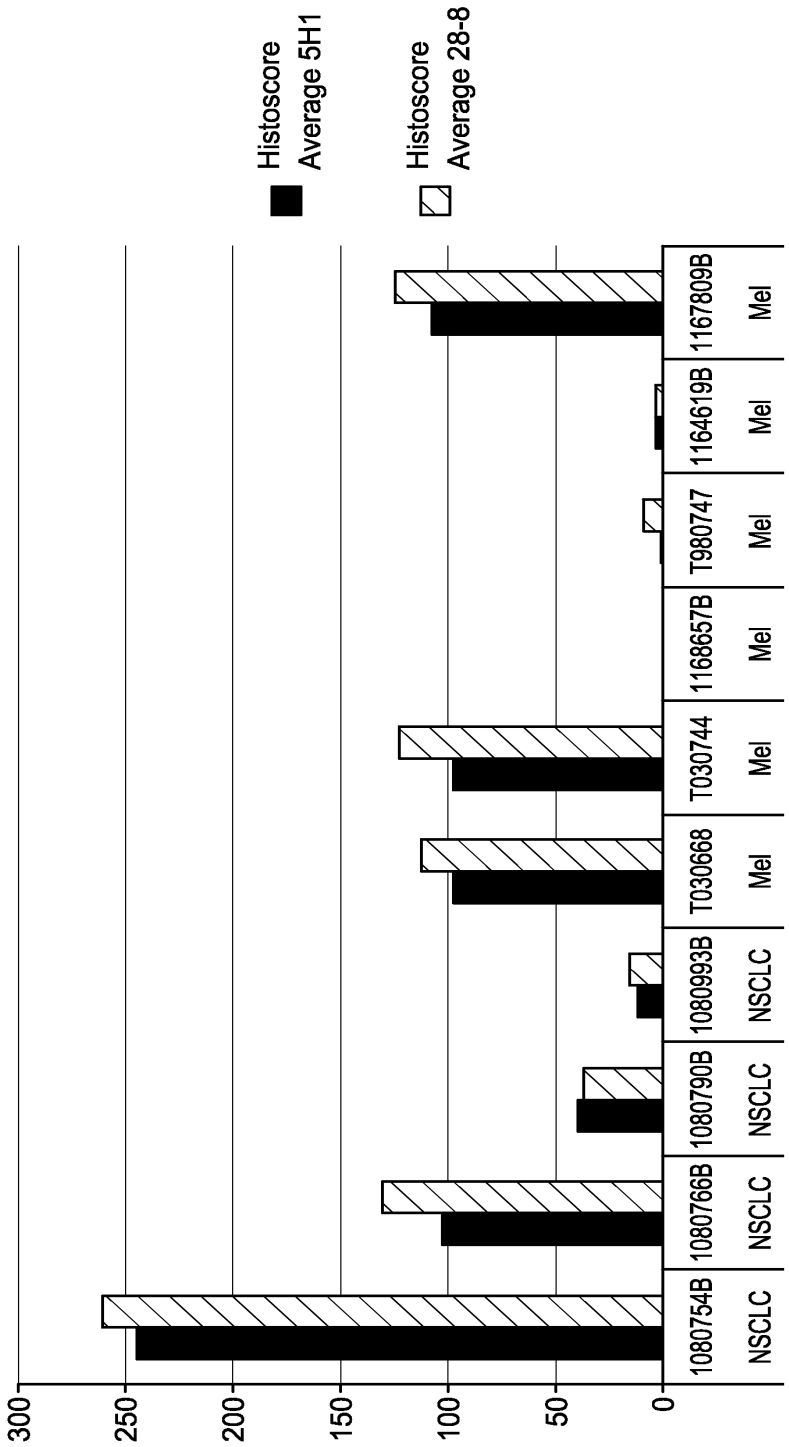


FIG. 9

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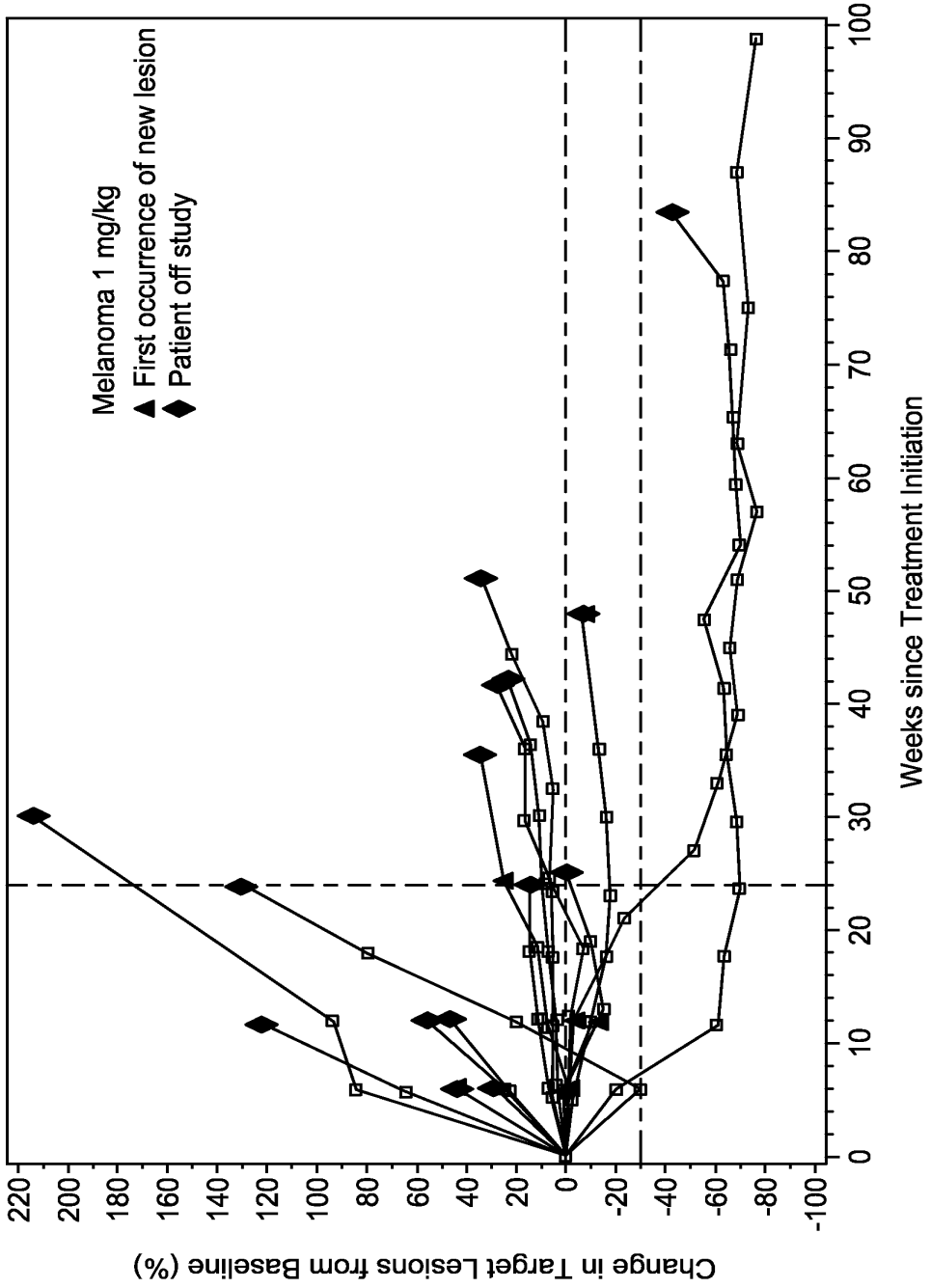

Melanoma 1 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
Weeks since Treatment Initiation
220
200
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100

FIG. 10A

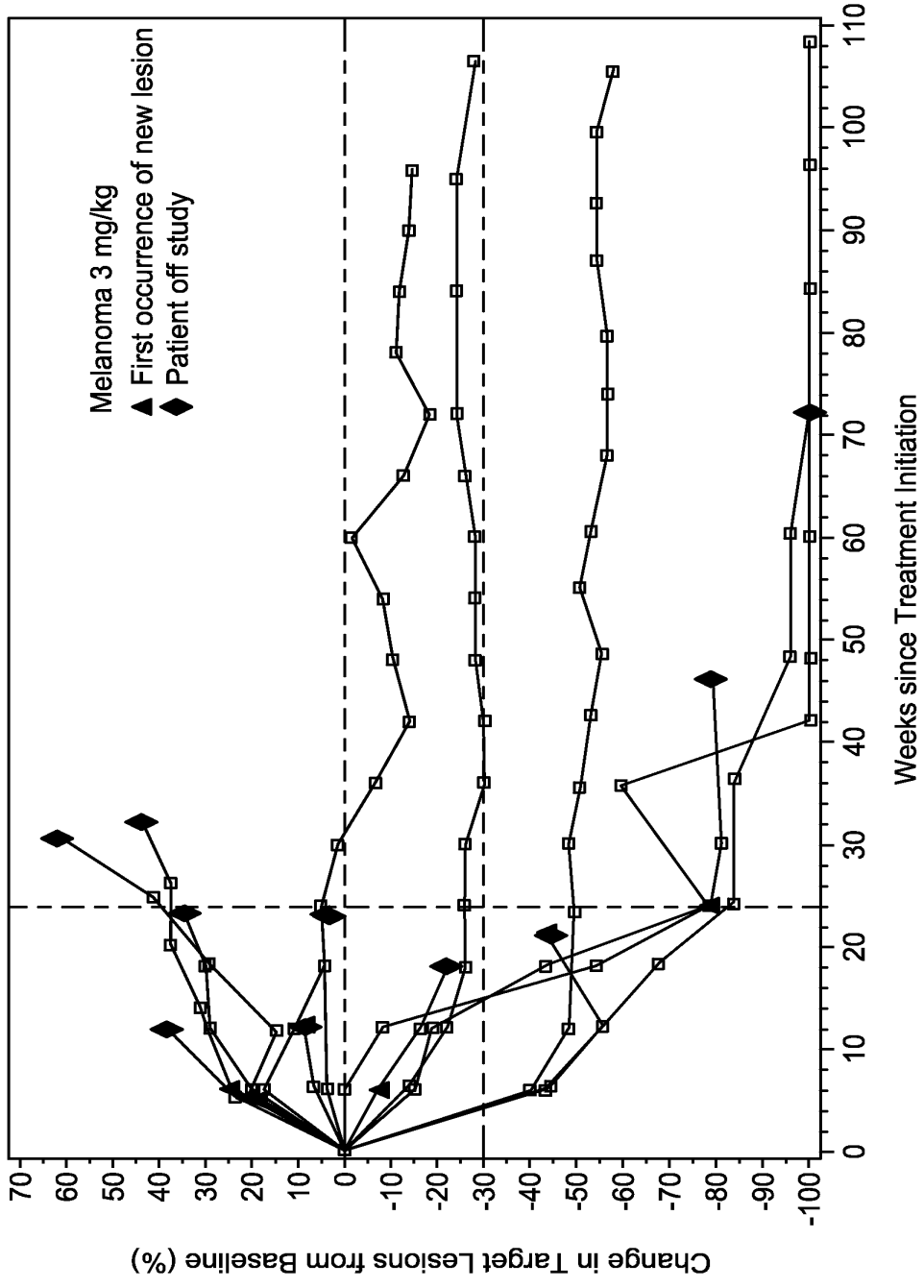

Melanoma 3 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
10
20
30
40
50
60
70
80
90
100
110
Weeks since Treatment Initiation

FIG. 10B

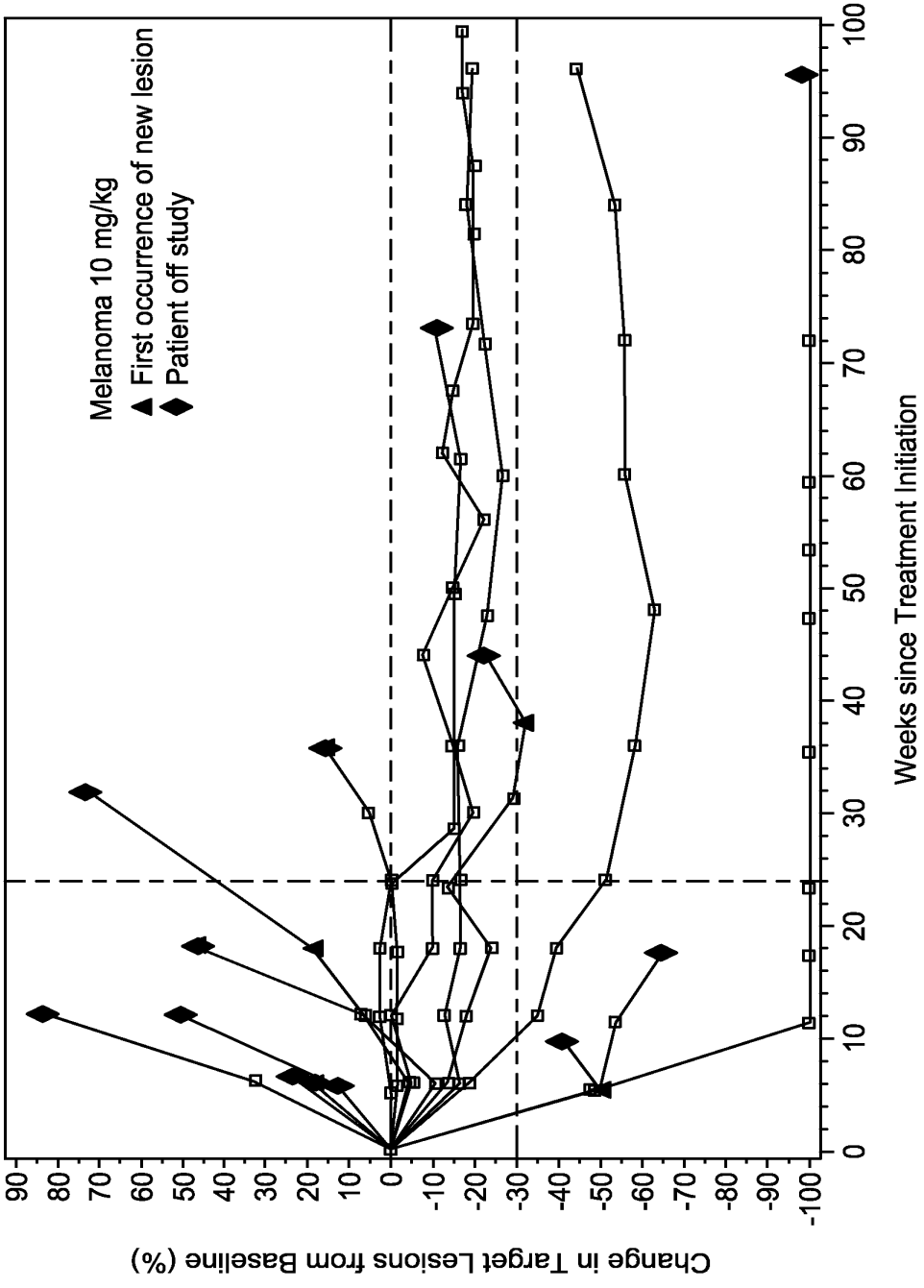

Melanoma 10 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
Weeks since Treatment Initiation
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
10
20
30
40
50
60
70
80
90
100

FIG. 10C

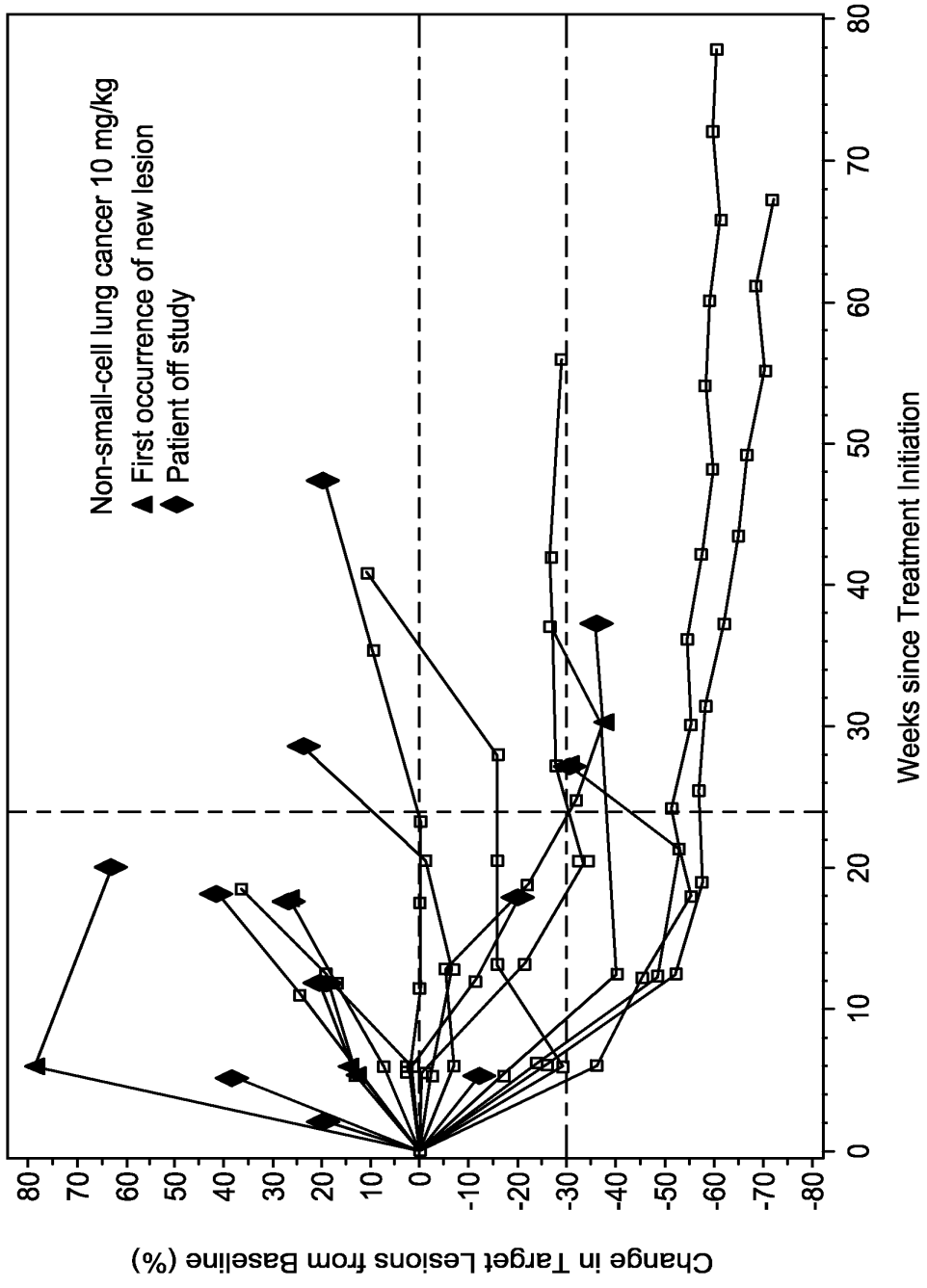

Non-small-cell lung cancer 10 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
Weeks since Treatment Initiation
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
0
10
20
30
40
50
60
70
80

FIG. 10D

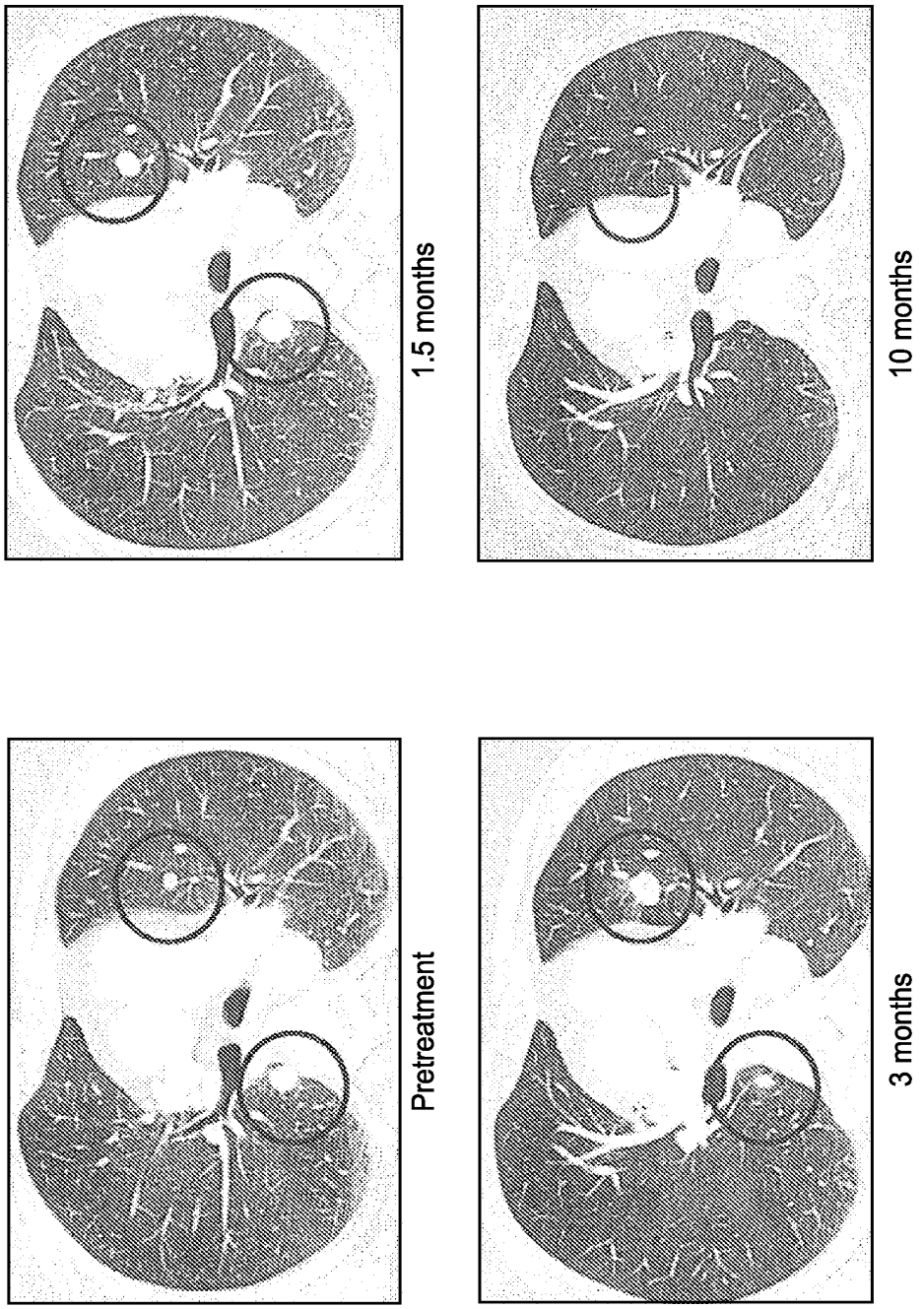


FIG. 11

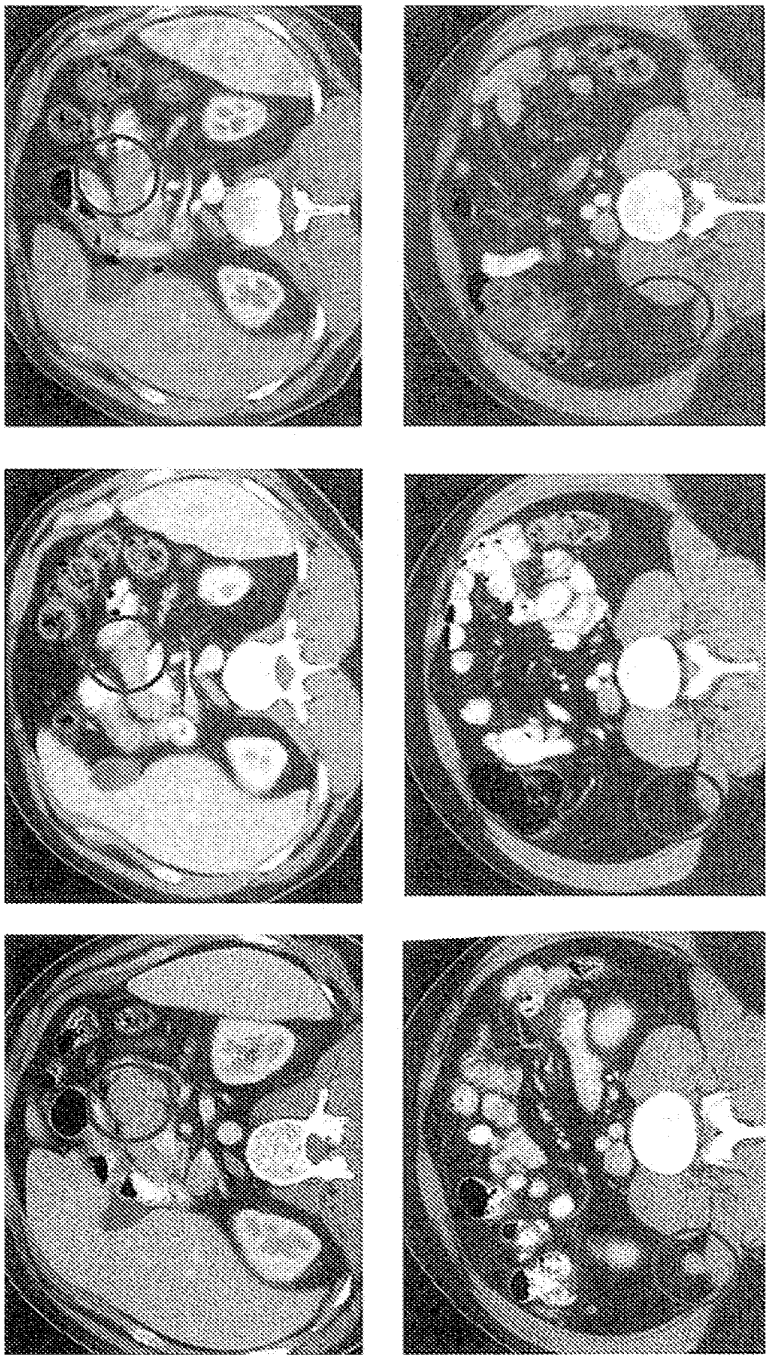


FIG. 12

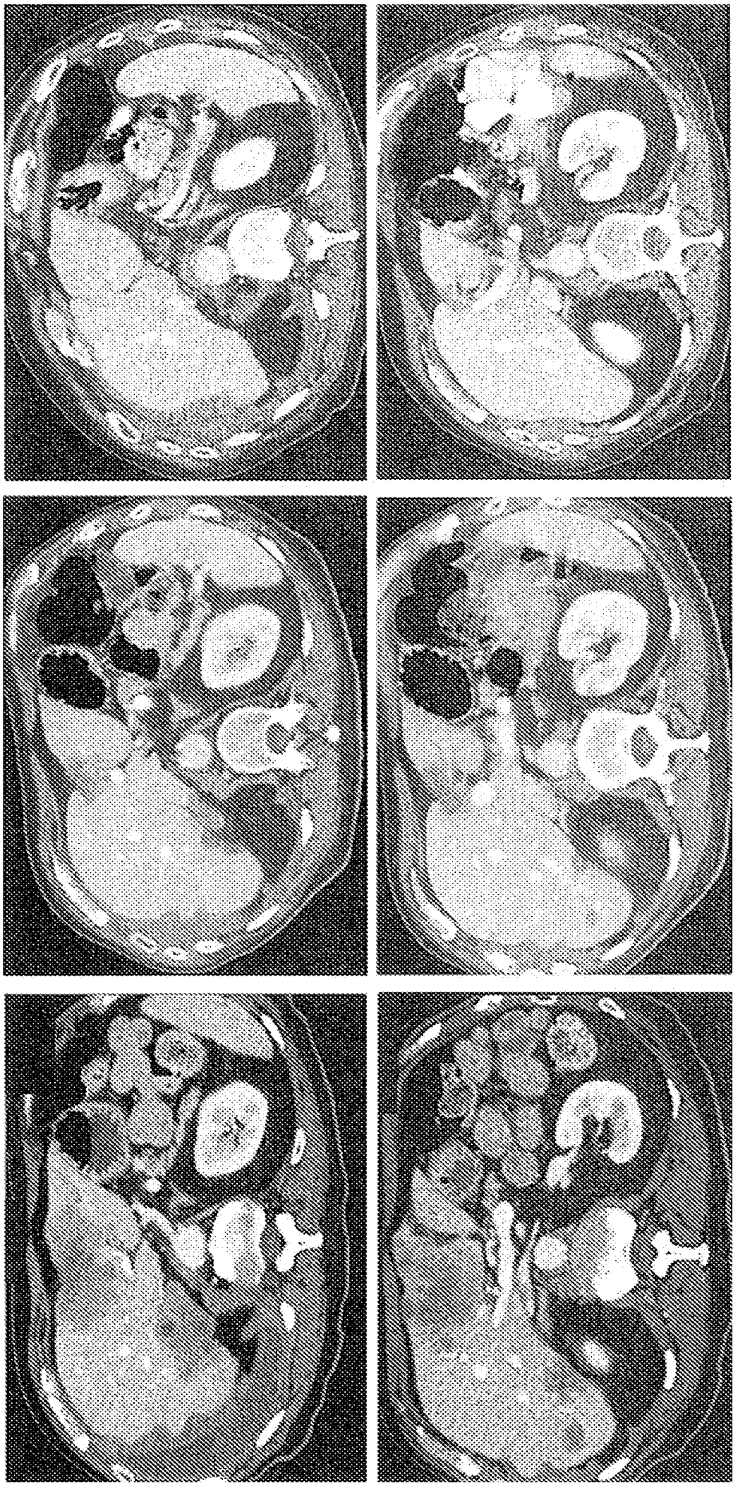


FIG. 13

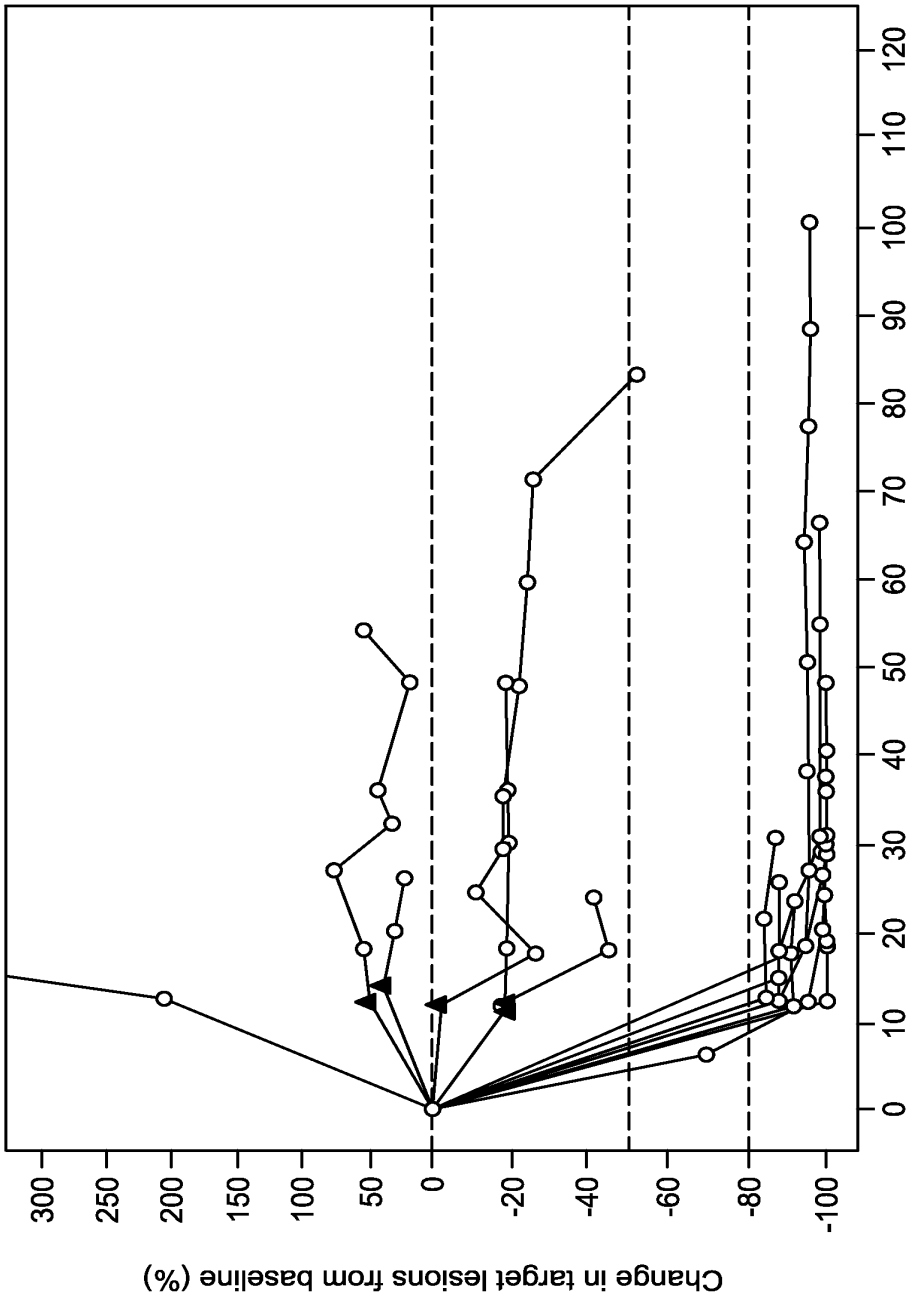

Change in target lesions from baseline (%)
300
250
200
150
100
50
0
-20
-40
-60
-80
-100
Weeks since treatment initiation
0
10
20
30
40
50
60
70
80
90
100
110
120

FIG. 14A

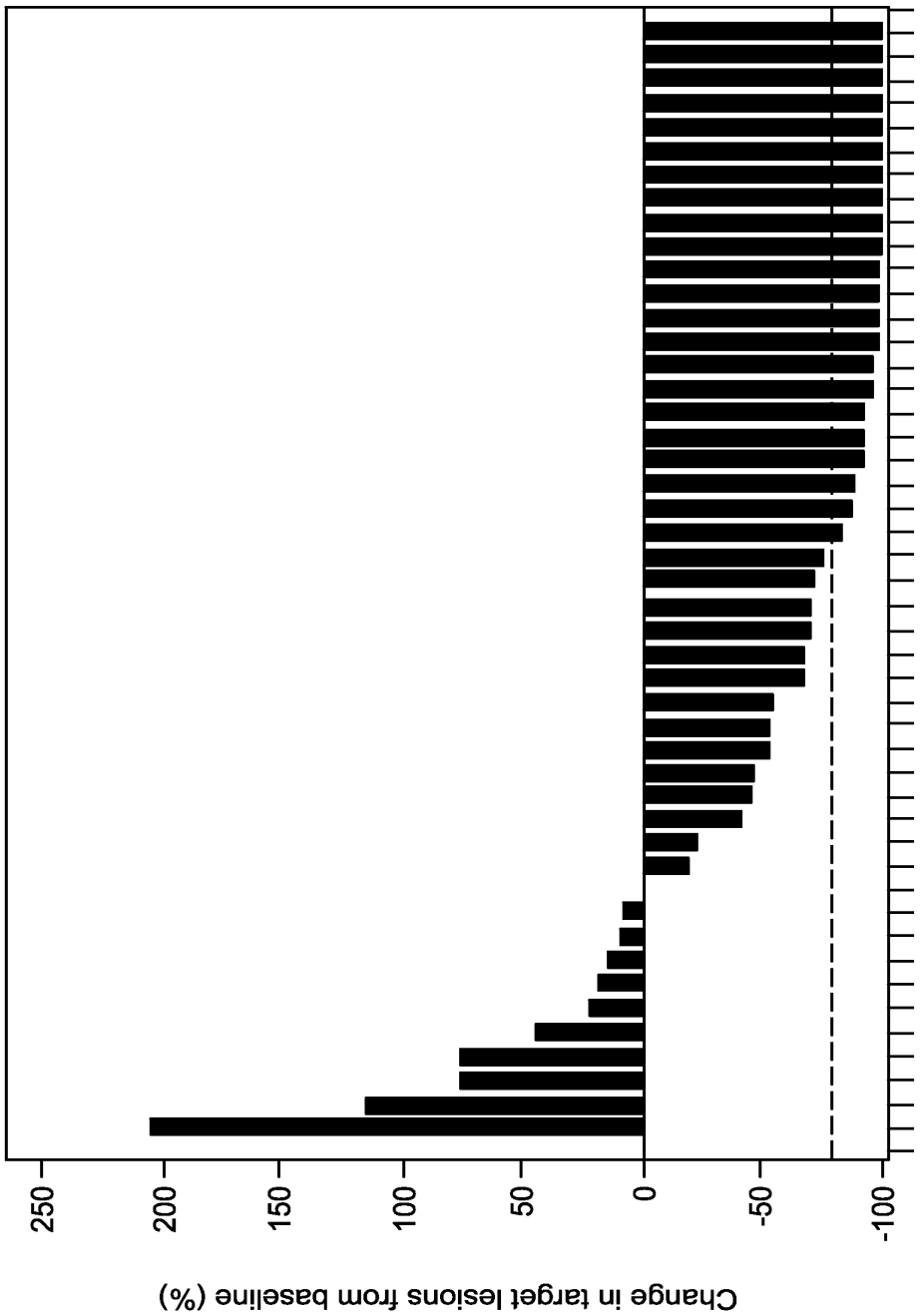

250
200
150
100
50
0
-50
-100
Change in target lesions from baseline (%)
Patients

FIG. 14B

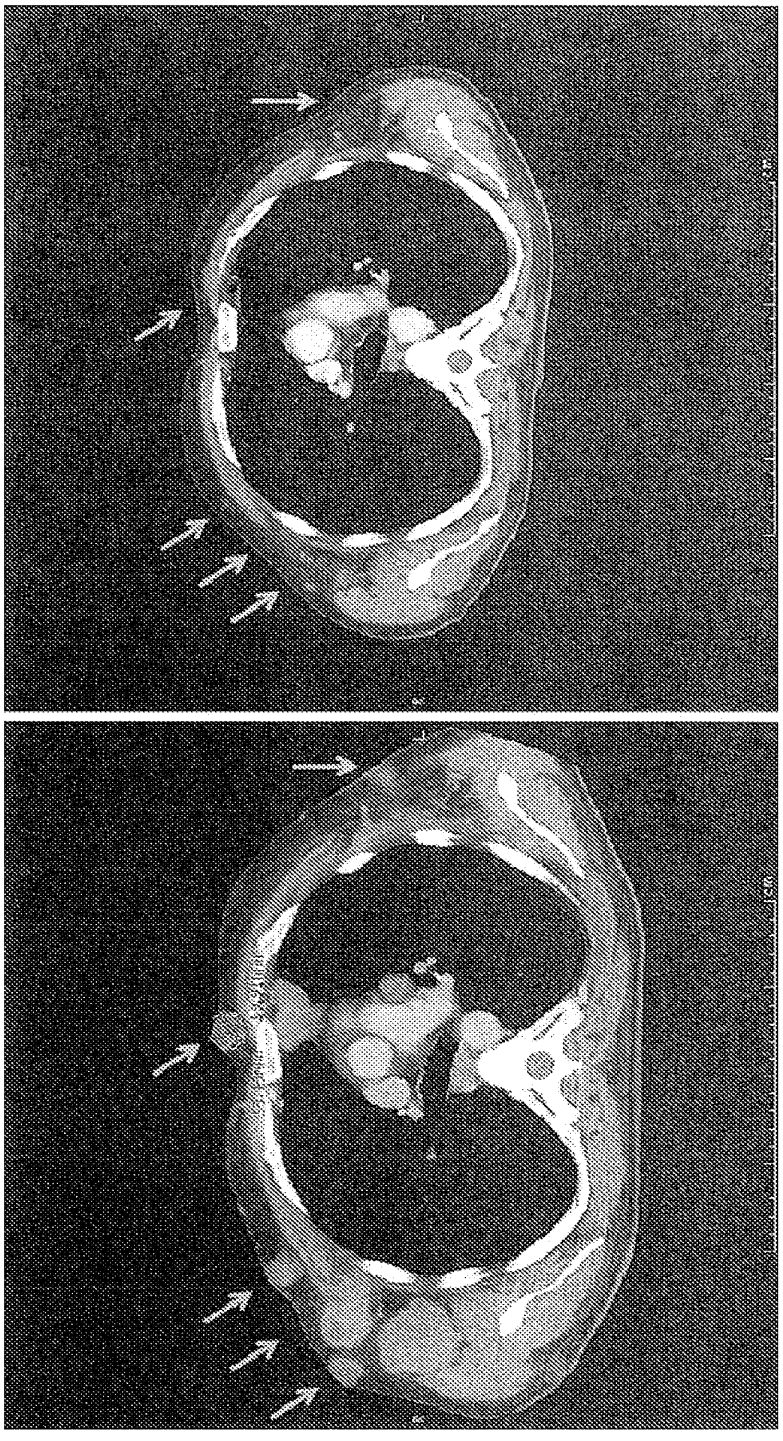


FIG. 15

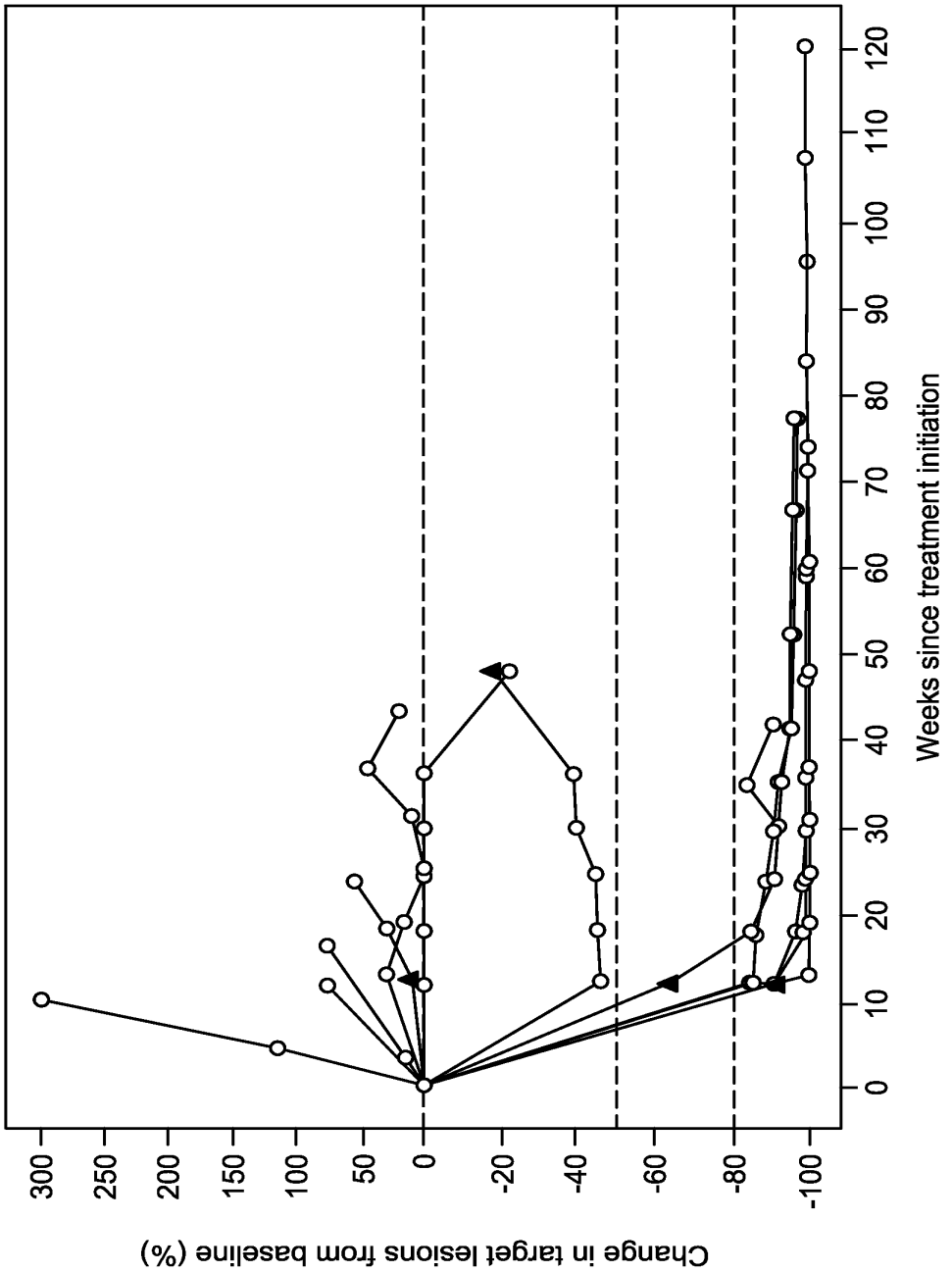

Change in target lesions from baseline (%)
300
250
200
150
100
50
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
Weeks since treatment initiation

FIG. 16A

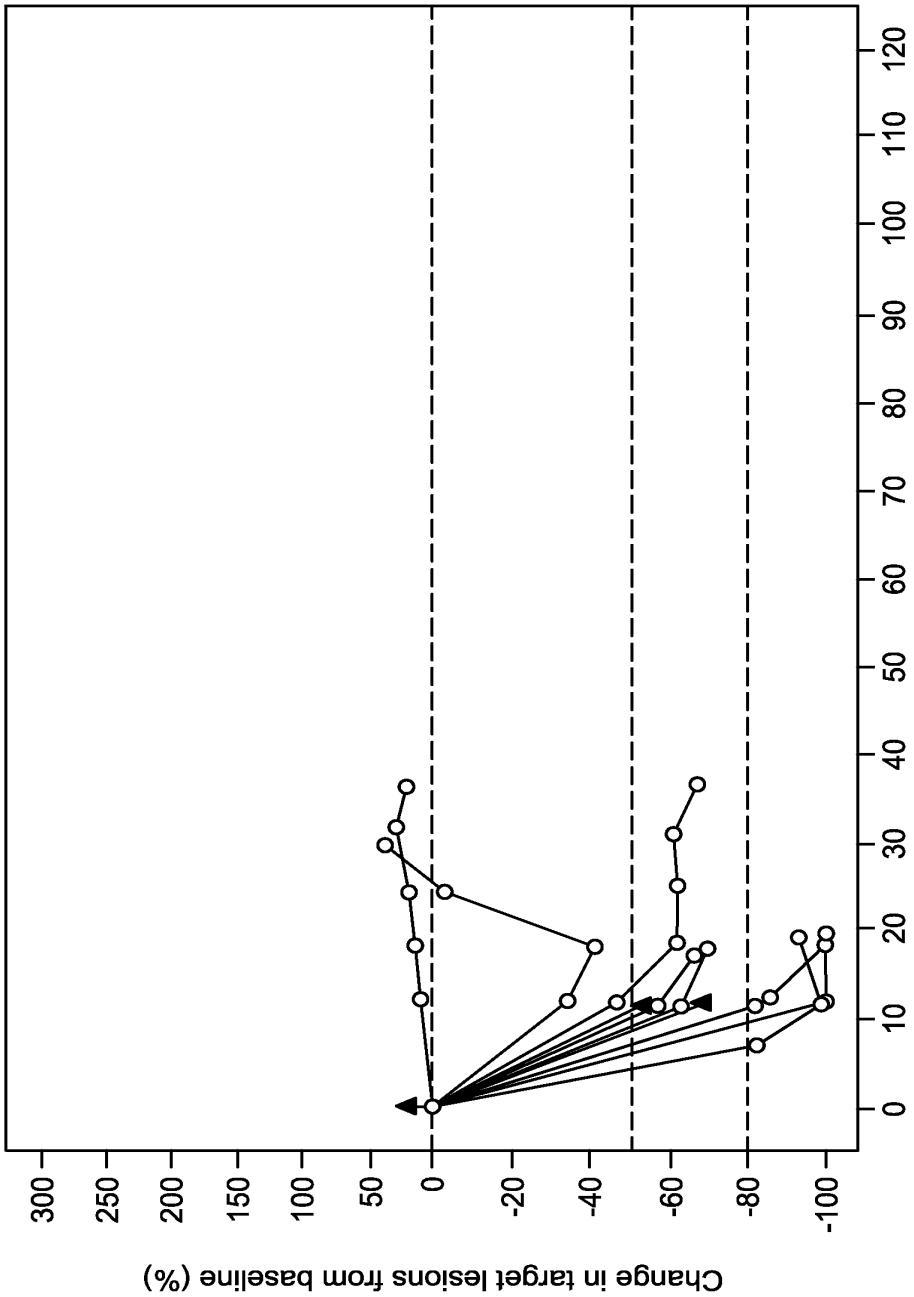

Change in target lesions from baseline (%)
300
250
200
150
100
50
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
Weeks since treatment initiation

FIG. 16B

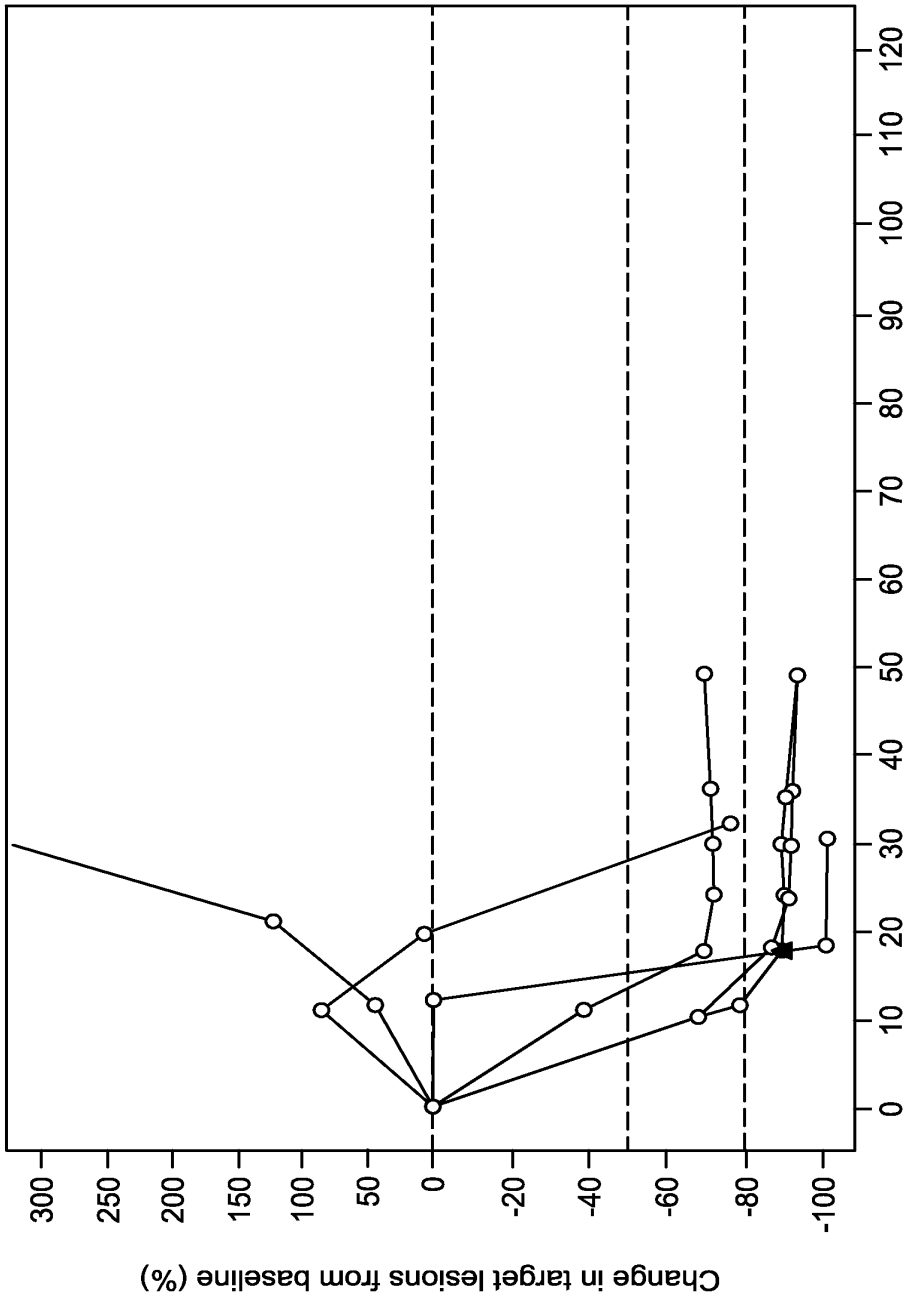

Change in target lesions from baseline (%)
300
250
200
150
100
50
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
Weeks since treatment initiation

FIG. 16C

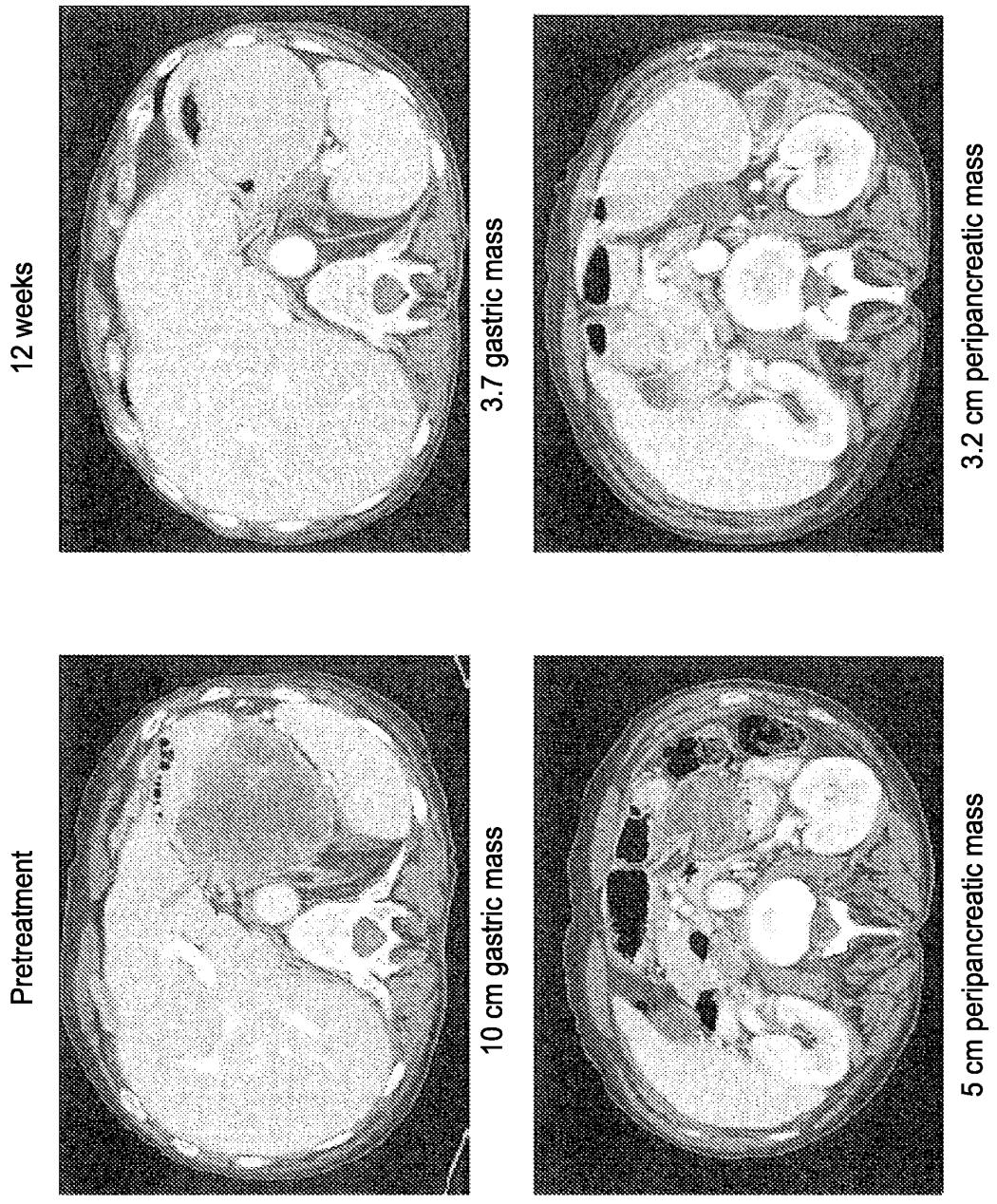

12 weeks
3.7 gastric mass
3.2 cm peripancreatic mass
Pretreatment
10 cm gastric mass
5 cm peripancreatic mass

FIG. 17

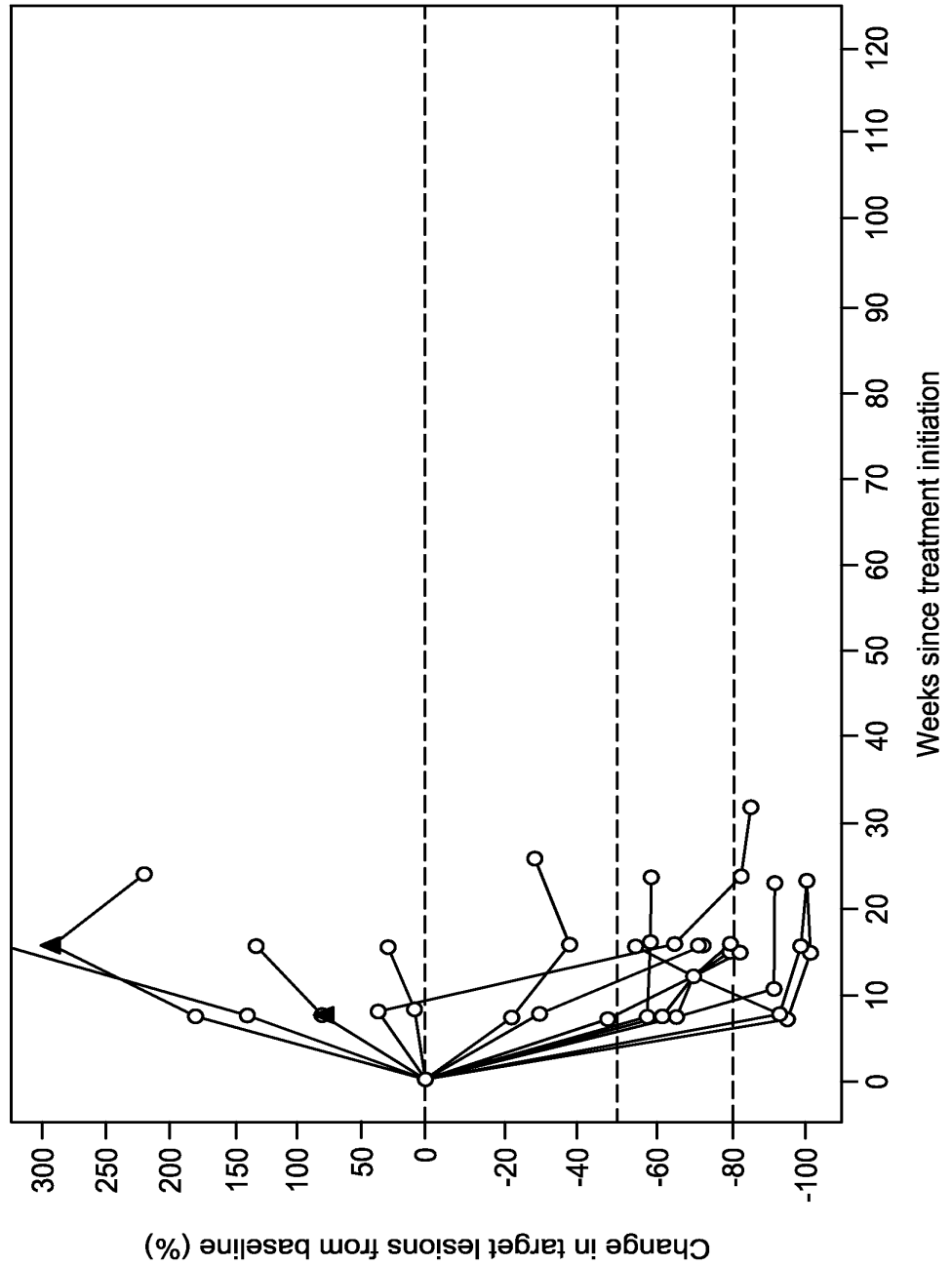

Change in target lesions from baseline (%)
300
250
200
150
100
50
0
-20
-40
-60
-80
-100
Weeks since treatment initiation
0
10
20
30
40
50
60
70
80
90
100
110
120

FIG. 18A

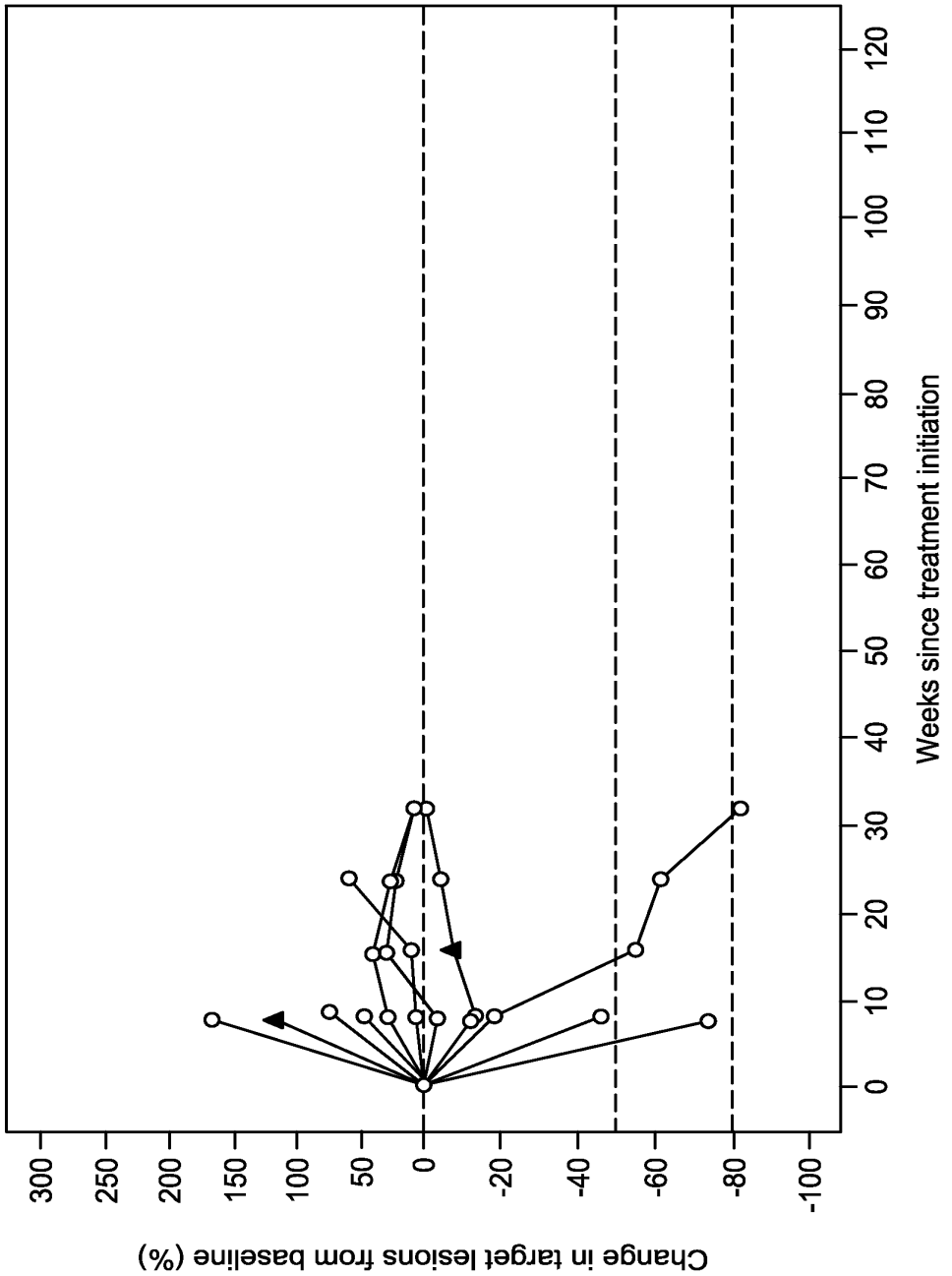

Change in target lesions from baseline (%)
300
250
200
150
100
50
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
Weeks since treatment initiation

FIG. 18B

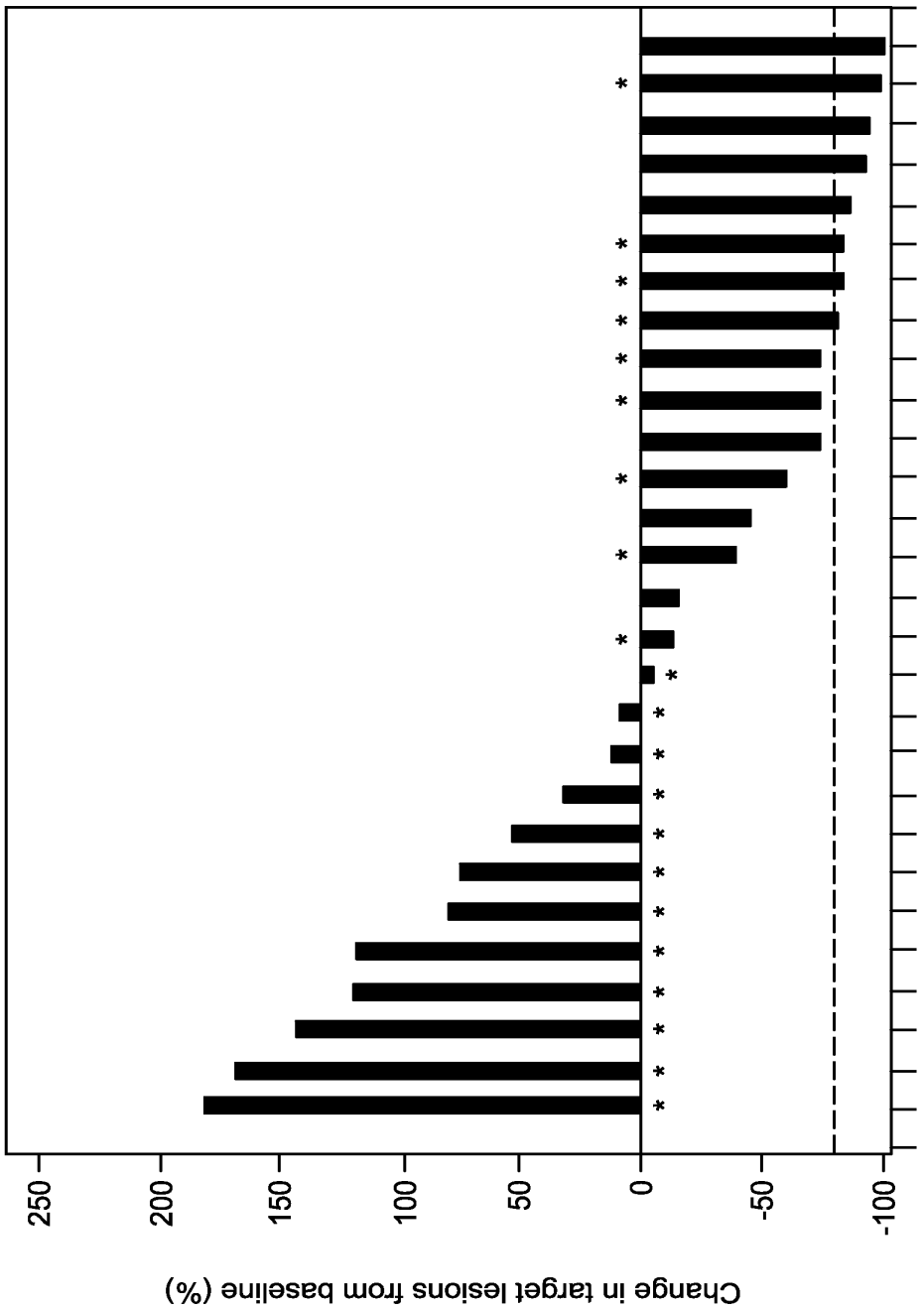

Change in target lesions from baseline (%)
250
200
150
100
50
0
-50
-100
Patients

FIG. 18C

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92*bis*.1 and Administrative Instructions, Section 422)

To:

DELAUNEY, Ashton J.
Bristol-Myers Squibb Company
P.O. Box 4000
Princeton, New Jersey 08543-4000
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)*
12 November 2014 (12.11.2014)

Applicant's or agent's file reference
11981-WO-PCT

IMPORTANT NOTIFICATION

International application No.
PCT/US2013/040764

International filing date *(day/month/year)*
13 May 2013 (13.05.2013)

1. The following indications appeared on record concerning:

☐ the applicant ☒ the inventor ☐ the agent ☐ the common representative

Name and Address
GUPTA, Ashok K.
c/o Bristol-Myers Squibb Company
Route 206 and Province Line Road
Princeton, New Jersey 08543
United States of America

State of Nationality | State of Residence

Telephone No.

Facsimile No.

E-mail address

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address
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Clarksburg, MD 20871
United States of America

State of Nationality | State of Residence

Telephone No.

Facsimile No.

E-mail address
☐ Notifications by e-mail authorized

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office
☐ the International Searching Authority
☐ the Authority(ies) specified for supplementary search
☐ the International Preliminary Examining Authority
☒ the designated Offices concerned
☐ the elected Offices concerned
☐ other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. +41 22 338 71 30

Authorized officer
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e-mail pt09.pct@wipo.int
Telephone No. +41 22 338 74 09

Form PCT/IB/306 (January 2009) 1/GDYVQ6GK4JJKY0

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92*bis*.1 and Administrative Instructions, Section 422)

To:

DELAUNEY, Ashton J.
Bristol-Myers Squibb Company
P.O. Box 4000
Princeton, New Jersey 08543-4000
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)*
12 November 2014 (12.11.2014)

Applicant's or agent's file reference
11981-WO-PCT

IMPORTANT NOTIFICATION

International application No.
PCT/US2013/040764

International filing date *(day/month/year)*
13 May 2013 (13.05.2013)

1. The following indications appeared on record concerning:

☐ the applicant ☒ the inventor ☐ the agent ☐ the common representative

Name and Address
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State of Nationality | State of Residence

Telephone No.

Facsimile No.

E-mail address

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

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United States of America

State of Nationality | State of Residence

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Facsimile No.

E-mail address
☐ Notifications by e-mail authorized

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office
☐ the International Searching Authority
☐ the Authority(ies) specified for supplementary search
☐ the International Preliminary Examining Authority
☒ the designated Offices concerned
☐ the elected Offices concerned
☐ other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. +41 22 338 71 30

Authorized officer
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Form PCT/IB/306 (January 2009) 1/SA6AMZ6JYZYUH0

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(19) World Intellectual Property Organization
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21 November 2013 (21.11.2013)

WIPO | PCT

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(25) Filing Language: English

(26) Publication Language: English

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61/790,747 15 March 2013 (15.03.2013) US

(71) Applicant: **BRISTOL-MYERS SQUIBB COMPANY** [US/US]; Route 206 and Province Line Road, Princeton, New Jersey 08543-4000 (US).

(72) Inventors: **COGSWELL, John P.**; c/o Bristol-Myers Squibb Company, Route 206 and Province Line Road, Princeton, New Jersey 08543 (US). **GOLDBERG, Stacie M.**; c/o Bristol-Myers Squibb Company, Route 206 and Province Line Road, Princeton, New Jersey 08543 (US). **GUPTA, Ashok K.**; c/o Bristol-Myers Squibb Company, Route 206 and Province Line Road, Princeton, New Jersey 08543 (US). **JURE-KUNKEL, Maria**; c/o Bristol-Myers Squibb Company, Route 206 and Province Line Road, Princeton, New Jersey 08543 (US). **WANG, Xi Tao**; c/o Bristol-Myers Squibb Company, Route 206 and Province Line Road, Princeton, New Jersey 08543 (US). **WIGGINTON, Jon M.**; c/o Bristol-Myers Squibb Company, Route 206 and Province Line Road, Princeton, New Jersey 08543 (US).

(74) Agents: **DELAUNEY, Ashton J.** et al.; Bristol-Myers Squibb Company, P.O. Box 4000, Princeton, New Jersey 08543-4000 (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, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, 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, 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, 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, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

[Continued on next page]

(54) Title: CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING

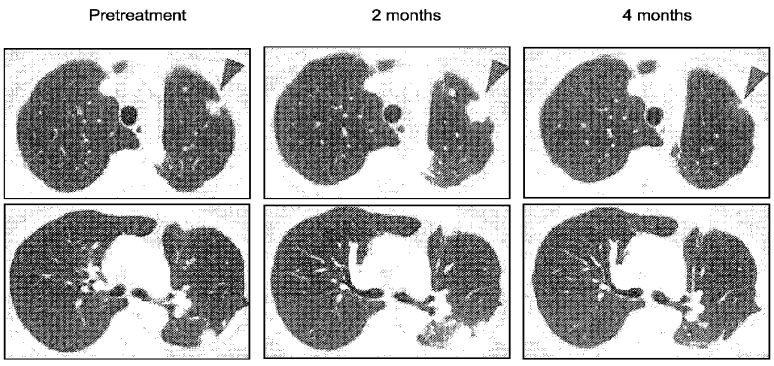


FIG. 7

(57) Abstract: The disclosure provides a method for immunotherapy of a cancer patient, comprises administering to the patient an Ab that inhibits signaling from the PD-1/PD-L1 signaling pathway, or a combination of such Ab and an anti-CTLA-4 Ab. This disclosure also provides a method for immunotherapy of a cancer patient comprising selecting a patient who is a suitable candidate for immunotherapy based on an assessment that the proportion of cells in a test tissue sample from the patient that express PD-L1 on the cell surface exceeds a predetermined threshold level, and administering an anti-PD-1 Ab to the selected subject. The disclosure additionally provides rabbit mAbs that bind specifically to a cell surface-expressed PD-L1 antigen in a FFPE tissue sample, and an automated IHC method for assessing cell surface expression in FFPE tissues using the provided anti-PD-L1 Abs.

WO 2013/173223 A1

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))*

— *with sequence listing part of description (Rule 5.2(a))*

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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/400,667	Filing Date 11/12/2014	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)		
FOR	NUMBER FILED	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 $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ 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	11/12/2014	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 22	Minus	** 22	= 0	X $80 =	0
	Independent (37 CFR 1.16(h))	* 5	Minus	***5	= 0	X $420 =	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 $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ 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.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** 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.

LIE
/RAMONA WILSON/

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.**

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