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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
61/647,442	05/15/2012		250	11981-US-PSP		

CONFIRMATION NO. 5263

CORRECTED FILING RECEIPT

OC000000064492306

23914
HENRY HADAD
BRISTOL-MYERS SQUIBB COMPANY
PATENT DEPARTMENT
P O BOX 4000
PRINCETON, NJ 08543-4000

Date Mailed: 10/24/2013

Receipt is acknowledged of this provisional patent application. It will not be examined for patentability and will become abandoned not later than twelve months after its filing date. 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;
Stacie M. Goldberg, Princeton, NJ;
Ashok K. Gupta, Plainsboro, NJ;
Maria Jure-Kunkel, Plainsboro, NJ;
Xi Tao Wang, Wellesley, MA;
Jon M. Wigginton, Collegeville, PA;

Applicant(s)

John P. Cogswell, Yardley, PA;
Stacie M. Goldberg, Princeton, NJ;
Ashok K. Gupta, Plainsboro, NJ;
Maria Jure-Kunkel, Plainsboro, NJ;
Xi Tao Wang, Wellesley, MA;
Jon M. Wigginton, Collegeville, PA;

Power of Attorney:
Ashton Delauney--64286

If Required, Foreign Filing License Granted: 06/15/2012

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 61/647,442**

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

Title

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

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications:

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.

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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).

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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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

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF **JOHN COGSWELL, ET AL**
FOR: **CANCER IMMUNOTHERAPY FOR DISRUPTING PD-1/PD-LI SIGNALING**
APPLICATION NO: **61/647,442** APPLICATION DATE: **15 May** 2012
ART UNIT: EXAMINER:
CONFIRMATION NO: 5263 USPTO CUSTOMER NO: **23914**

TRANSMITTED VIA EFS-WEB

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

LETTER CORRECTING OFFICIAL FILING RECEIPT
ACCOMPANIED BY APPLICATION DATA SHEET

Sir:

Please issue a corrected filing receipt listing the inventors as follows:

John P. Cogswell, Yardley, PA
Stacie M. Goldberg, Princeton, NJ
Ashok K. Gupta, Plainsboro, NJ
Maria Jure-Kunkel, Plainsboro, NJ
Xi Tao Wang, Wellesley, MA
Jon M. Wigginton, Collegeville, PA

A copy of the filing receipt with the correction noted is enclosed. Also enclosed is an Application Data Sheet with the correction underlined. Applicant respectfully requests that the Application Data Sheet be entered into the file.

Applicant believes that no fee is due, if however any additional fees in association with this request are due, please charge to Deposit Account No. 19-3880 in the name of Bristol-Myers Squibb Company.

Respectfully submitted,

A J Delauney

ASHTON J. DELAUNEY
Attorney for Applicant
Reg. No. 64,286
Phone: 609-252-4560
Date: October 17, 2013

Bristol-Myers Squibb Company
Patent Department
P.O. Box 4000
Princeton, NJ 08543-4000

UNITED STATES PATENT AND TRADEMARK OFFICE

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CONFIRMATION NO. 5263

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23914
HENRY HADAD
BRISTOL-MYERS SQUIBB COMPANY
PATENT DEPARTMENT
P O BOX 4000
PRINCETON, NJ 08543-4000

Date Mailed: 05/23/2013

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Inventor(s)

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Stacie M. Goldberg, Princeton, NJ;
Ashok K. Gupta, Plainsboro, NJ;
~~Maria-Jure Kunkel, Plainsboro, NJ;~~ Maria-Jure Kunkel, Plainsboro, NJ
Xi Tao Wang, Wellesley, MA;
Jon M. Wigginton, Collegeville, PA;

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Stacie M. Goldberg, Princeton, NJ;
Ashok K. Gupta, Plainsboro, NJ;
~~Maria-Jure Kunkel, Plainsboro, NJ;~~ Maria-Jure Kunkel, Plainsboro, NJ
Xi Tao Wang, Wellesley, MA;
Jon M. Wigginton, Collegeville, PA;

Power of Attorney:
Ashton Delauney--64286

If Required, Foreign Filing License Granted: 06/15/2012

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 61/647,442**

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

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	P.
Family Name	Cogswell
Residence City	Yardley
Residence State/Province	PA
Country of Residence	US
Mailing Address	Route 206 and Province Line Road
Mailing Address City	Princeton
Mailing Address Postal Code	08543
Mailing Address State/Province	NJ
Mailing Address Country	US

Inventor Information

Authority Type	Inventor
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Middle Name/Initial	M.
Family Name	Goldberg
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Residence State/Province	New Jersey
Country of Residence	United States of America
Mailing Address	Route 206 and Province Line Road
Mailing Address City	Princeton
Mailing Address Postal Code	08543
Mailing Address State/Province	New Jersey
Mailing Address Country	United States of America

Dana-Farber EX1011
Dana-Farber v. BMS, IPR2023-00252
Page 6 of 167

Application Data Sheet 37 CFR 1.76

Inventor Information

Authority Type	Inventor
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Middle Name/Initial	K.
Family Name	Gupta
Residence City	Plainsboro
Residence State/Province	New Jersey
Country of Residence	United States of America
Mailing Address	Route 206 and Province Line Road
Mailing Address City	Princeton
Mailing Address Postal Code	08543
Mailing Address State/Province	New Jersey
Mailing Address Country	United States of America

Inventor Information

Authority Type	Inventor
Primary Citizenship Country	US
Given Name	Maria
Middle Name/Initial	
Family Name	~~Jurk-Kunkel~~ **Jure-Kunkel**
Residence City	Plainsboro
Residence State/Province	NJ
Country of Residence	US
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Mailing Address City	Princeton
Mailing Address Postal Code	08543
Mailing Address State/Province	NJ
Mailing Address Country	US

Inventor Information

Authority Type	Inventor
Given Name	Xi Tao
Middle Name/Initial	
Family Name	Wang
Residence City	Wellesley
Residence State/Province	Massachusetts
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Mailing Address	Route 206 and Province Line Road
Mailing Address City	Princeton
Mailing Address Postal Code	08540
Mailing Address State/Province	New Jersey
Mailing Address Country	United States of America

Dana-Farber EX1011
Dana-Farber v. BMS, IPR2023-00252
Page 7 of 167

Application Data Sheet 37 CFR 1.76

Inventor Information

Authority Type	Inventor
Given Name	Jon
Middle Name/Initial	M.
Family Name	Wigginton
Residence City	Collegeville
Residence State/Province	Pennsylvania
Country of Residence	United States of America
Mailing Address	Route 206 and Province Line Road
Mailing Address City	Princeton
Mailing Address Postal Code	08543
Mailing Address State/Province	New Jersey
Mailing Address Country	United States of America

Correspondence Information:

Customer Number	23914
Main E-Mail address	patents@bms.com

Representative Information:

Customer Number	23914

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	October 17, 2013
Attorney/Agent Name	Ashton J. Delauney
Registration Number	64,286
Telephone Number	609-252-4560

Electronic Acknowledgement Receipt

EFS ID:	17154925
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 P. Cogswell
Customer Number:	23914
Filer:	Ashton J. Delauney/Lisa Swidra
Filer Authorized By:	Ashton J. Delauney
Attorney Docket Number:	11981-US-PSP
Receipt Date:	17-OCT-2013
Filing Date:	15-MAY-2012
Time Stamp:	14:54:55
Application Type:	Provisional

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	Request for Corrected Filing Receipt	20131017_REQ_11981USPSP.pdf	286922 7794996bbb680170bdcc773dd73aebc19857e92d	no	5

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Total Files Size (in bytes):	286922

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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.

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SelectUSA

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF **JOHN P. COGSWELL, ET AL.**
FOR: **CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/OD-LI SIGNALING**
APPLICATION NO: **61/647,442** APPLICATION DATE: **May 15, 2012**
ART UNIT: **NOT ASSIGNED** EXAMINER: **NOT ASSIGNED**
CONFIRMATION NO: **5263** USPTO CUSTOMER NO: **23914**

TRANSMITTED VIA EFS-WEB

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

CORRECTION OF INVENTORSHIP UNDER 37 C.F.R. § 1.48 (d)

Sir:

I hereby request the following individual be added as an inventor to the above provisional application:

Inventor	Residence
STACIE M. GOLDBERG	**PRINCETON, NJ**
ASHOK K. GUPTA	**PLAINSBORO, NJ**
XI TAO WANG	**WELLESLEY, MA**
JON M. WIGGINTON	**COLLEGEVILLE, PA**

Listed below is the complete updated list of inventors:

Inventor	Residence
JOHN P. COGSWELL	**YARDLEY, PA**
STACIE M. GOLDBERG	**PRINCETON, NJ**
ASHOK K. GUPTA	**PLAINSBORO, NJ**
MARIA JURK-KUNKEL	**PLAINSBORO, NJ**
XI TAO WANG	**WELLESLEY, MA**
JON M. WIGGINTON	**COLLEGEVILLE, PA**

The Commissioner is authorized to charge the fee of $50.00, set forth under 37 CFR §1.17(q), or any additional fees required to Account No. 19-3880 in the name of Bristol-Myers Squibb Company.

Respectfully submitted,

ASHTON J. DELAUNEY
Agent for Applicant
Reg. No. 64,286
Phone: 609-252-4560
Date: May 15, 2013

Bristol-Myers Squibb Company
Patent Department
P.O. Box 4000
Princeton, NJ 08543-4000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF **COGSWELL, ET AL**
FOR: **CANCER IMMUNOTHERAPY FOR DISRUPTING PD-1/PD-LI SIGNALING**
APPLICATION NO: **61/647,442** APPLICATION DATE: **May 15, 2012**
ART UNIT: EXAMINER:
CONFIRMATION NO: 5263 USPTO CUSTOMER NO: **23914**

TRANSMITTED VIA EFS-WEB

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL APPLICATION DATA SHEET
ALONG WITH LETTER CORRECTING OFFICIAL FILING RECEIPT

Sir:

Please issue a corrected filing receipt listing the inventors as follows:

John P. Cogswell, Yardley, PA
Stacie M. Goldberg, Princeton, NJ
Ashok K. Gupta, Plainsboro, NJ
Maria Jurk-Kunkel, Plainsboro, NJ
Xi Tao Wang, Wellesley, MA
Jon M. Wigginton, Collegeville, PA

A copy of the filing receipt with the correction noted is enclosed. Also enclosed is a Supplemental Application Data Sheet with the above corrections underlined. Applicant respectfully requests that the Supplemental Application Data Sheet be entered into the file.

Applicant believes that no fee is due, if however any additional fees in association with this request are due, please charge to Deposit Account No. 19-3880 in the name of Bristol-Myers Squibb Company.

Respectfully submitted,

ASHTON J. DELAUNEY
Attorney for Applicant
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Date: May 15, 2013

Bristol-Myers Squibb Company
Patent Department
P.O. Box 4000
Princeton, NJ 08543-4000

Supplemental 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
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Middle Name/Initial	**P.**
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Residence State/Province	PA
Country of Residence	US
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Mailing Address City	~~Yardley~~ **Princeton**
Mailing Address Postal Code	~~19067~~ **08543**
Mailing Address State/Province	~~PA~~ **NJ**
Mailing Address Country	US

Inventor Information

Authority Type	Inventor
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Mailing Address State/Province	New Jersey
Mailing Address Country	United States of America

Dana-Farber EX1011
Dana-Farber v. BMS, IPR2023-00252
Page 17 of 167

Supplemental Application Data Sheet 37 CFR 1.76

Inventor Information

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Mailing Address City	Princeton
Mailing Address Postal Code	08543
Mailing Address State/Province	New Jersey
Mailing Address Country	United States of America

Inventor Information

Authority Type	Inventor
Primary Citizenship Country	US
Given Name	Maria
Middle Name/Initial	
Family Name	Jure-Kunkel
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Residence State/Province	NJ
Country of Residence	US
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Mailing Address City	~~Plainsboro~~ **Princeton**
Mailing Address Postal Code	~~08536~~ **08543**
Mailing Address State/Province	NJ
Mailing Address Country	US

Inventor Information

Authority Type	Inventor
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Middle Name/Initial	
Family Name	Wang
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Residence State/Province	Massachusetts
Country of Residence	United States of America
Mailing Address	Route 206 and Province Line Road
Mailing Address City	Princeton
Mailing Address Postal Code	08540
Mailing Address State/Province	New Jersey
Mailing Address Country	United States of America

Supplemental Application Data Sheet 37 CFR 1.76

Inventor Information

Authority Type	Inventor
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Middle Name/Initial	M.
Family Name	Wigginton
Residence City	Collegeville
Residence State/Province	Pennsylvania
Country of Residence	United States of America
Mailing Address	Route 206 and Province Line Road
Mailing Address City	Princeton
Mailing Address Postal Code	08543
Mailing Address State/Province	New Jersey
Mailing Address Country	United States of America

Correspondence Information:

Customer Number	23914
Main E-Mail address	patents@bms.com

Representative Information:

Customer Number	23914

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	A Delauney
Date	May 15, 2013
Attorney/Agent Name	Ashton J. Delauney
Registration Number	64,286
Telephone Number	609-252-4560

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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
61/647,442	05/15/2012		250	11981-US-PSP		

CONFIRMATION NO. 5263

FILING RECEIPT

23914
HENRY HADAD
BRISTOL-MYERS SQUIBB COMPANY
PATENT DEPARTMENT
P O BOX 4000
PRINCETON, NJ 08543-4000

OC000000054892837

Date Mailed: 06/20/2012

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Applicant(s)

~~John Cogswell, Plainsboro, PA;~~ John P. Cogswell, Yardley, PA
Maria Jure-Kunkel, Plainsboro, NJ;
Stacie M. Goldberg, Princeton, NJ
Ashok K. Gupta, Plainsboro, NJ
Xi Tao Wang, Wellesley, MA
Jon M. Wigginton, Collegeville, PA

Power of Attorney:
Ashton Delauney--64286

If Required, Foreign Filing License Granted: 06/15/2012

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 61/647,442**

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

Title

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

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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

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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 (1-866-999-4158).

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Title 37, Code of Federal Regulations, 5.11 & 5.15

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Electronic Patent Application Fee Transmittal

Application Number:	61647442
Filing Date:	15-May-2012
Title of Invention:	CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING
First Named Inventor/Applicant Name:	John Cogswell
Filer:	Ashton J. Delauney/Lisa Swidra
Attorney Docket Number:	11981-US-PSP

Filed as Large Entity

Provisional Filing Fees

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($)
Miscellaneous:				
Processing Fee for Provis. Applications	1807	1	50	50
		Total in USD ($)		**50**

Electronic Acknowledgement Receipt

EFS ID:	15780658
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/Lisa Swidra
Filer Authorized By:	Ashton J. Delauney
Attorney Docket Number:	11981-US-PSP
Receipt Date:	15-MAY-2013
Filing Date:	15-MAY-2012
Time Stamp:	11:38:30
Application Type:	Provisional

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$50
RAM confirmation Number	9076
Deposit Account	193880
Authorized User	

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Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
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Warnings:					
Information:					
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Information:					
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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.

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<u>New International Application Filed with the USPTO as a Receiving Office</u>
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United States Patent and Trademark Office
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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
61/647,442	05/15/2012		250	11981-US-PSP		

CONFIRMATION NO. 5263

FILING RECEIPT

OC000000054892837

23914
HENRY HADAD
BRISTOL-MYERS SQUIBB COMPANY
PATENT DEPARTMENT
P O BOX 4000
PRINCETON, NJ 08543-4000

Date Mailed: 06/20/2012

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Applicant(s)

John Cogswell, Plainsboro, PA;
Maria Jure-Kunkel, Plainsboro, NJ;

Power of Attorney:
Ashton Delauney--64286

If Required, Foreign Filing License Granted: 06/15/2012

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 61/647,442**

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

Title

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

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

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Title 37, Code of Federal Regulations, 5.11 & 5.15

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Sequence Listing was accepted.
If you need help call the Patent Electronic Business Center at (866) 217-9197 (toll free).
Reviewer: Durreshwar Anjum
Timestamp: [year=2012; month=5; day=22; hr=13; min=7; sec=50; ms=985;]
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Validated By CRFValidator v 1.0.3

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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 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 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 IgGl) 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_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 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 SEO 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 ≥ 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 110ºC (P1) for 10 min, then moved to the next step (P2 FAN ON at 98ºC; FAN OFF at 90 ºC). 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 ≥ 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.

Dana-Farber EX1011
Dana-Farber v. BMS, IPR2023-00252
Page 134 of 167

FIG. 1A

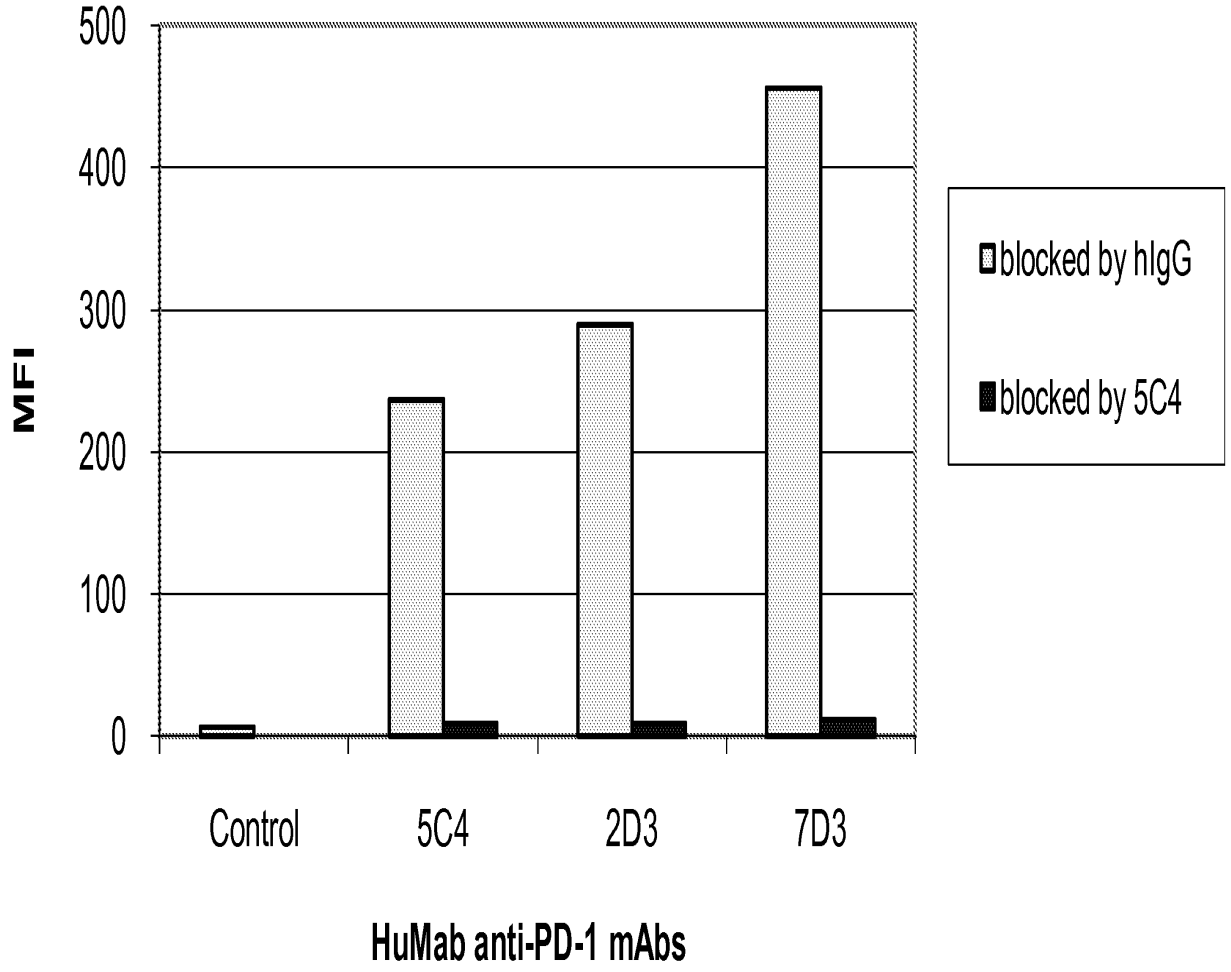

500
400
300
200
100
0
MFI
blocked by hIgG
blocked by 5C4
Control
5C4
2D3
7D3
HuMab anti-PD-1 mAbs

FIG. 1B

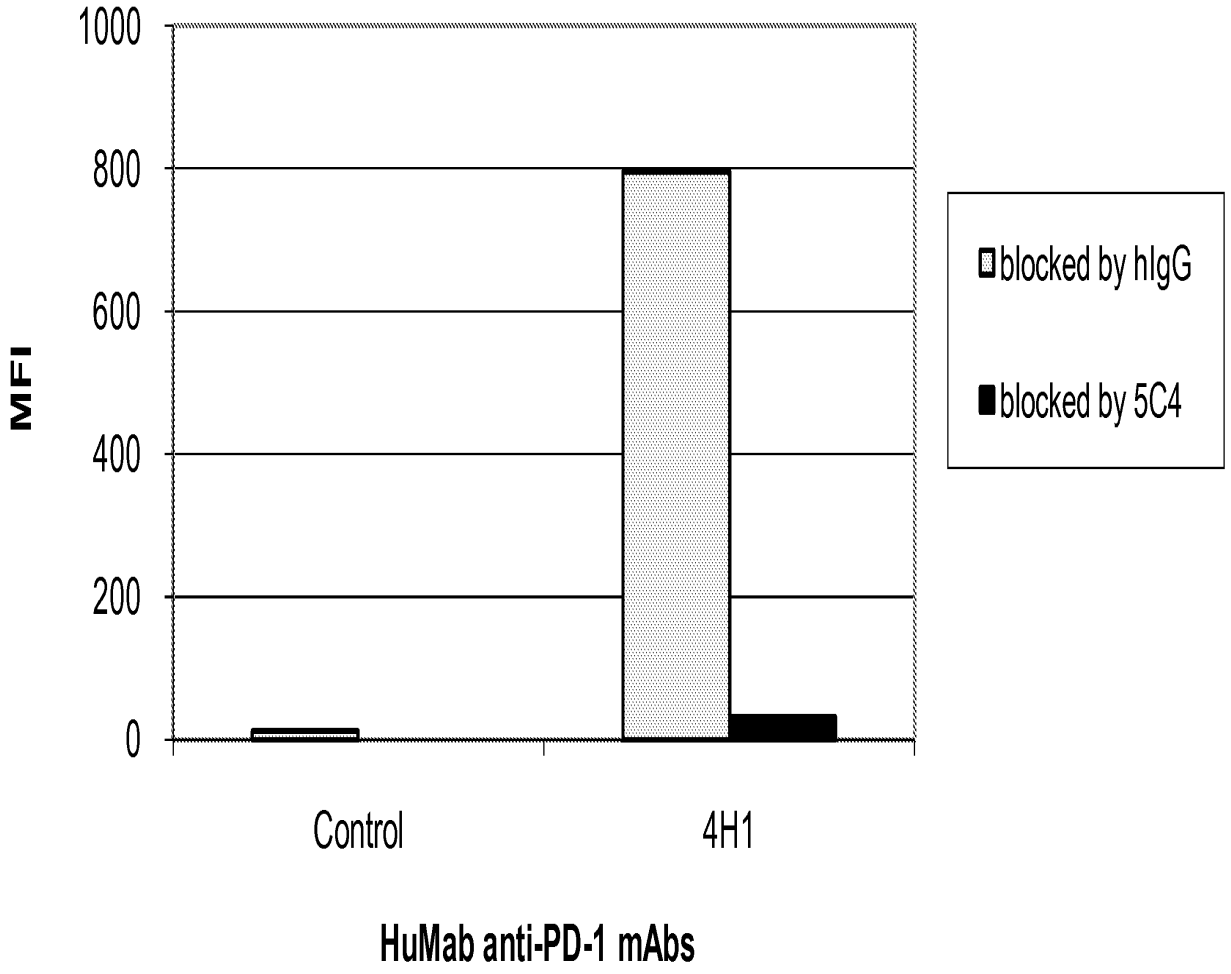

1000
800
600
400
200
0
MFI
blocked by hIgG
blocked by 5C4
Control
4H1
HuMab anti-PD-1 mAbs

FIG. 1C

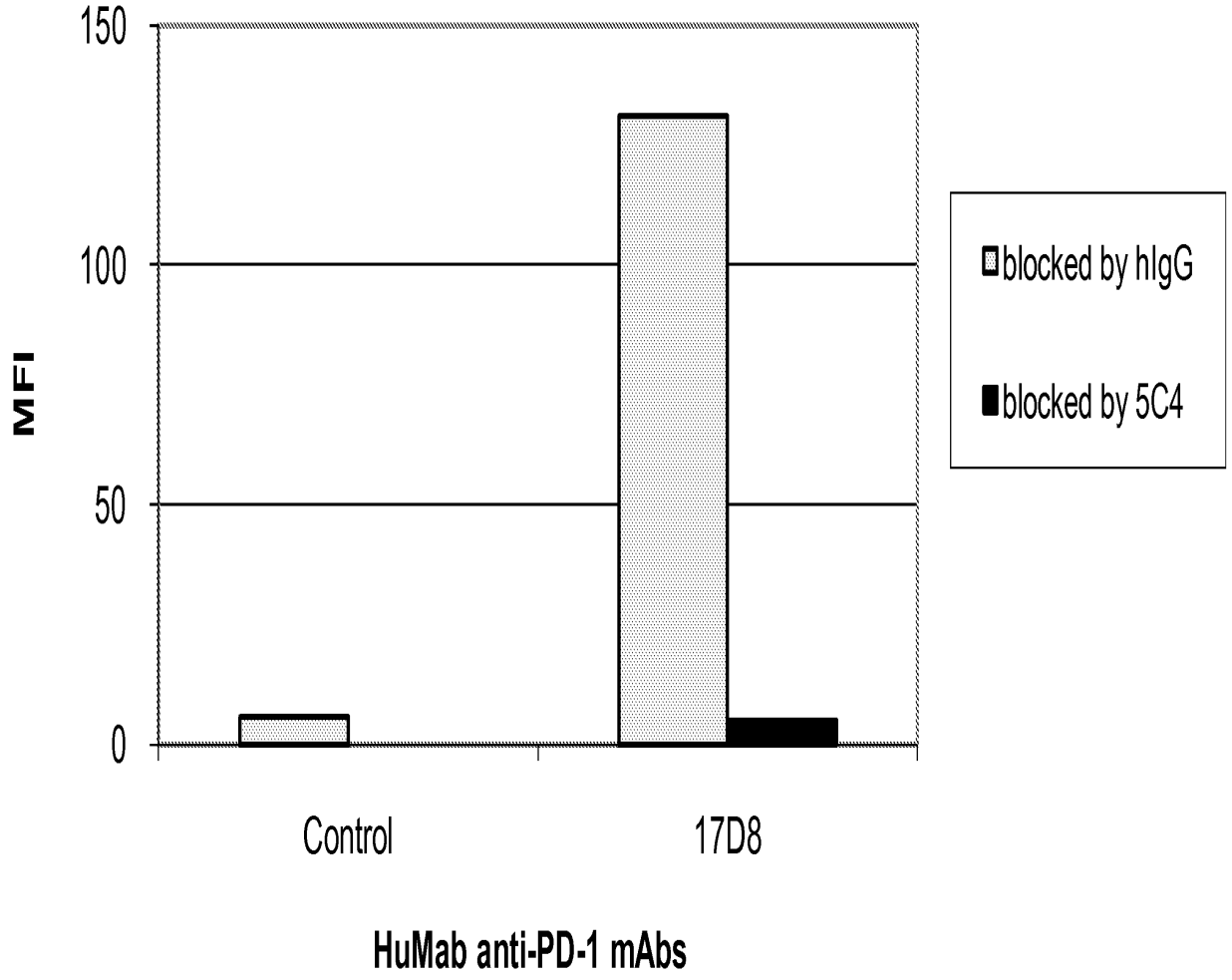

150
100
50
0
MFI
blocked by hIgG
blocked by 5C4
Control
17D8
HuMab anti-PD-1 mAbs

FIG. 2A

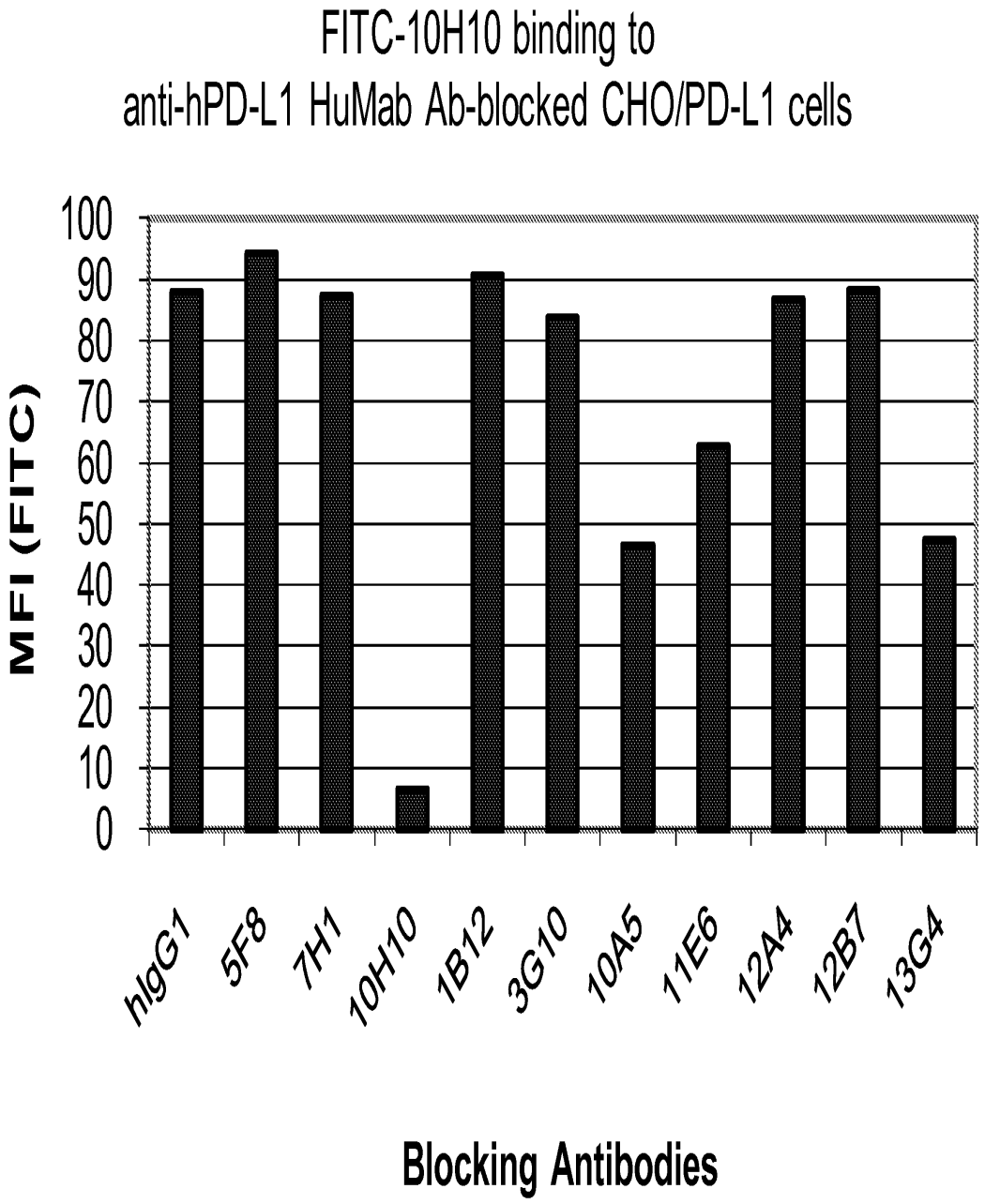

FITC-10H10 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
100
90
80
70
60
50
40
30
20
10
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2B

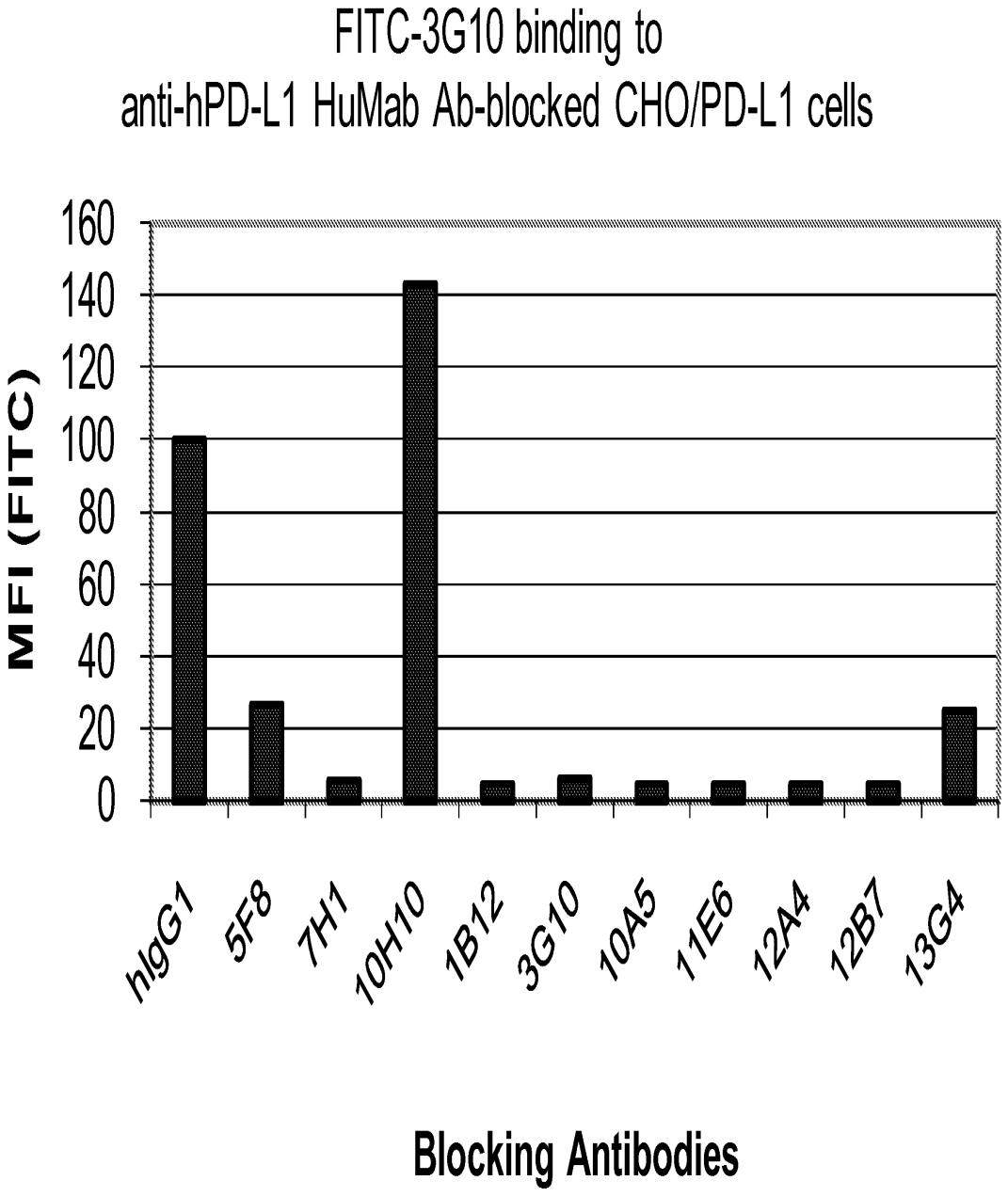

FITC-3G10 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
160
140
120
100
80
60
40
20
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2C

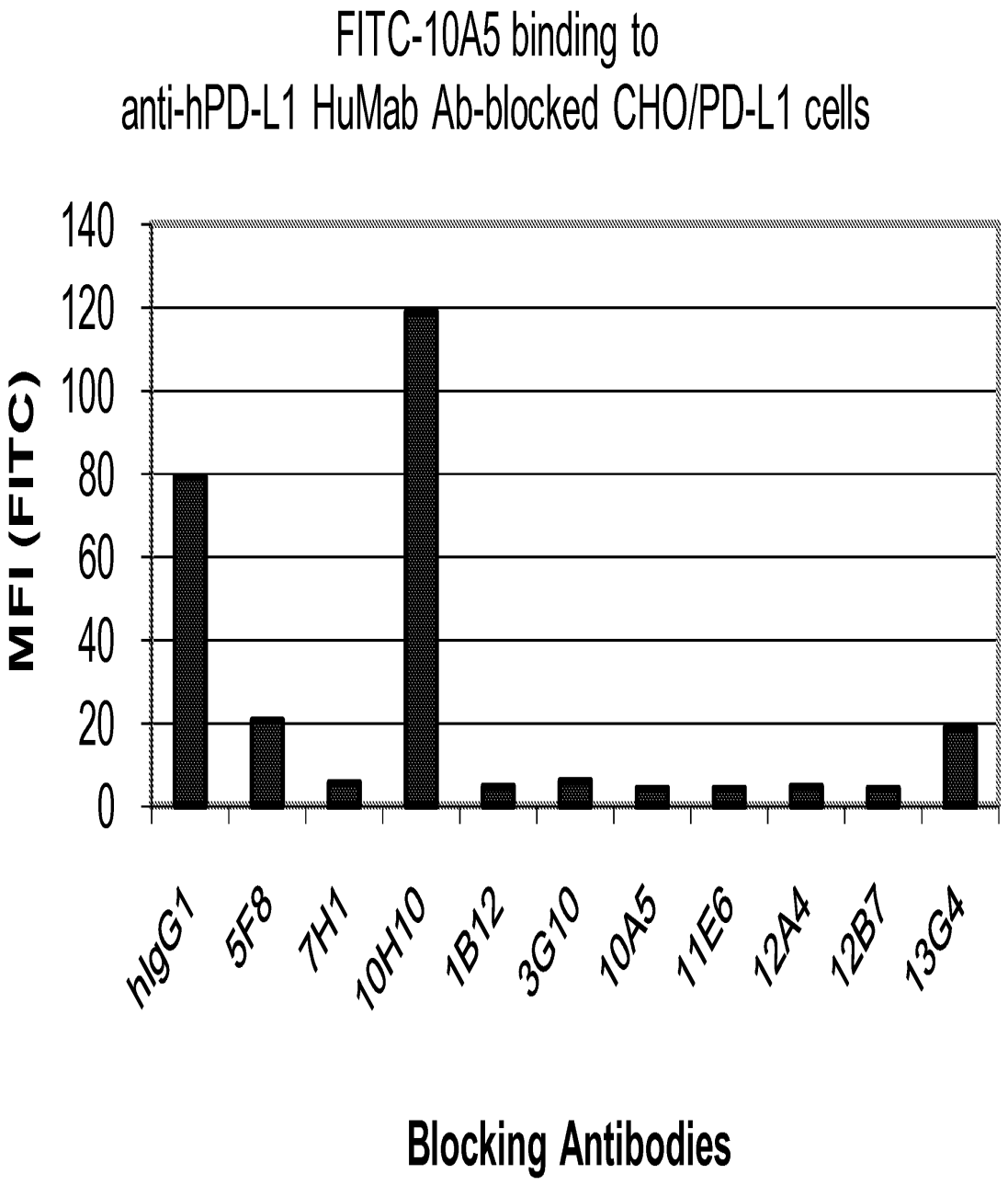

FITC-10A5 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
140
120
100
80
60
40
20
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2D

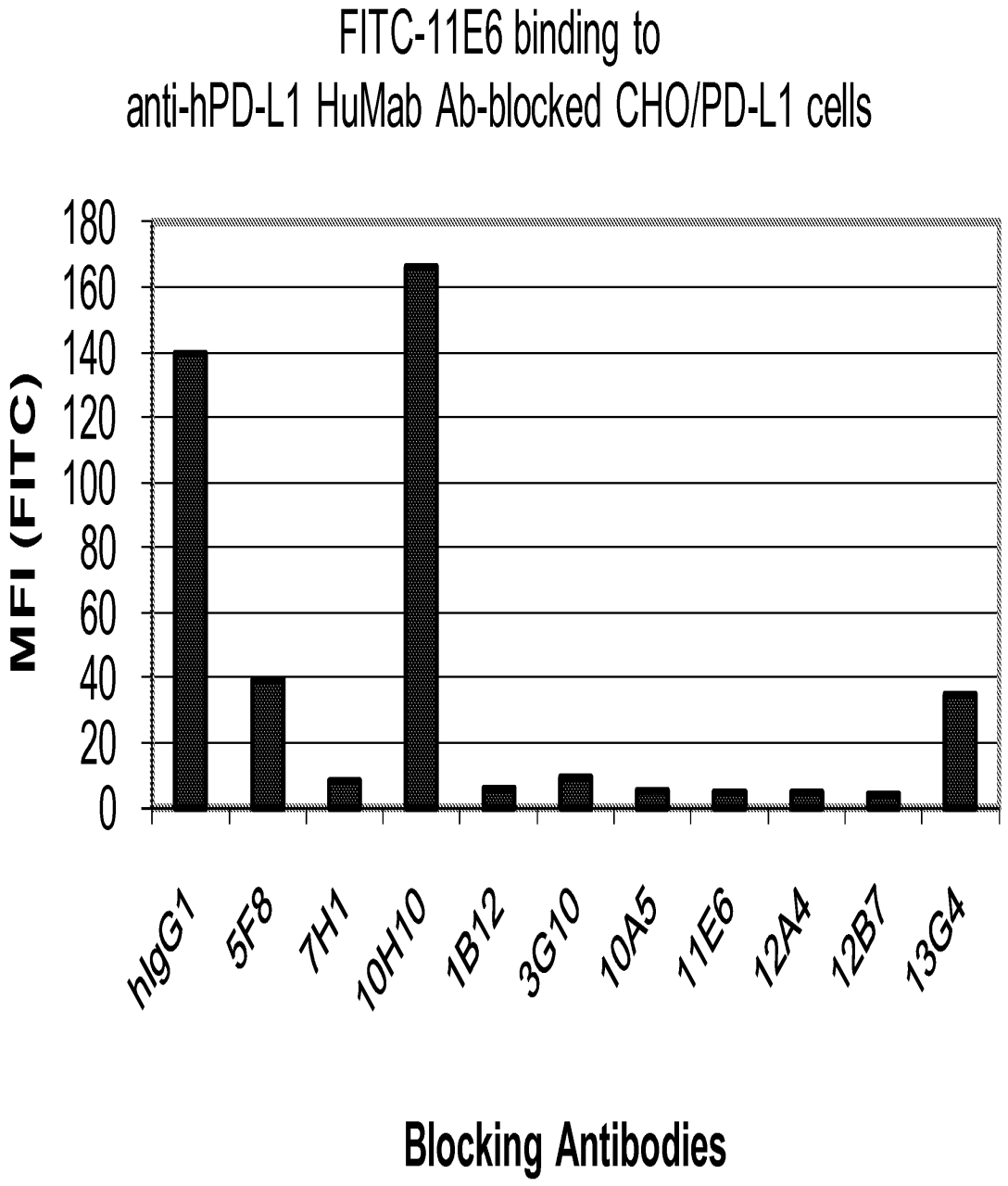

FITC-11E6 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
180
160
140
120
100
80
60
40
20
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2E

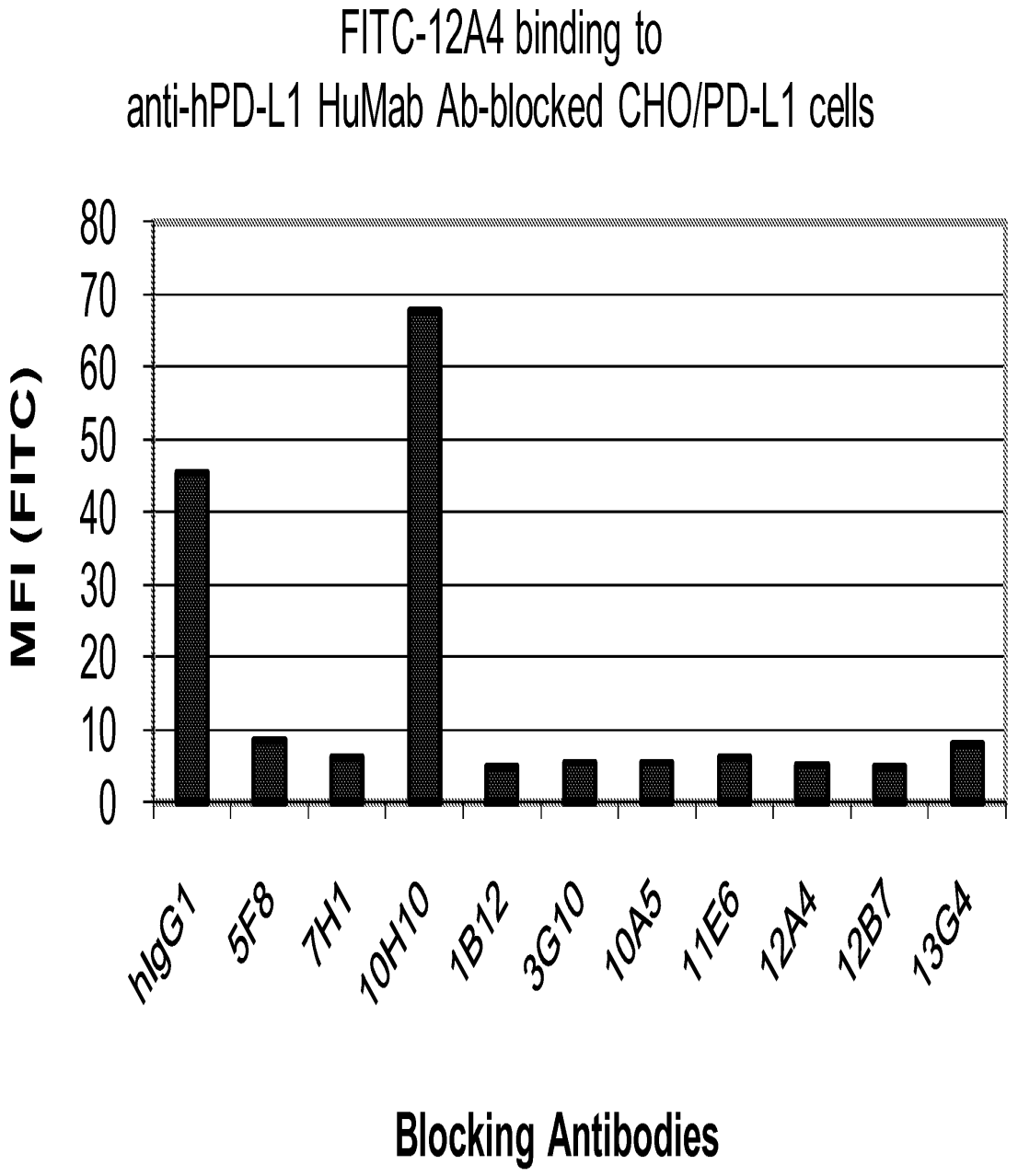

FITC-12A4 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
80
70
60
50
40
30
20
10
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2F

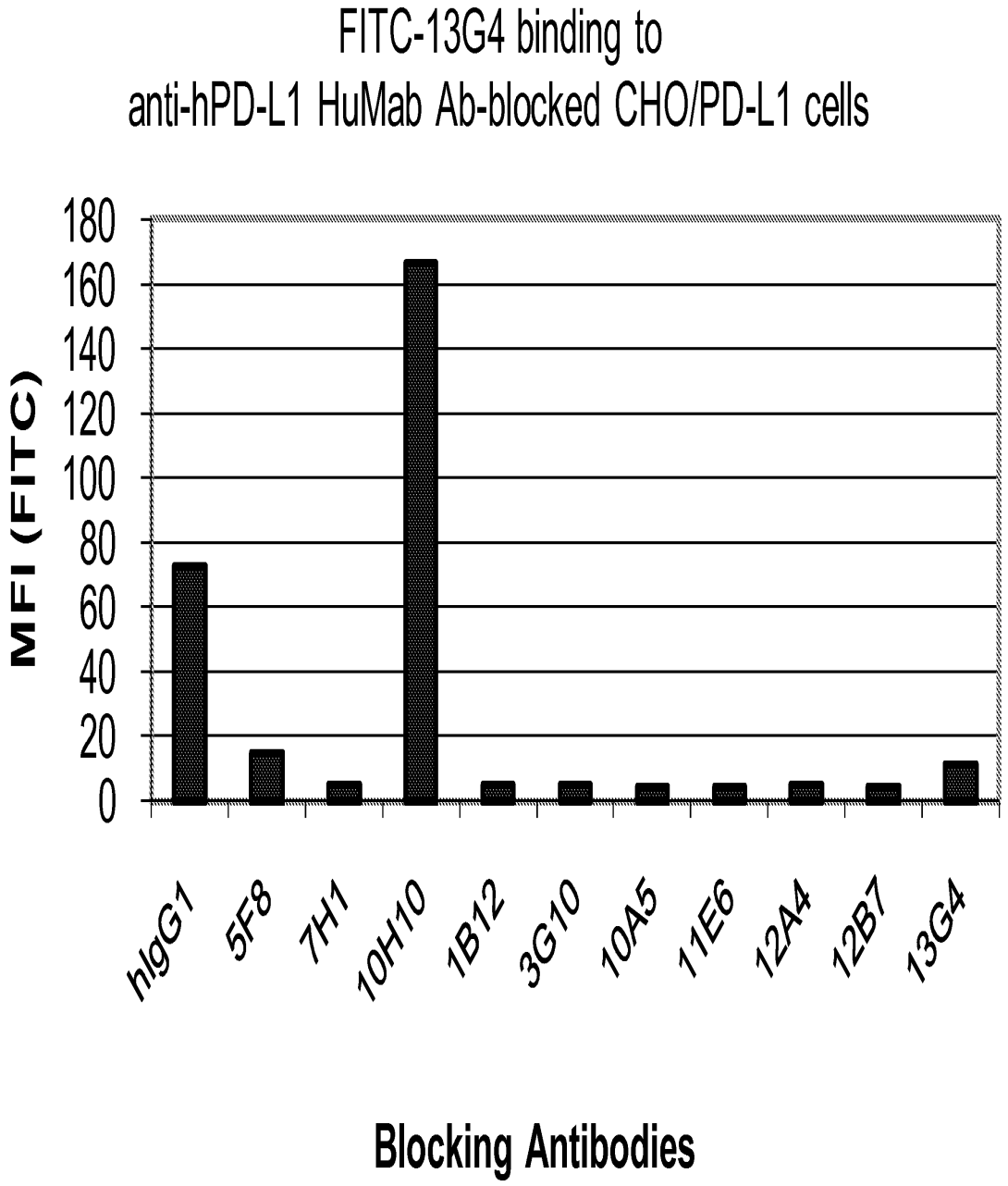

FITC-13G4 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
180
160
140
120
100
80
60
40
20
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 3

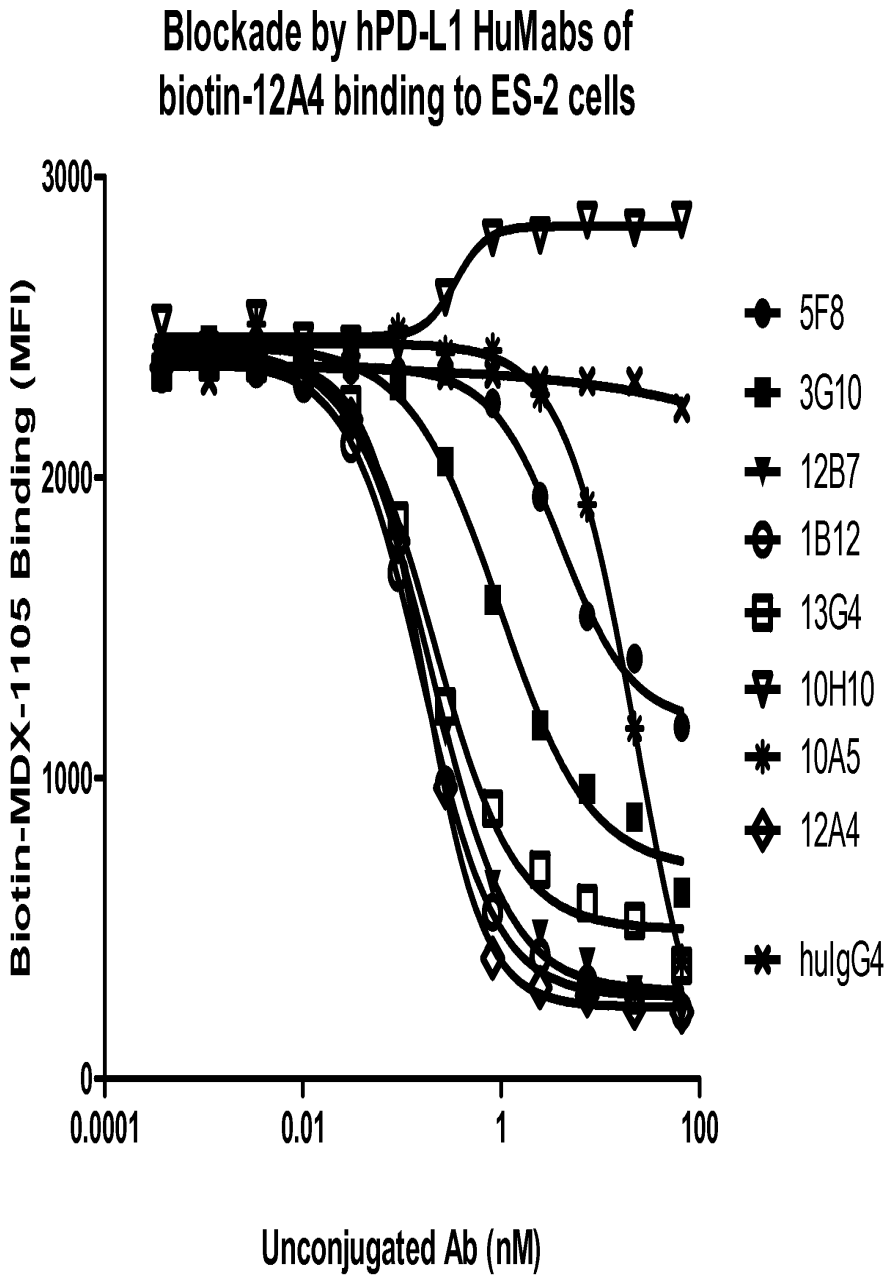

Blockade by hPD-L1 HuMabs of
biotin-12A4 binding to ES-2 cells
Biotin-MDX-1105 Binding (MFI)
3000
2000
1000
0
0.0001
0.01
1
100
Unconjugated Ab (nM)
5F8
3G10
12B7
1B12
13G4
10H10
10A5
12A4
hulgG4

FIG. 4

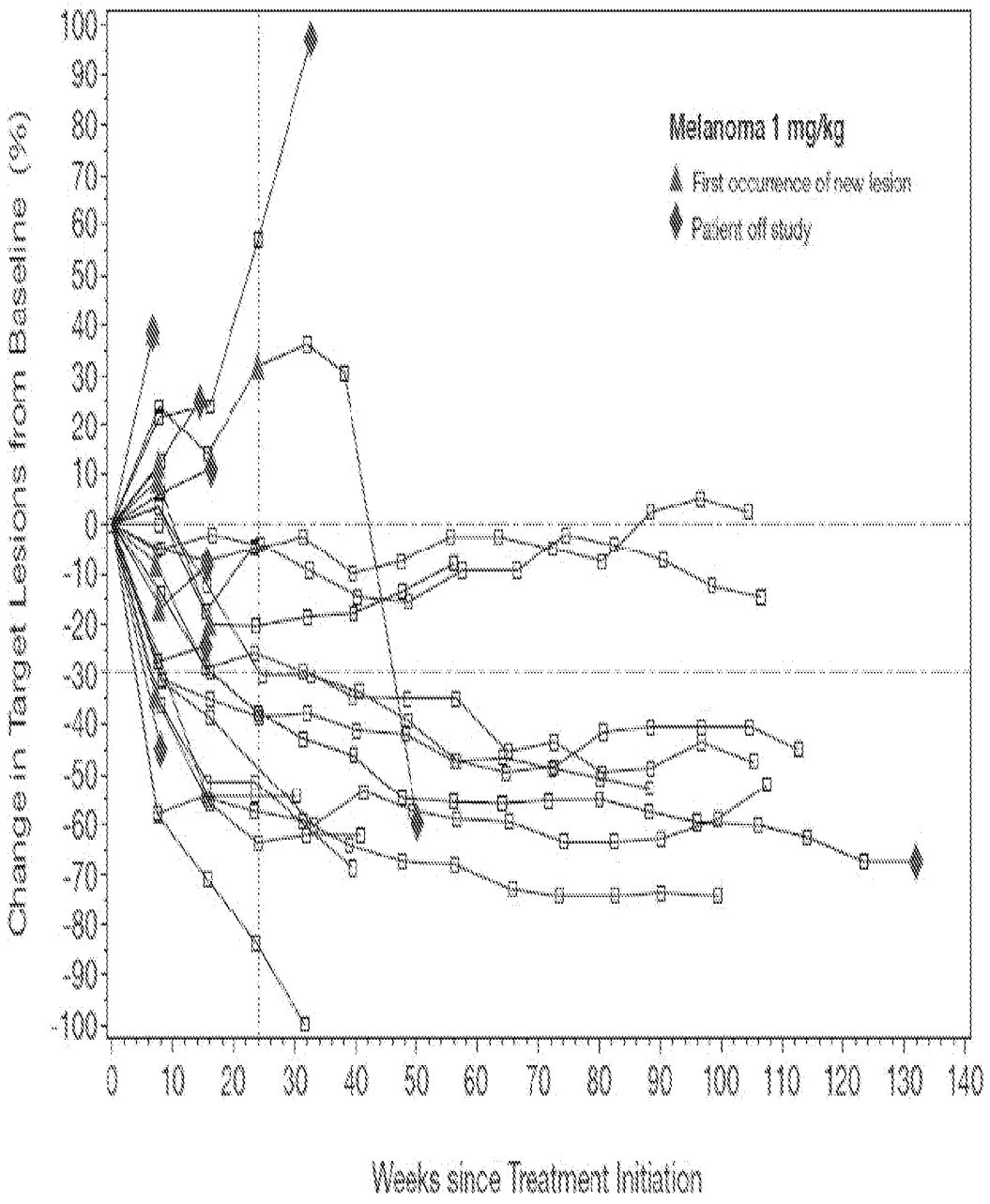

Melanoma 1 mg/kg
First occurence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
Weeks since Treatment Initiation

FIG. 5

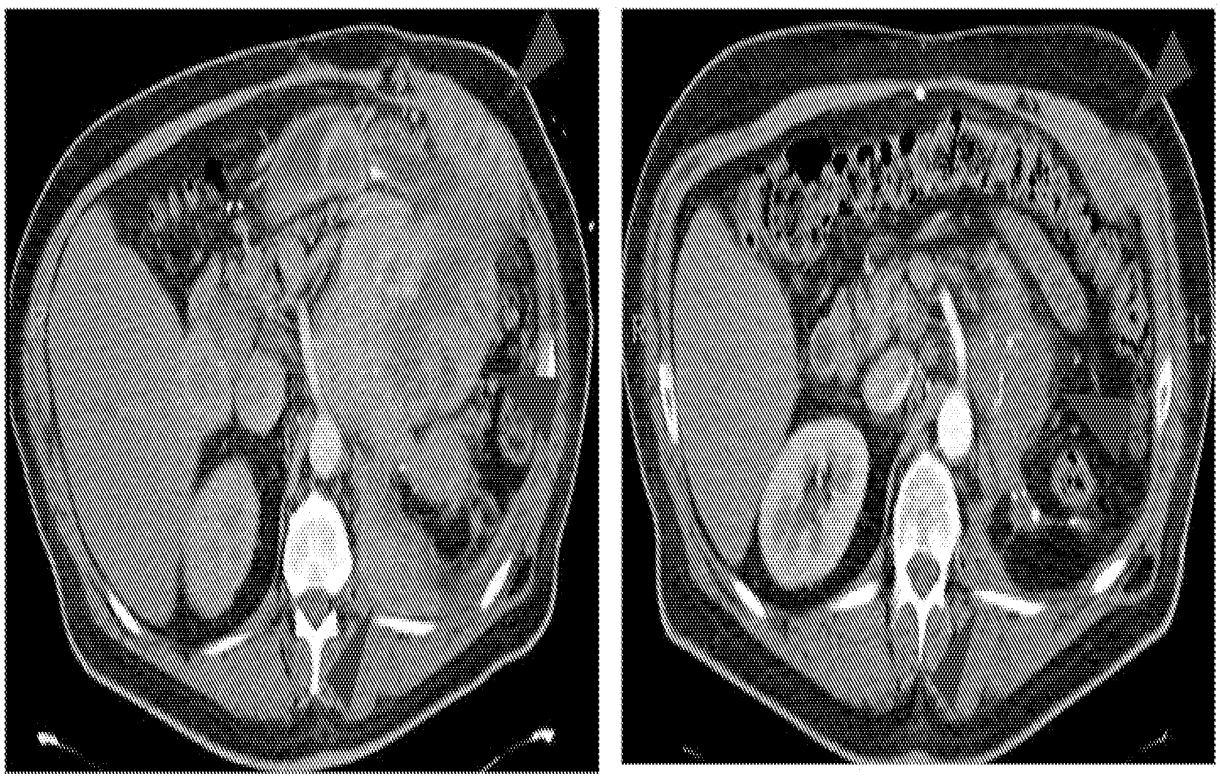


FIG. 6

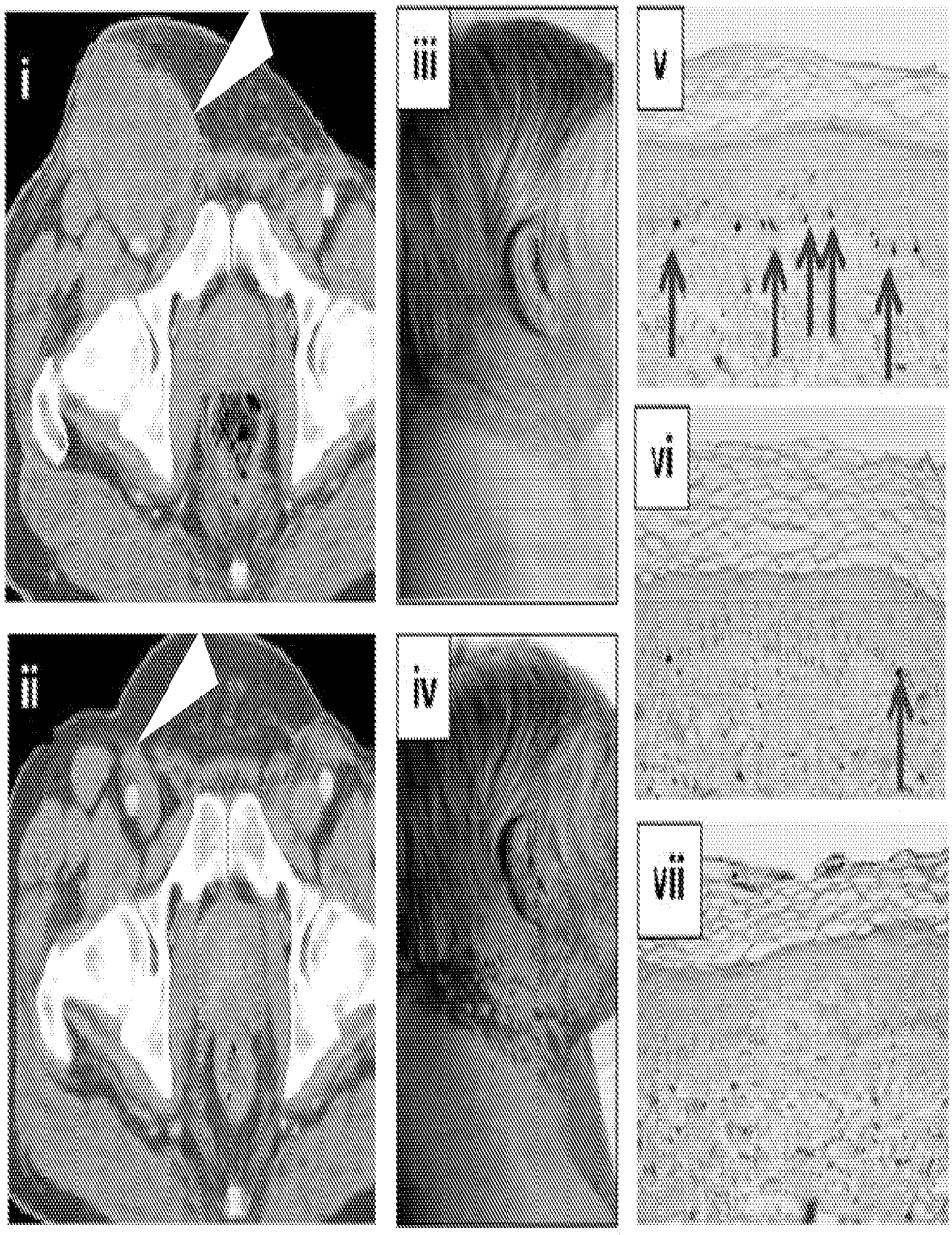


FIG. 7

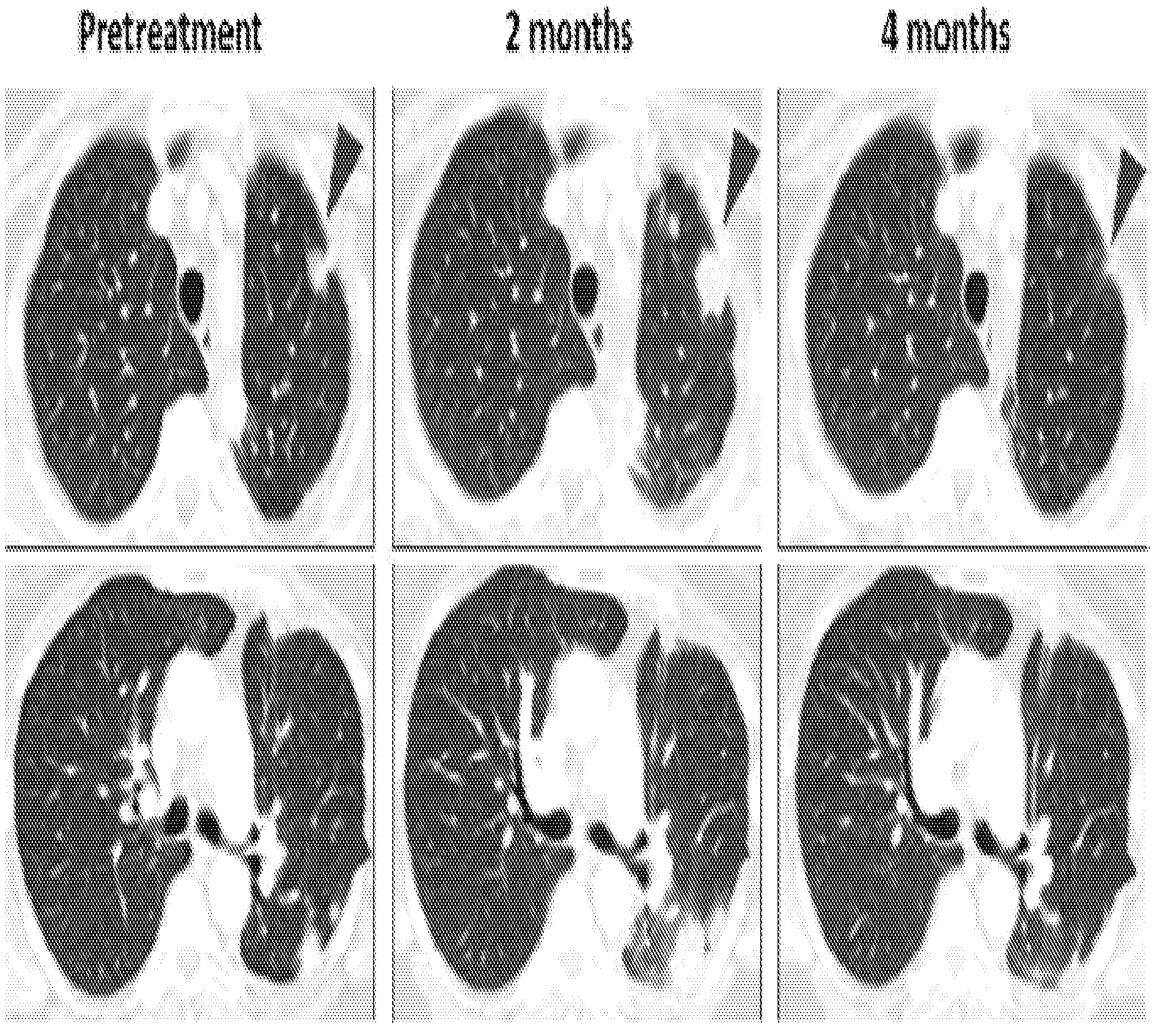
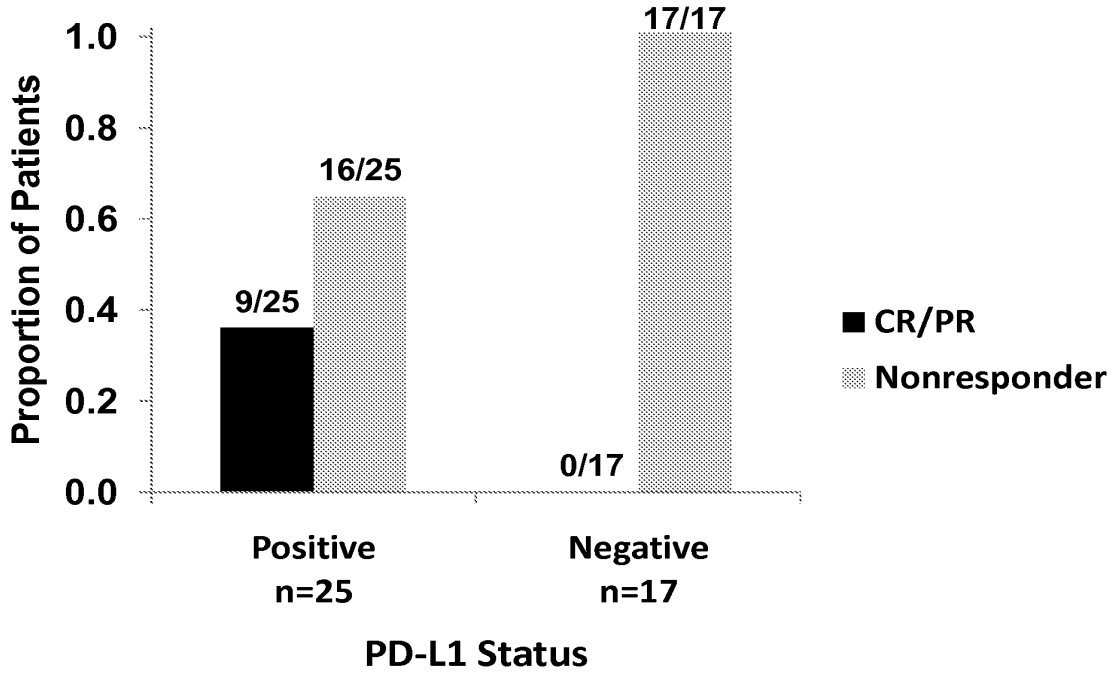


FIG. 8A



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

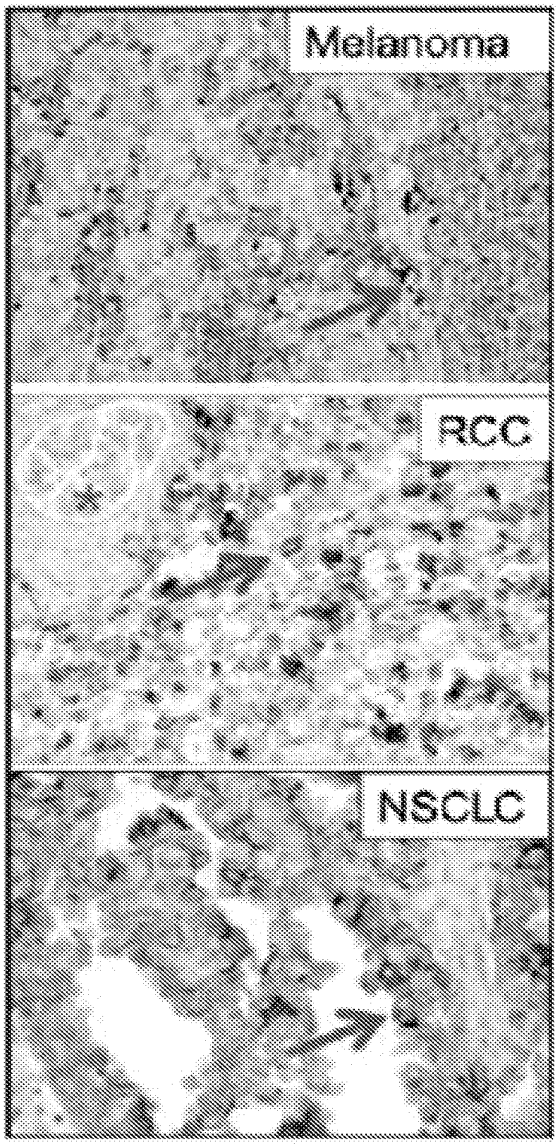


FIG. 9

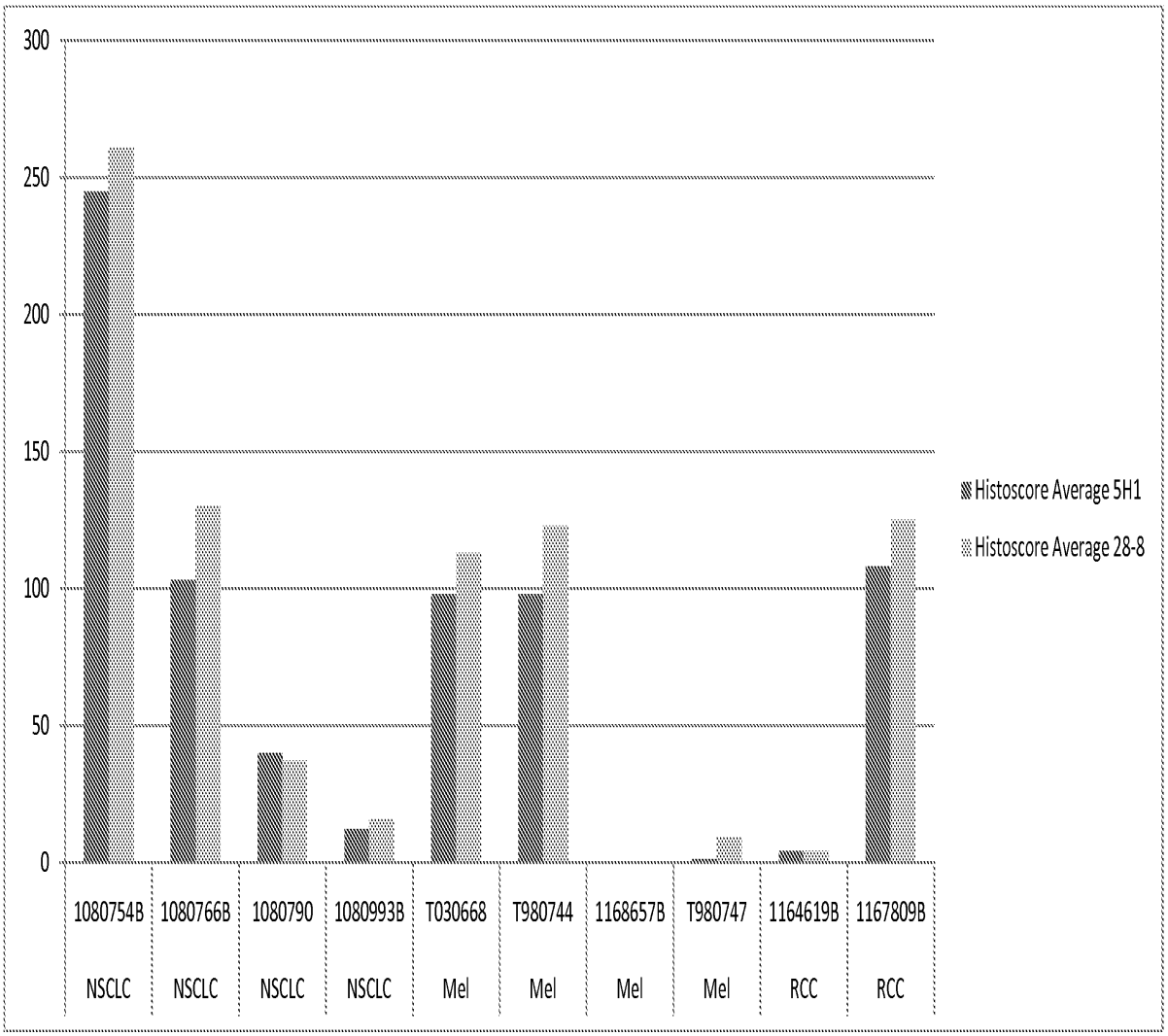

300
250
200
150
100
50
0
Histoscore Average 5H1
Histoscore Average 28-8
1080754B
1080766B
1080790
1080993B
T030668
T980744
1168657B
T980747
1164619B
1167809B
NSCLC
NSCLC
NSCLC
NSCLC
Mel
Mel
Mel
Mel
RCC
RCC

FIG. 10A

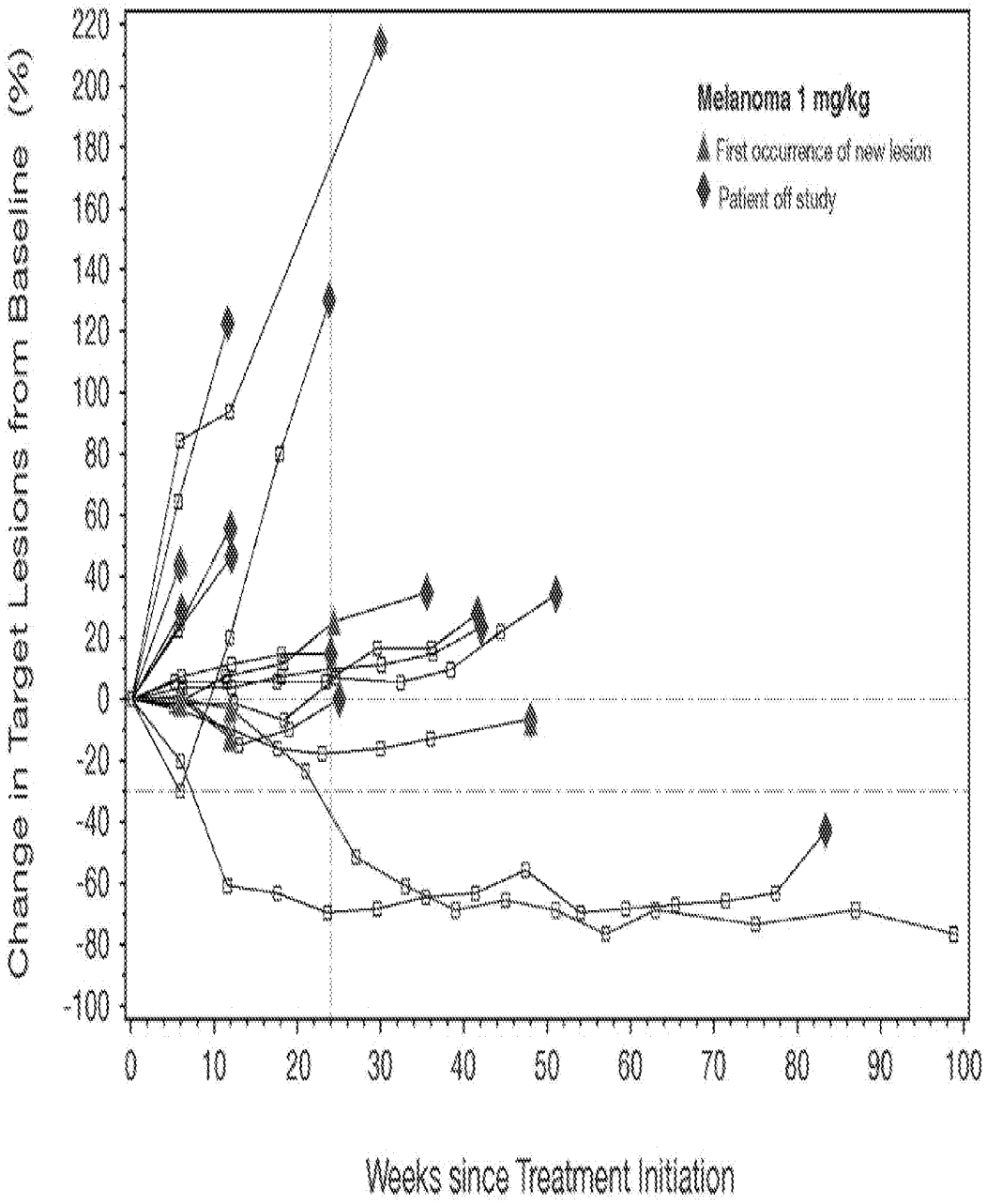

Melanoma 1 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
220
200
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
Weeks since Treatment Initiation

FIG. 10B

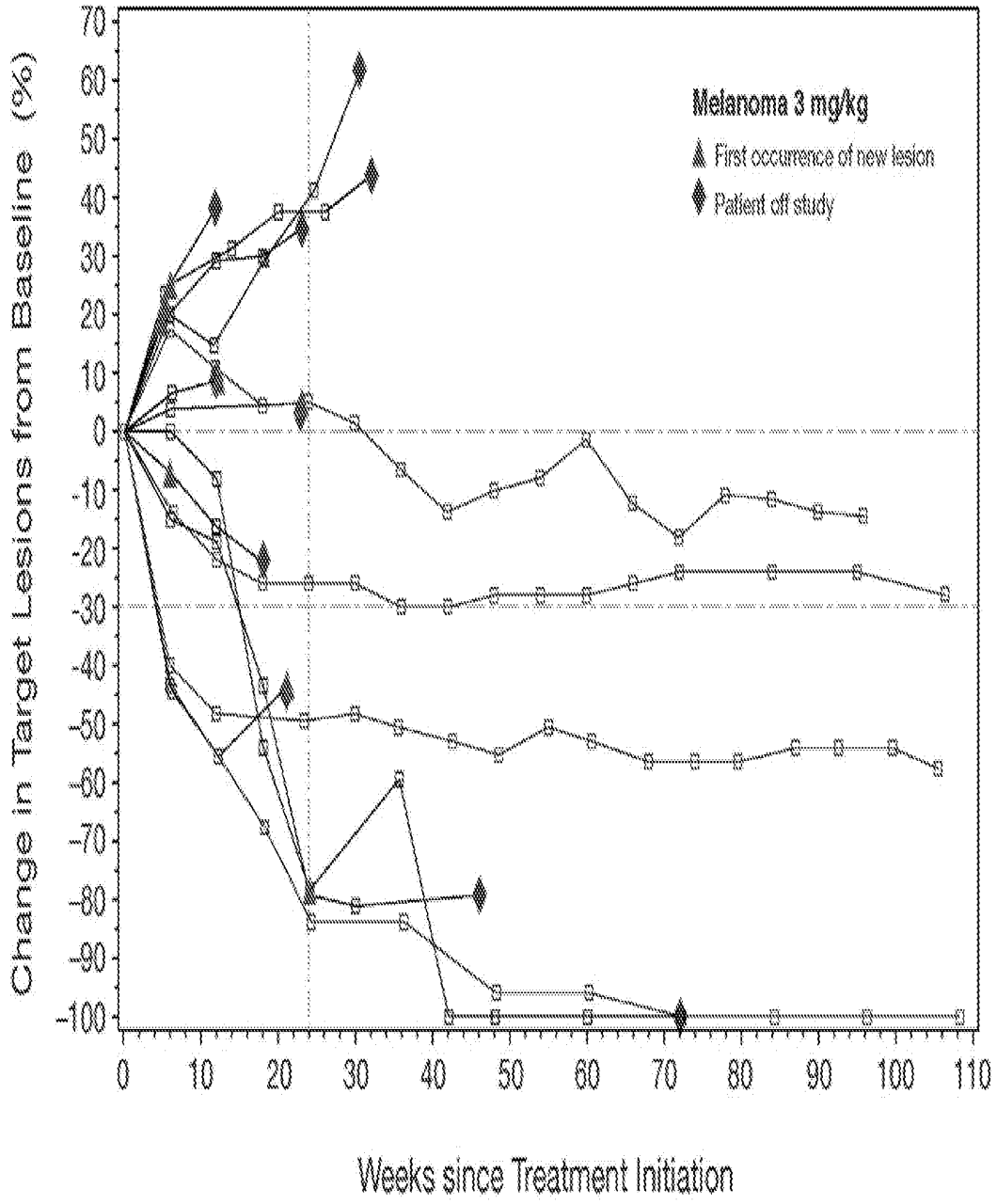

Melanoma 3 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
10
20
30
40
50
60
70
80
90
100
110
Weeks since Treatment Initiation

FIG. 10C

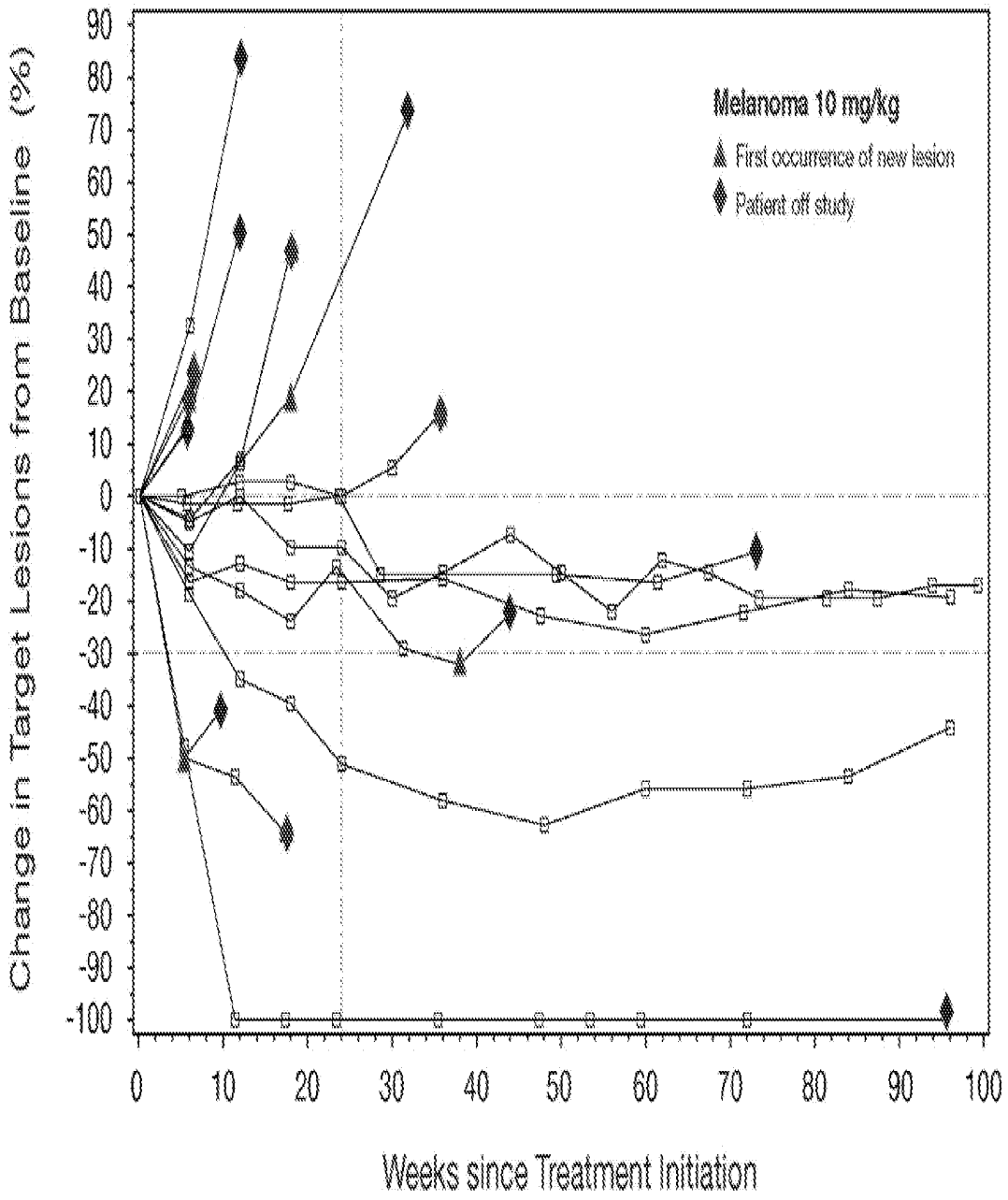

Melanoma 10 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
10
20
30
40
50
60
70
80
90
100
Weeks since Treatment Initiation

FIG. 10D

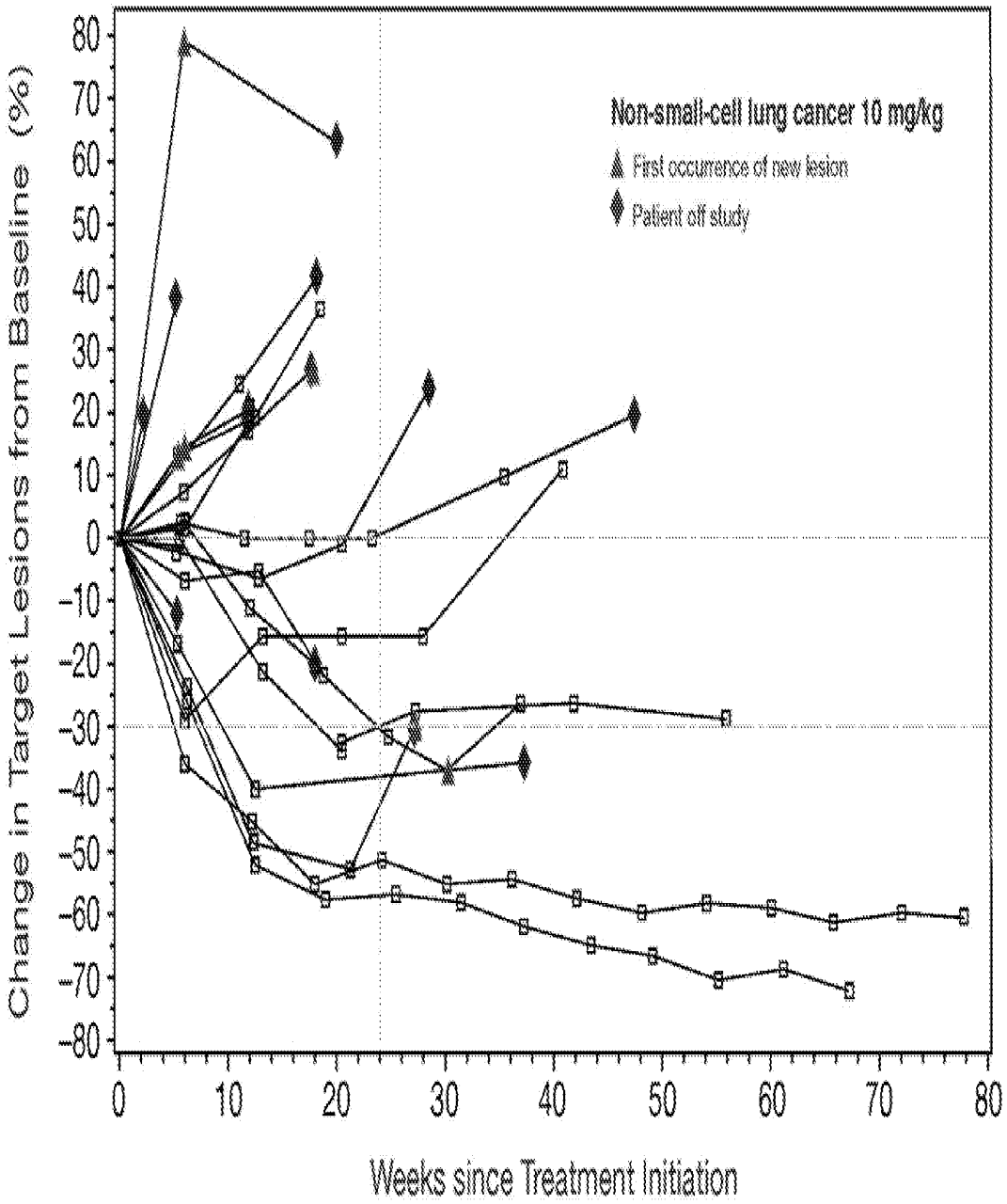

Non-small-cell lung cancer 10 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
0
10
20
30
40
50
60
70
80
Weeks since Treatment Initiation

FIG. 11

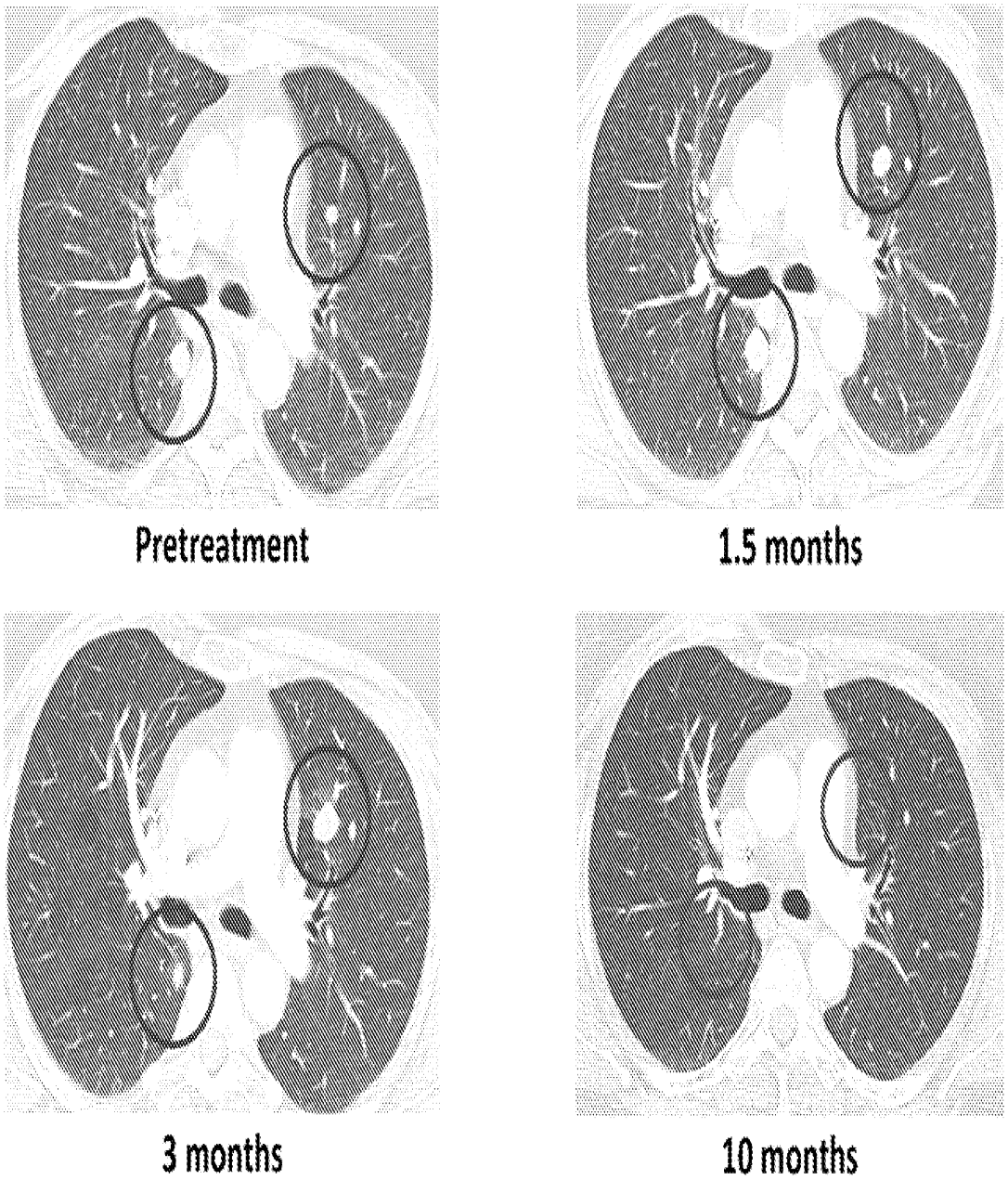


FIG. 12

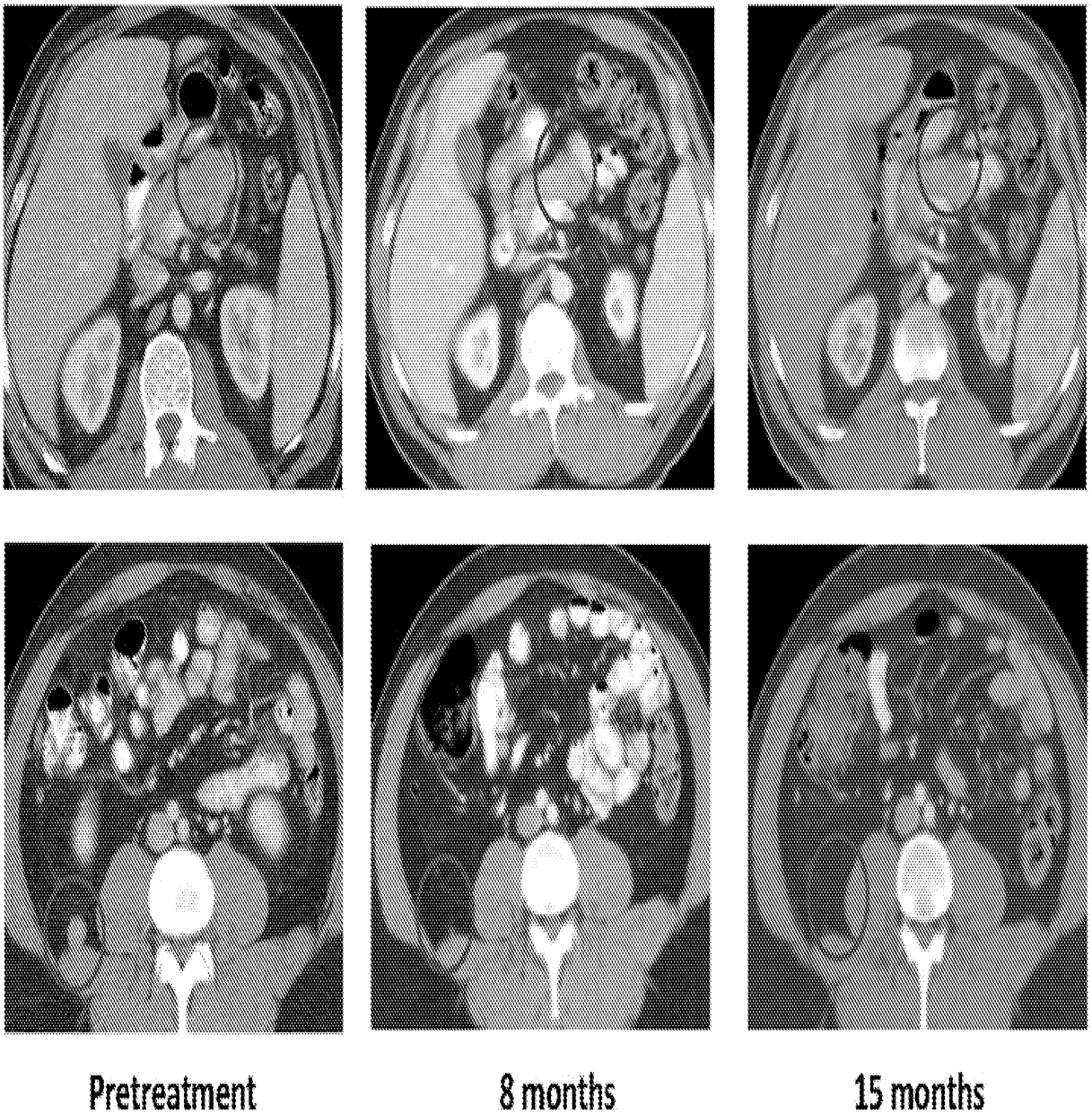
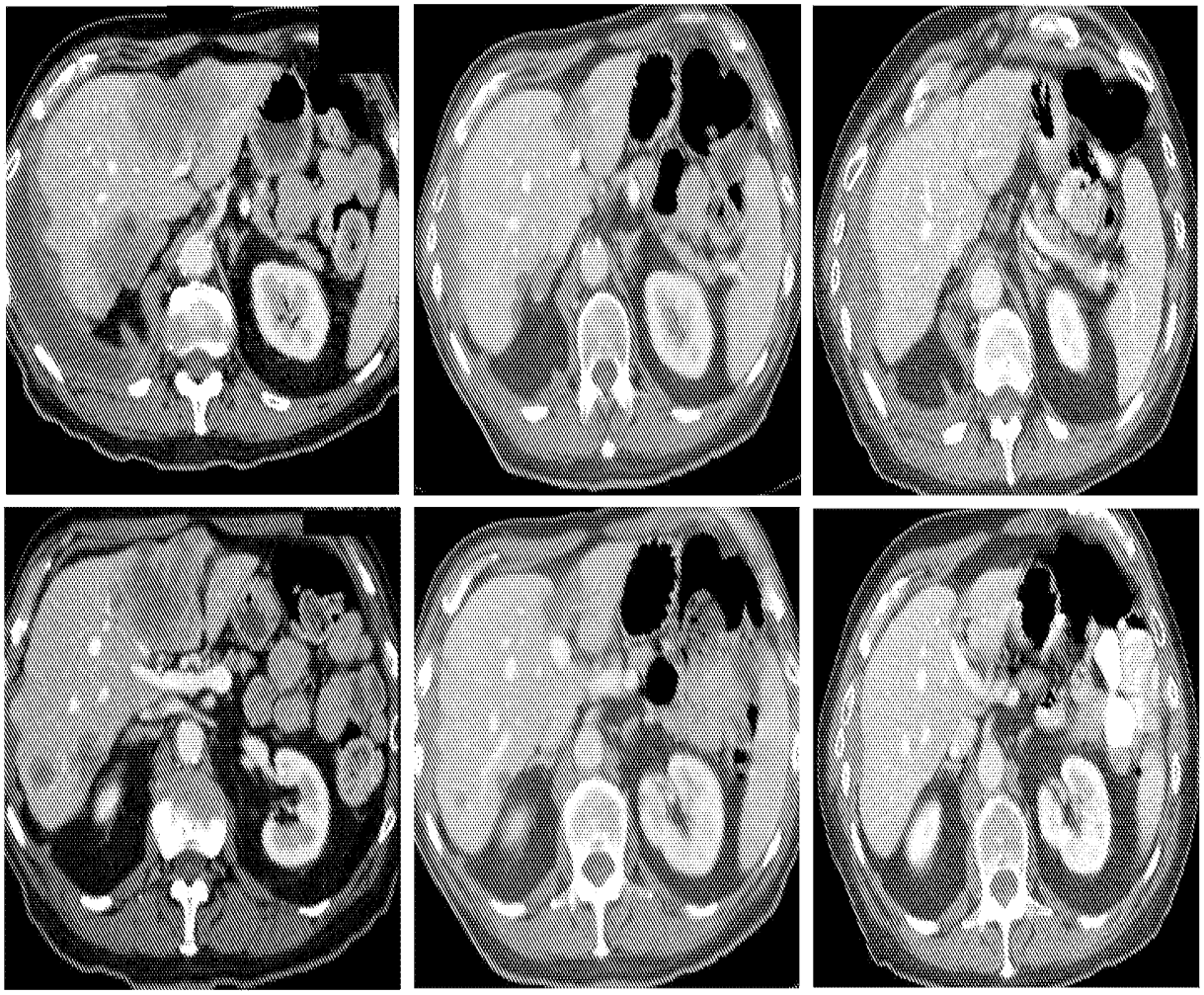


FIG. 13



Pretreatment 3 months 15 months

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	
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Residence State/Province	PA
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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
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Middle Name/Initial	
Family Name	Jure-Kunkel
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Residence State/Province	NJ
Country of Residence	US
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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

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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 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 Cogswell
Filer:	Ashton J. Delauney/Hilda Franco
Attorney Docket Number:	11981-US-PSP

Filed as Large Entity

Provisional Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Provisional application filing	1005	1	250	250
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($)
Miscellaneous:				
			Total in USD ($)	**250**

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
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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 0061d18d8d1ec36ca8da5f70ad580d27f2703b2a	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
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DocCode – SCORE

SCORE Placeholder Sheet for IFW Content

Application Number: 61647442 Document Date: 05/15/2012

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Form Revision Date: February 8, 2006

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SCORE Placeholder Sheet for IFW Content

Application Number: 61647442 Document Date: 05/15/2012

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Form Revision Date: February 8, 2006