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May 18, 2016

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APPLICATION NUMBER: *62/153,973*
FILING DATE: *April 28, 2015*
RELATED PCT APPLICATION NUMBER: *PCT/US16/29877*

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS *US62/153,973*

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Michelle K. Lee

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Fig. 1 – Patient Flow Diagram: Enrollment, Randomization, and Follow-up of Patients in the Study

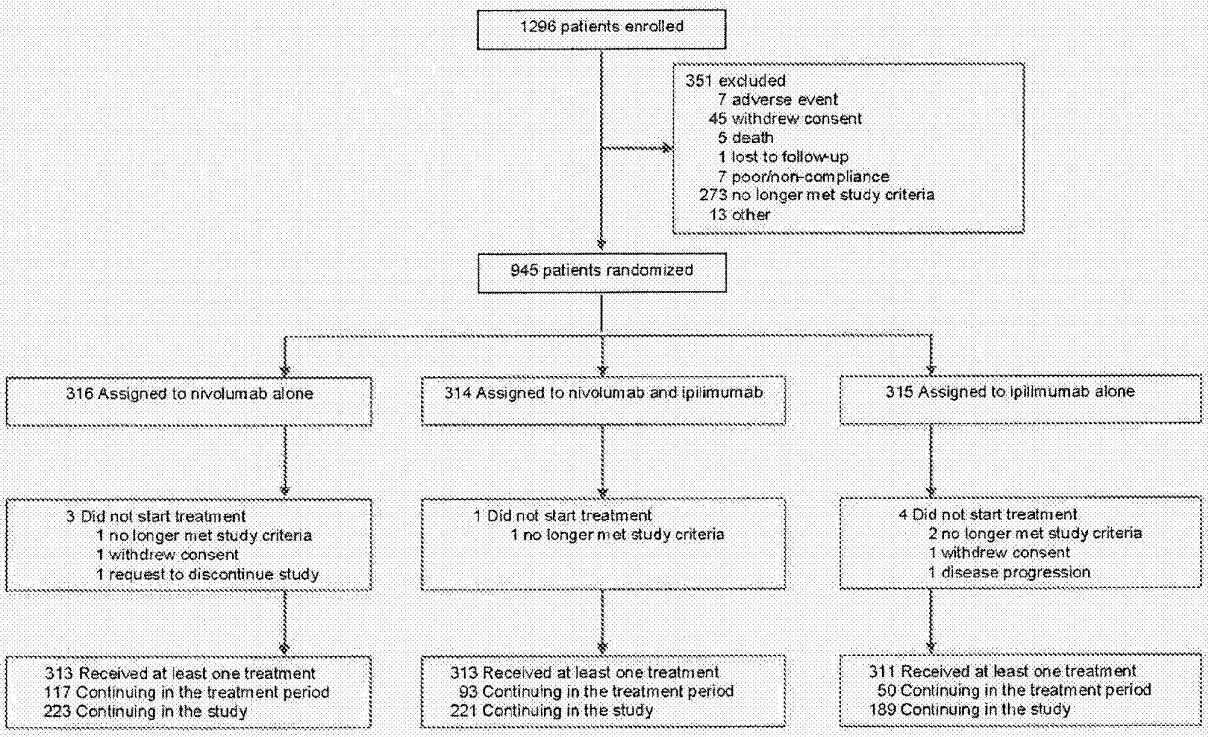
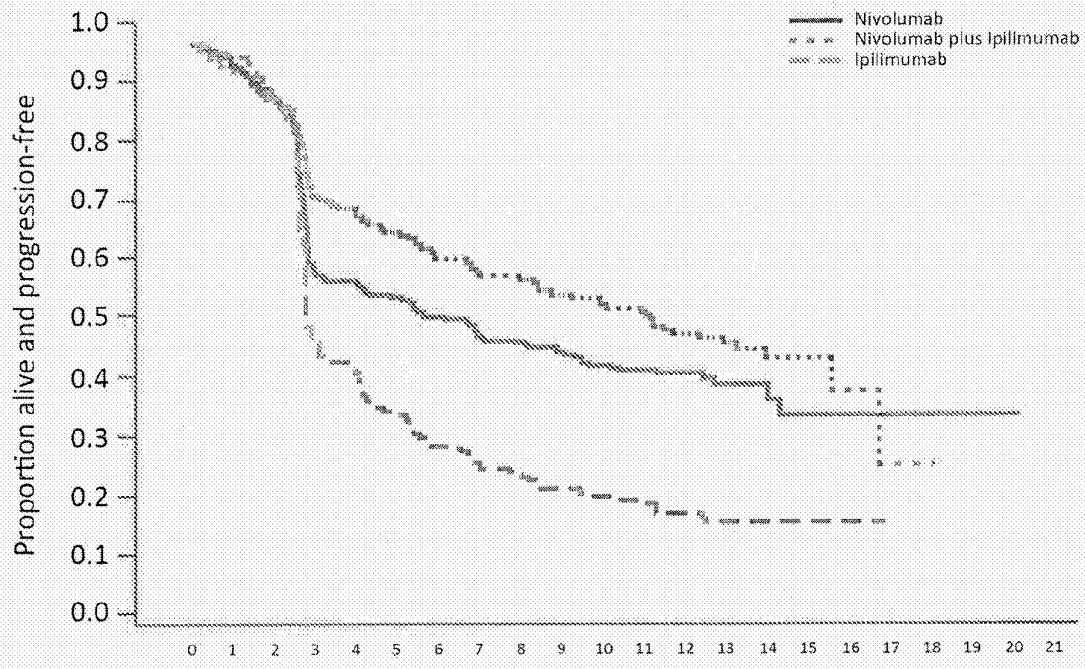
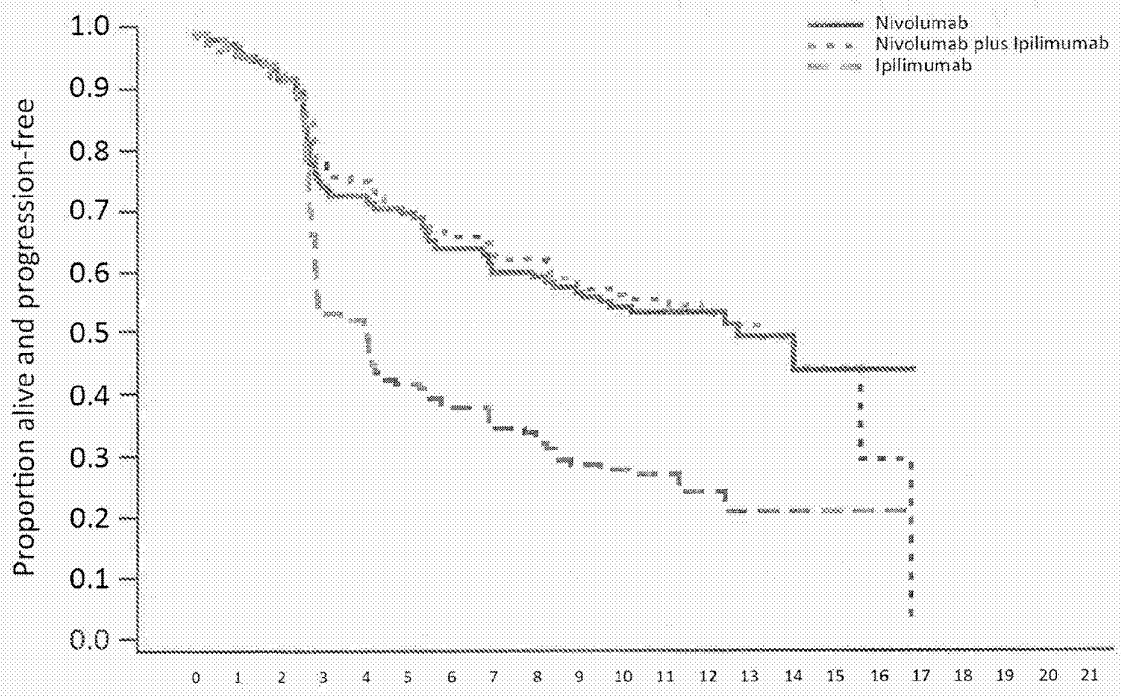


Fig. 2A – Progression-free Survival (Intention-to-Treat Population)



No. at Risk																					
Nivolumab	316	292	271	177	170	160	147	136	132	124	106	86	50	38	14	9	6	2	1	1	1	0
Nivolumab plus Ipilimumab	314	293	275	219	208	191	173	164	163	151	137	116	65	54	18	11	7	2	1	0	0	0
Ipilimumab	315	285	265	137	118	95	77	68	63	54	47	42	24	17	7	4	3	0	0	0	0	0

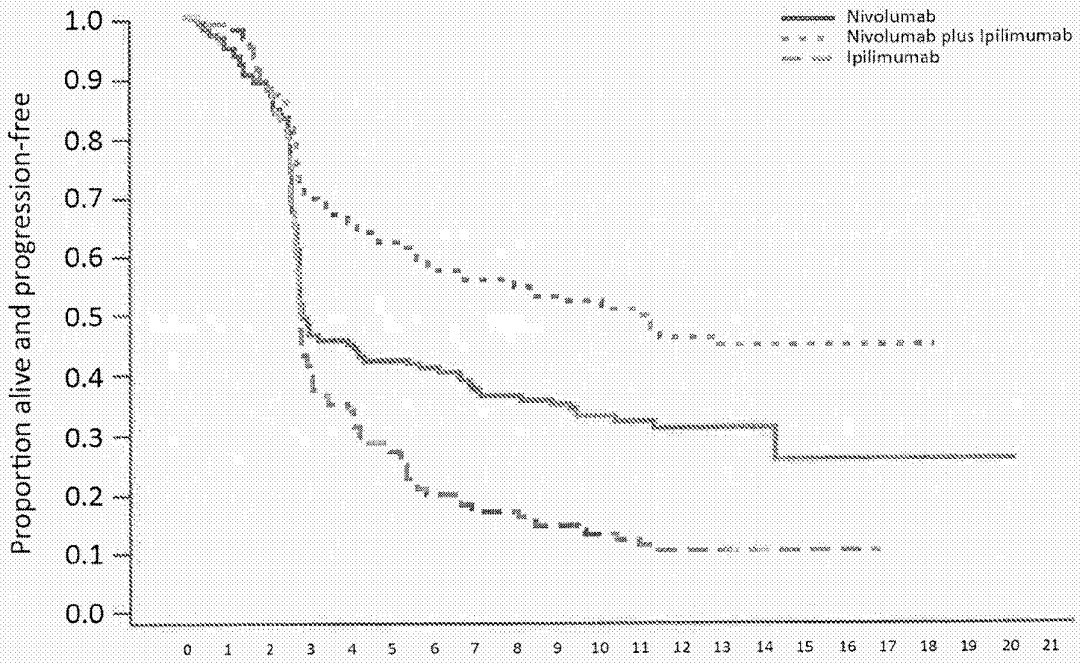
Fig. 2B – Progression-free Survival in Patients with Positive PD-L1 Status



No. at Risk

Nivolumab	143	134	126	101	98	92	83	77	76	70	63	52	28	22	8	6	3	0	0	0	0	0
Nivolumab plus Ipilimumab	144	137	131	107	103	93	85	80	80	73	67	53	32	27	7	4	2	0	0	0	0	0
Ipilimumab	144	131	125	73	66	53	46	42	38	33	29	26	15	11	5	3	2	0	0	0	0	0

Fig. 2C – Progression-free Survival in Patients with Negative PD-L1 Status



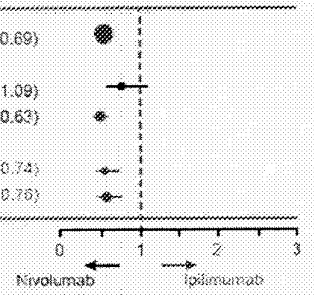
No. at risk

Nivolumab	165	150	137	72	68	64	60	55	52	50	40	32	20	15	6	3	3	2	1	1	1	0
Nivolumab plus Ipilimumab	154	142	131	101	95	88	79	76	75	70	64	57	31	25	11	7	5	2	1	0	0	0
Ipilimumab	162	146	132	60	48	38	27	23	23	19	16	14	8	5	2	1	1	0	0	0	0	0

Fig. 3 – Subgroup Analyses of Progression-free Survival

(A) Nivolumab versus ipilimumab

	N	Nivolumab N of events (N of patients)	Nivolumab Median PFS (95% CI)	Ipilimumab N of events (N of patients)	Ipilimumab Median PFS (95% CI)	Unstratified Hazard Ratio (95% CI)
Overall	945	174 (316)	6.87 (4.34, 9.46)	234 (315)	2.89 (2.79, 3.42)	0.57 (0.47, 0.69)
BRAF Mutation Status						
Mutant	300	57 (98)	5.62 (2.79, 9.46)	66 (100)	4.04 (2.79, 5.52)	0.77 (0.54, 1.09)
Wild-type	645	117 (218)	7.89 (4.86, 12.68)	168 (215)	2.83 (2.76, 3.09)	0.50 (0.39, 0.63)
M Stage at Study Entry						
M0/M1a/M1b	386	63 (131)	9.30 (5.62, N.A.)	92 (126)	4.17 (2.96, 5.52)	0.54 (0.39, 0.74)
M1c	559	111 (185)	5.39 (2.83, 8.87)	142 (189)	2.79 (2.73, 2.83)	0.59 (0.46, 0.76)



(B) Nivolumab plus ipilimumab versus ipilimumab

	N	Nivolumab + Ipilimumab N of events (N of patients)	Nivolumab + Ipilimumab Median PFS (95% CI)	Ipilimumab N of events (N of patients)	Ipilimumab Median PFS (95% CI)	Unstratified Hazard Ratio (95% CI)
Overall	945	151 (314)	11.50 (8.90, 16.72)	234 (315)	2.89 (2.79, 3.42)	0.43 (0.35, 0.53)
BRAF Mutation Status						
Mutant	300	48 (102)	11.73 (8.02, N.A.)	66 (100)	4.04 (2.79, 5.52)	0.47 (0.32, 0.68)
Wild-type	645	103 (212)	11.24 (8.34, N.A.)	168 (215)	2.83 (2.76, 3.09)	0.41 (0.32, 0.53)
M Stage at Study Entry						
M0/M1a/M1b	386	51 (129)	15.54 (11.17, N.A.)	92 (126)	4.17 (2.96, 5.52)	0.35 (0.25, 0.50)
M1c	559	100 (185)	8.51 (5.52, 12.35)	142 (189)	2.79 (2.73, 2.83)	0.48 (0.37, 0.62)

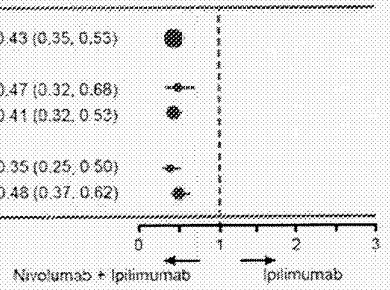
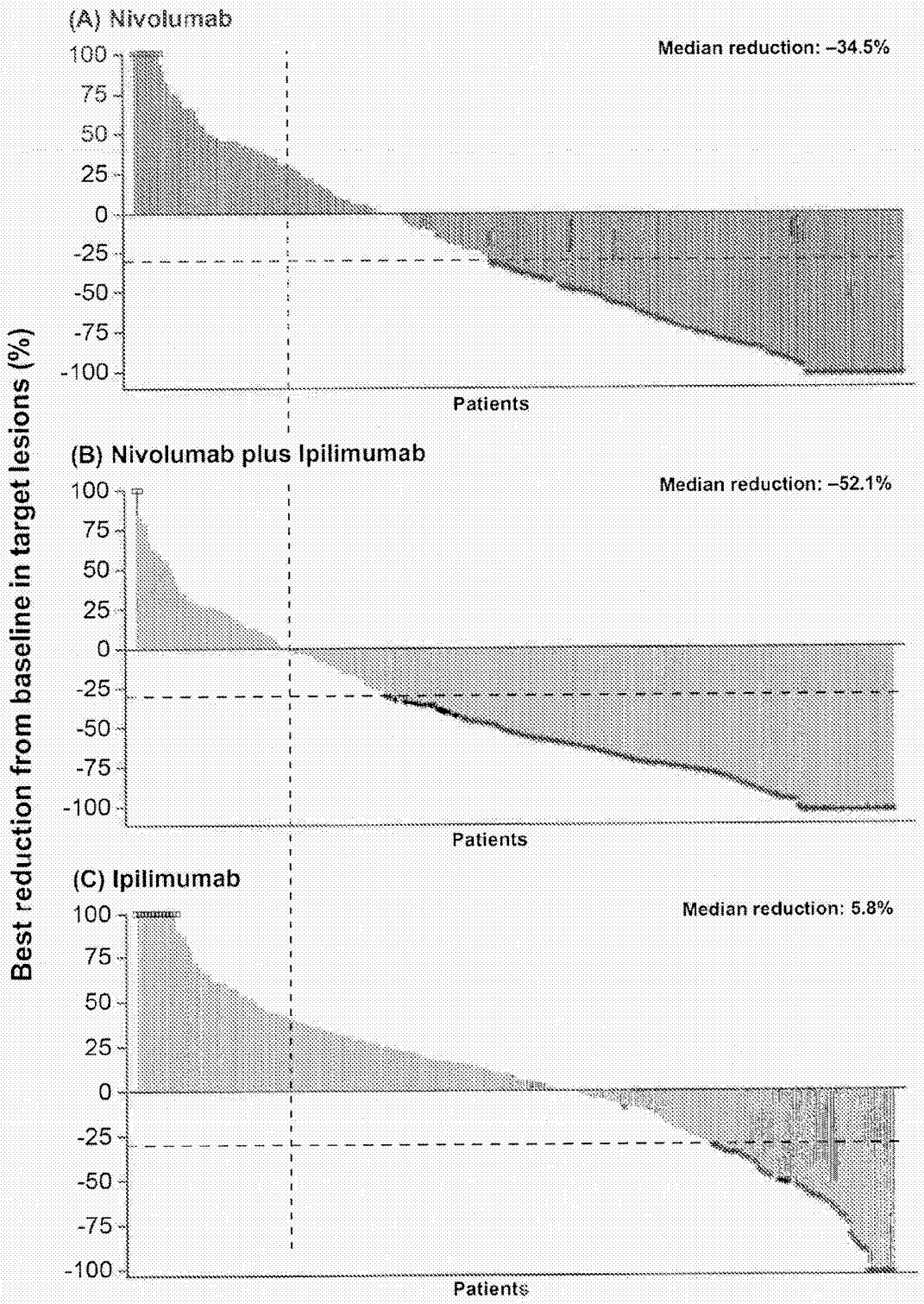


Fig. 4 – Tumor Burden Change in Target Lesions



ABSTRACT

The invention provides a method of treating a melanoma comprising (i) identifying a patient having a PD-L1-negative melanoma and (ii) administering to the patient a combination of an anti-PD-1 antibody or an antigen-binding portion thereof and an anti-CTLA-4 antibody or an antigen-binding portion thereof. The methods of the invention can extend progression-free survival for over 8 months and/or reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	3338.0330000
	Application Number	
Title of Invention	TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

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☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Arvin		YANG	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

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All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

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Title of the Invention	TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY		
Attorney Docket Number	3338.0330000	**Small Entity Status Claimed**	☐
Application Type	Provisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	6	**Suggested Figure for Publication (if any)**	

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Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

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When referring to the current application, please leave the application number blank.

Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	3338.0330000
	Application Number	
Title of Invention	TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY	

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

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Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

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

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization to Permit Access:

☒ Authorization to Permit Access to the Instant Application by the Participating Offices

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In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date o f filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

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If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

○ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
◉ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :	

If the Applicant is an Organization check here. ☒

Organization Name	BRISTOL-MYERS SQUIBB CO.

Mailing Address Information For Applicant:

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Address 2			
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Country	US	Postal Code	08543
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Email Address	

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Assignee 1

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If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

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City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

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Signature				Date (YYYY-MM-DD)	2015-04-28
First Name	Ji-Eun	Last Name	Kim	Registration Number	68504

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This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

What is claimed is:

1. A method for treating a melanoma comprising:

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

(ii) administering to the patient:

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

(b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

2. A method for treating a melanoma comprising administering to a patient afflicted with a melanoma tumor:

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

(b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4,

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

3. A method for extending a progression-free survival period for over 8 months in a patient afflicted with a melanoma tumor comprising administering to the patient:

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

(b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4,

wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the patient demonstrates progression-free survival for over 8 months.

4. The method of claim 3, wherein the progression-free survival of the patient is extended after the administration for over about 11 months, about 12 months, about 13 months, about 14

months, about 15 months, about 16 months, about 17 months, about 18 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, or about 10 years.

5. The method of claim 4, wherein the progression-free survival of the patient is extended for over 11 months.

6. A method for reducing a tumor size at least by 10% in a patient afflicted with a melanoma tumor comprising administering to the patient:

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

(b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4,

wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration.

7. The method of any one of claims 2 to 6, further comprising identifying the patient as having a melanoma tumor that does not express PD-L1 prior to the administration.

8. The method of any one of claims 1 to 7, wherein the patient is characterized by (i) extended progression-free survival for over 8 months, (ii) tumor size reduction at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration, or (iii) both.

9. A method for increasing an objective response rate to be higher than 40% in a patient population, each of whom is afflicted with a melanoma tumor, in a cancer treatment comprising administering to the patient:

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

(b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4,

wherein each patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the objective response rate is higher than 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%.

10. The method of claim 9, further comprising identifying each patient of the patient population as having a PD-L1-negative melanoma tumor prior to the administration.

11. The method of claim 9 or 10, wherein each patient of the patient population is further characterized by (i) extended progression-free survival for over 8 months, (ii) tumor size reduction at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration, or (iii) both.

12. The method of any one of claims 9 to 11, wherein the patient population comprises at least about 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 patients having a PD-L1-negative melanoma tumor.

13. A method for selecting a patient suitable for an anti-PD-1 antibody and anti-CTLA-4 antibody combination therapy comprising:

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

(ii) instructing a healthcare provider to administer to the patient:

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

(b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

14. The method of any one of claims 1, 7, 10, and 13, wherein the identifying comprises measuring a PD-L1 expression on the melanoma tumor.

15. The method of any one of claims 1 to 14, wherein the administration treats the melanoma tumor.

16. The method of claim 15 or 16, wherein the measuring comprises providing a test tissue sample obtained from the patient, the test tissue sample comprising tumor cells and/or tumor-infiltrating inflammatory cells.

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

18. The method of claim 17, wherein the test tissue sample is a formalin-fixed paraffin-embedded (FFPE) tissue sample.

19. The method of claim 18, wherein the presence of PD-L1 is determined using an automated IHC assay.

20. The method of claim 19, wherein the IHC assay is performed using an anti-PD-L1 monoclonal antibody that specifically binds to the PD-L1 and wherein the anti-PD-L1 monoclonal antibody is selected from the group consisting of 28-8, 28-1, 28-12, 29-8, 5H1, and any combination thereof.

21. The method of any one of claims 1 to 20, wherein the PD-L1-negative melanoma tumor is characterized by having less than about 5%, about 4%, about 3%, about 2%, about 1%, or about 0% of tumor cells showing binding to the anti-PD-L1 antibody or an antigen-binding portion thereof.

22. The method of any one of claims 1 to 21, wherein the anti-PD-1 antibody or antigen-binding portion thereof cross-competes with nivolumab for binding to human PD-1.

23. The method of any one of claims 1 to 22, wherein the anti-PD-1 antibody or antigen-binding portion thereof is a chimeric, humanized or human monoclonal antibody or a portion thereof.

24. The method of any one of claims 1 to 23, wherein the anti-PD-1 antibody or antigen-binding portion thereof comprises a heavy chain constant region which is of a human IgG1 or IgG4 isotype.

25. The method of any one of claims 1 to 24, wherein the anti-PD-1 antibody is nivolumab.

26. The method of any one of claims 1 to 24, wherein the anti-PD-1 antibody is pembrolizumab.

27. The method of any one of claims 1 to 26, wherein the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose ranging from 0.1 to 10.0 mg/kg body weight once every 2, 3 or 4 weeks.

28. The method of claim 27, wherein the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of 1 or 3 mg/kg body weight once every 3 weeks.

29. The method of any one of claims 1 to 28, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof cross-competes with ipilimumab for binding to human CTLA-4.

30. The method of any one of claims 1 to 29, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof is a chimeric, humanized or human monoclonal antibody or a portion thereof.

31. The method of any one of claims 1 to 30, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof comprises a heavy chain constant region which is of a human IgG1 isotype.

32. The method of any one of claims 1 to 31, wherein the anti-CTLA-4 antibody is ipilimumab.

33. The method of any one of claims 1 to 31, wherein the anti-CTLA-4 antibody is tremelimumab.

34. The method of any one of claims 1 to 33, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof is administered at a dose ranging from 0.1 to 10.0 mg/kg body weight once every 2, 3 or 4 weeks.

35. The method of claim 34, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof is administered at a dose of 1 or 3 mg/kg body weight once every 3 weeks.

36. The method of claim 35, wherein the anti-PD-1 antibody or an antigen-binding portion thereof is administered at a dose of 1 mg/kg body weight once every 3 weeks and the anti-

CTLA-4 antibody or an antigen-binding portion thereof is administered at a dose of 3 mg/kg body weight once every 3 weeks.

37. The method of any of claims 1 to 36, wherein the anti-PD-1 antibody or an antigen-binding portion thereof is administered concurrently with the anti-CTLA-4 antibody or an antigen-binding portion thereof.

38. The method of any of claims 1 to 36, wherein the anti-PD-1 antibody or an antigen-binding portion thereof is administered prior to or after the anti-CTLA-4 antibody or an antigen-binding portion thereof.

39. The method of any of claims 1 to 36, wherein the anti-PD-1 antibody or an antigen-binding portion thereof and the anti-CTLA-4 antibody or an antigen-binding portion thereof are in a single pharmaceutical composition.

40. The method of claim 39, wherein the pharmaceutical composition comprises the anti-PD-1 antibody and the anti-CTLA-4 antibody at a ratio of 50:1 to 1:50.

41. The method of claim 39, wherein the pharmaceutical composition comprises the anti-PD-1 antibody and the anti-CTLA-4 antibody at a ratio of 10:1 to 1:10.

42. The method of claim 39, wherein the pharmaceutical composition comprises the anti-PD-1 antibody and the anti-CTLA-4 antibody at a ratio of 3:1 to 1:3.

43. The method of claim 39, wherein the pharmaceutical composition comprises the anti-PD-1 antibody and the anti-CTLA-4 antibody at a ratio of 1:3.

44. The method of claim 39, wherein the pharmaceutical composition comprises 80 mg of the anti-PD-1 antibody and 240 mg of the anti-CTLA-4 antibody.

45. The method of claim any of claims 1 to 38, wherein the anti-PD-1 and anti-CTLA-4 antibodies are administered within 30 minutes of each other.

46. The method of any one of claims 1 to 45, further comprising administering an anti-cancer agent.

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

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

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

(c) instructions for using the anti-PD-1 antibody or the antigen-binding portion thereof and the anti-CTLA-4 antibody or the antigen-binding portion thereof in the method of any one of claims 1 to 46.

Electronic Acknowledgement Receipt	
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International Application Number:	
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Title of Invention:	TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY
First Named Inventor/Applicant Name:	Arvin YANG
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Document Description	Start	End
Miscellaneous Incoming Letter	1	2
Provisional Cover Sheet (SB16)	3	5
Authorization for Extension of Time all replies	6	6
Application Data Sheet	7	12

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Document Description	Start	End
Specification	1	53
Claims	54	60
Abstract	61	61
Drawings-only black and white line drawings	62	67

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4	Fee Worksheet (SB06)	fee-info.pdf	30217 1bc77bfd4fa2042839c71e5ce271f08e4d0b6a09	no	2
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Application Number	To Be Assigned
Filing Date	Herewith
First Named Inventor	Arvin YANG
Title	TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY
Art Unit	To Be Assigned
Examiner Name	To Be Assigned
Attorney Docket Number	3338.0330000

SIGNATURE of Applicant or Patent Practitioner

Signature	Ji-Eun Kim	Date (Optional)	April 28, 2015
Name	Ji-Eun Kim	Registration Number	68,504
Title (if Applicant is a juristic entity)	Attorney for Applicant		
Applicant Name (if Applicant is a juristic entity)	Bristol-Myers Squibb Co.		

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[ ] Inventor or Joint Inventor

[ ] Legal Representative of a Deceased or Legally Incapacitated Inventor

[X] Assignee or Person to Whom the Inventor is Under an Obligation to Assign

[ ] Person Who Otherwise Shows Sufficient Proprietary Interest (*e.g.*, a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document)

SIGNATURE of Applicant for Patent

Signature	I. Clauss	Date	9/20/13
Name	Isabelle M. Clauss, Ph.D., Esq.	Telephone	609-252-6254
Title and Company	Senior Counsel, Patents - Bristol-Myers Squibb Company		

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

[0001] Throughout this application, various publications are referenced in parentheses by author name and date, or by Patent No. or Patent Publication No. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications are hereby incorporated in their entireties by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein. However, the citation of a reference herein should not be construed as an acknowledgement that such reference is prior art to the present invention.

FIELD OF THE INVENTION

[0002] This invention relates to a method of treating PD-L1-negative melanoma comprising administering a combination of an anti-PD-1 antibody and an anti-CTLA-4 antibody.

BACKGROUND OF THE INVENTION

[0003] Human cancers harbor numerous genetic and epigenetic alterations, generating neoantigens potentially recognizable by the immune system (Sjoblom et al., 2006). The adaptive immune system, comprised of T and B lymphocytes, has powerful anti-cancer potential, with a broad capacity and exquisite specificity to respond to diverse tumor antigens. Further, the immune system demonstrates considerable plasticity and a memory component. The successful harnessing of all these attributes of the adaptive immune system would make immunotherapy unique among all cancer treatment modalities.

[0004] Recently, several immune checkpoint pathway inhibitors have begun to provide new immunotherapeutic approaches for treating cancer, including the development of an antibody (Ab), ipilimumab (YERVOY®), that binds to and inhibits Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) for the treatment of patients with advanced melanoma and the development of Abs such as nivolumab and pembrolizumab (formerly

lambrolizumab; USAN Council Statement, 2013) that bind specifically to the Programmed Death-1 (PD-1) receptor and block the inhibitory PD-1/PD-1 ligand pathway.

[0005] The promise of the emerging field of personalized medicine is that advances in pharmacogenomics will increasingly be used to tailor therapeutics to defined sub-populations, and ultimately, individual patients in order to enhance efficacy and minimize adverse effects. Recent successes include, for example, the development of imatinib mesylate (GLEEVEC®), a protein tyrosine kinase inhibitor that inhibits the bcr-abl tyrosine kinase, to treat Philadelphia chromosome-positive chronic myelogenous leukemia (CML); crizotinib (XALKORI®) to treat the 5% of patients with late-stage non-small cell lung cancers who express a mutant anaplastic lymphoma kinase (ALK) gene; and vemurafenib (ZELBORAF®), an inhibitor of mutated B-RAF protein (V600E-BRAF) which is expressed in around half of melanoma tumors. However, unlike the clinical development of small molecule agents that target discrete activating mutations found in select cancer populations, a particular challenge in cancer immunotherapy has been the identification of mechanism-based predictive biomarkers to enable patient selection and guide on-treatment management.

SUMMARY OF THE INVENTION

[0006] The present disclosure provides a method for treating a melanoma comprising (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4. The present disclosure also provides a method for treating a melanoma comprising administering to a patient afflicted with a melanoma tumor (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration.

[0007] The present disclosure further provides a method for extending a progression-free survival period for over 8 months in a patient afflicted with a melanoma tumor

comprising administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the patient demonstrates progression-free survival for over 8 months. In certain embodiments, the progression-free survival of the patient is extended after the administration for over about 11 months, about 12 months, about 13 months, about 14 months, about 15 months, about 16 months, about 17 months, about 18 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, or about 10 years. In one particular embodiment, the progression-free survival of the patient is extended for over 11 months.

[0008] The present disclosure also provides a method for reducing a tumor size at least by 10% in a patient afflicted with a melanoma tumor comprising administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration.

[0009] The present disclosure also provides a method for increasing an objective response rate to be higher than 40% in a patient population, each of whom is afflicted with a melanoma tumor, in a cancer treatment comprising administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein each patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the objective response rate is higher than 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%.

[0010] The present disclosure also provides a method for selecting a patient suitable for an anti-PD-1 antibody and anti-CTLA-4 antibody combination therapy comprising (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) instructing a healthcare provider to administer to the patient (a) an anti-PD-1 antibody or an antigen-

binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

[0011] In certain embodiments, the methods disclosed herein further comprise identifying the patient as having a melanoma tumor that does not express PD-L1 prior to the administration. In some embodiments, the patient is characterized by (i) extended progression-free survival for over 8 months, (ii) tumor size reduction at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration, or (iii) both.

[0012] In some embodiments, the methods disclosed herein further comprise measuring a PD-L1 expression on the melanoma tumor. In certain embodiments, the measuring further comprises assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface. In one particular embodiment, the presence of PD-L1 is determined using an automated IHC assay.

[0013] The present disclosure further provides a kit for treating a patient afflicted with a melanoma tumor, the kit comprising (a) a dosage ranging from 0.1 to 10 mg/kg body weight of an anti-PD-1 antibody or an antigen-binding portion thereof; (b) a dosage ranging from 0.1 to 10 mg/kg body weight of an anti-CTLA-4 antibody or an antigen-binding portion thereof; and (c) instructions for using the anti-PD-1 antibody or the antigen-binding portion thereof and the anti-CTLA-4 antibody or the antigen-binding portion thereof in any of the methods disclosed herein.

BRIEF DESCRIPTION OF THE FIGURES

[0014] Figure 1 shows a patient flow diagram of the randomized, double-blind, multicenter, phase 3 trial.

[0015] Figures 2A-C show progression-free survival data in the intention-to-treat population (Figure 2A), in patients with positive PD-L1 status (Figure 2B), and Negative PD-L1 Status (Figure 2C). Each graph shows the progression free survival for patients treated with nivolumab alone (solid line), ipilimumab alone (dashed line), or the combination of nivolumab and ipilimumab (dotted line) in months (Figures 2A-C). The number at risk in months for each of nivolumab, nivolumab plus ipilimumab, and

ipilimumab is shown below each x-axis (Figures 2A-C). PD-L1 expression status is based on verified PD-L1 assay data (Figures 2B-C).

[0016] Figures 3A-B show subgroup analyses of progression-free survival among patients treated with nivolumab alone compared to ipilimumab alone (Figure 3A) and patients treated with nivolumab plus ipilimumab compared to ipilimumab alone (Figure 3B).

[0017] Figures 4A-C show the tumor burden change in target lesions in patients treated with nivolumab alone (Figure 4A), nivolumab plus ipilimumab (Figure 4B), and ipilimumab alone (Figure 4C). In each graph, the y-axis shows the best reduction from baseline in target lesions (%) and the x-axis represents each patient (Figures 4A-C).

DETAILED DESCRIPTION OF THE INVENTION

[0018] The present invention relates to identifying an optimal strategy to treat a patient having a PD-L1-negative melanoma. The present invention shows that in a patient having a PD-L1-negative melanoma tumor, a combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody provides a better response (e.g., progression-free survival) than a monotherapy of either an anti-PD-1 antibody or an anti-CTLA-4 antibody. Furthermore, the combination therapy when given to a population of patients can increase the objective response rate compared to the monotherapy.

Definitions

[0019] In order that the present disclosure may be more readily understood, certain terms are first defined. As used in this application, except as otherwise expressly provided herein, each of the following terms shall have the meaning set forth below. Additional definitions are set forth throughout the application.

[0020] The term "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects:

A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0021] It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0022] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0023] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. The headings provided herein are not limitations of the various aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0024] "Administering" refers to the physical introduction of a composition comprising a therapeutic agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. Preferred routes of administration for the formulations disclosed herein include intravenous, intramuscular, subcutaneous, intraperitoneal, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as *in vivo* electroporation. In some embodiments, the formulation is administered via a non-parenteral route, preferably orally. Other non-parenteral routes include a topical,

epidermal or mucosal route of administration, for example, intranasally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

[0025] An "adverse event" (AE) as used herein is any unfavorable and generally unintended or undesirable sign (including an abnormal laboratory finding), symptom, or disease associated with the use of a medical treatment. For example, an adverse event may be associated with activation of the immune system or expansion of immune system cells (*e.g.*, T cells) in response to a treatment. A medical treatment may have one or more associated AEs and each AE may have the same or different level of severity. Reference to methods capable of "altering adverse events" means a treatment regime that decreases the incidence and/or severity of one or more AEs associated with the use of a different treatment regime.

[0026] An "antibody" (Ab) shall include, without limitation, a glycoprotein immunoglobulin which binds specifically to an antigen and comprises at least two heavy (H) chains and two light (L) chains interconnected by disulfide bonds, or an antigen-binding portion thereof. Each H chain comprises a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region comprises three constant domains, C_{H1}, C_{H2} and C_{H3}. Each light chain comprises a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprises one constant domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L comprises three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the Abs may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system.

[0027] An immunoglobulin may derive from any of the commonly known isotypes, including but not limited to IgA, secretory IgA, IgG and IgM. IgG subclasses are also

well known to those in the art and include but are not limited to human IgG1, IgG2, IgG3 and IgG4. "Isotype" refers to the Ab class or subclass (*e.g.*, IgM or IgG1) that is encoded by the heavy chain constant region genes. The term "antibody" includes, by way of example, both naturally occurring and non-naturally occurring Abs; monoclonal and polyclonal Abs; chimeric and humanized Abs; human or nonhuman Abs; wholly synthetic Abs; and single chain Abs. A nonhuman Ab may be humanized by recombinant methods to reduce its immunogenicity in man. Where not expressly stated, and unless the context indicates otherwise, the term "antibody" also includes an antigen-binding fragment or an antigen-binding portion of any of the aforementioned immunoglobulins, and includes a monovalent and a divalent fragment or portion, and a single chain Ab.

[0028] The term "monoclonal antibody" ("mAb") refers to a non-naturally occurring preparation of Ab molecules of single molecular composition, *i.e.*, Ab molecules whose primary sequences are essentially identical, and which exhibits a single binding specificity and affinity for a particular epitope. A mAb is an example of an isolated Ab. MAbs may be produced by hybridoma, recombinant, transgenic or other techniques known to those skilled in the art.

[0029] A "human" antibody (HuMAb) refers to an Ab having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the Ab contains a constant region, the constant region is also derived from human germline immunoglobulin sequences. The human Abs of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody," as used herein, is not intended to include Abs in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. The terms "human" Abs and "fully human" Abs and are used synonymously.

[0030] A "humanized antibody" refers to an Ab in which some, most or all of the amino acids outside the CDR domains of a non-human Ab are replaced with corresponding amino acids derived from human immunoglobulins. In one embodiment of a humanized form of an Ab, some, most or all of the amino acids outside the CDR domains have been

replaced with amino acids from human immunoglobulins, whereas some, most or all amino acids within one or more CDR regions are unchanged. Small additions, deletions, insertions, substitutions or modifications of amino acids are permissible as long as they do not abrogate the ability of the Ab to bind to a particular antigen. A "humanized" Ab retains an antigenic specificity similar to that of the original Ab.

[0031] A "chimeric antibody" refers to an Ab in which the variable regions are derived from one species and the constant regions are derived from another species, such as an Ab in which the variable regions are derived from a mouse Ab and the constant regions are derived from a human Ab.

[0032] An "anti-antigen" Ab refers to an Ab that binds specifically to the antigen. For example, an anti-PD-1 Ab binds specifically to PD-1 and an anti-CTLA-4 Ab binds specifically to CTLA-4.

[0033] An "antigen-binding portion" of an Ab (also called an "antigen-binding fragment") refers to one or more fragments of an Ab that retain the ability to bind specifically to the antigen bound by the whole Ab.

[0034] A "cancer" refers a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth results in the formation of malignant tumors that invade neighboring tissues and may also metastasize to distant parts of the body through the lymphatic system or bloodstream. A "cancer" or "cancer tissue" can include a tumor.

[0035] "Cytotoxic T-Lymphocyte Antigen-4" (CTLA-4) refers to an immunoinhibitory receptor belonging to the CD28 family. CTLA-4 is expressed exclusively on T cells *in vivo*, and binds to two ligands, CD80 and CD86 (also called B7-1 and B7-2, respectively). The term "CTLA-4" as used herein includes human CTLA-4 (hCTLA-4), variants, isoforms, and species homologs of hCTLA-4, and analogs having at least one common epitope with hCTLA-4. The complete hCTLA-4 sequence can be found under GenBank Accession No. AAB59385.

[0036] The term “progression-free survival,” which can be abbreviated as PFS, as used herein refers to the length of time during and after the treatment of a solid tumor (*i.e.*, melanoma) that a patient lives with the disease but it does not get worse.

[0037] "Dosing interval," as used herein, means the amount of time that elapses between multiple doses of a formulation disclosed herein being administered to a subject. Dosing interval can thus be indicated as ranges.

[0038] The term "dosing frequency" as used herein refers to the frequency of administering doses of a formulation disclosed herein in a given time. Dosing frequency can be indicated as the number of doses per a given time, *e.g.*, once a week or once in two weeks.

[0039] The use of the term "fixed dose" with regard to a composition of the invention means that two or more different antibodies in a single composition are present in the composition in particular (fixed) ratios with each other. In some embodiments, the fixed dose is based on the weight (*e.g.*, mg) of the antibodies. In certain embodiments, the fixed dose is based on the concentration (*e.g.*, mg/ml) of the antibodies. In some embodiments, the ratio is at least about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9, about 1:10, about 1:15, about 1:20, about 1:30, about 1:40, about 1:50, about 1:60, about 1:70, about 1:80, about 1:90, about 1:100, about 1:120, about 1:140, about 1:160, about 1:180, about 1:200, about 200:1, about 180:1, about 160:1, about 140:1, about 120:1, about 100:1, about 90:1, about 80:1, about 70:1, about 60:1, about 50:1, about 40:1, about 30:1, about 20:1, about 15:1, about 10:1, about 9:1, about 8:1, about 7:1, about 6:1, about 5:1, about 4:1, about 3:1, or about 2:1 mg first antibody to mg second antibody. For example, the 3:1 ratio of a first antibody and a second antibody can mean that a vial can contain about 240 mg of the first antibody and 80 mg of the second antibody or about 3 mg/ml of the first antibody and 1 mg/ml of the second antibody.

[0040] The use of the term "flat dose" with regard to the composition of the invention means a dose that is administered to a patient without regard for the weight or body surface area (BSA) of the patient. The flat dose is therefore not provided as a mg/kg dose, but rather as an absolute amount of the agent (*e.g.,* the anti-CTLA-4 antibody and/or anti-PD-1 antibody). For example, a 60 kg person and a 100 kg person would receive the same dose of the composition (*e.g.*, 240 mg of an anti-PD-1 antibody and 80 mg of an anti-CTLA-4 antibody in a single fixed dosing formulation vial containing both 240 mg of an anti-PD-1 antibody and 80 mg of an anti-CTLA-4 antibody (or two fixed dosing

formulation vials containing 120 mg of an anti-PD-1 antibody and 40 mg of an anti-CTLA-4 antibody, etc.)).

[0041] The term "weight based dose" as referred to herein means that a dose that is administered to a patient is calculated based on the weight of the patient. For example, when a patient with 60 kg body weight requires 3 mg/kg of an anti-PD-1 antibody in combination with 1 mg/kg of an anti-CTLA-4 antibody, one can draw the appropriate amounts of the anti-PD-1 antibody (*i.e.*, 180 mg) and the anti-CTLA-4 antibody (*i.e.*, 60 mg) at once from a 3:1 ratio fixed dosing formulation of an anti-PD1 antibody and an anti-CTLA-4 antibody.

[0042] An "immune response" refers to the action of a cell of the immune system (for example, T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells and neutrophils) and soluble macromolecules produced by any of these cells or the liver (including Abs, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from a vertebrate's body of invading pathogens, cells or tissues infected with pathogens, cancerous or other abnormal cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

[0043] 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.

[0044] 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.

[0045] "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.

[0046] A "predetermined threshold value," relating to cell surface PD-L1 expression, refers to the proportion of cells in a test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells above which the sample is scored as being positive for cell surface PD-L1 expression and below which the sample is scored as being negative 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 some 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. In some embodiments, the predetermined threshold value is at least about 5%. In certain embodiments, the predetermined threshold value is at least about 1%, or in the range of 1-5%.

[0047] "Programmed Death-1 (PD-1)" refers to an immunoinhibitory receptor belonging to the CD28 family. PD-1 is expressed predominantly on previously activated T cells *in vivo*, and binds to two ligands, PD-L1 and PD-L2. The term "PD-1" as used herein includes human PD-1 (hPD-1), variants, isoforms, and species homologs of hPD-1, and analogs having at least one common epitope with hPD-1. The complete hPD-1 sequence can be found under GenBank Accession No. U64863.

[0048] "Programmed Death Ligand-1 (PD-L1)" is one of two cell surface glycoprotein ligands for PD-1 (the other being PD-L2) that down-regulate T cell activation and cytokine secretion upon binding to PD-1. The term "PD-L1" as used herein includes human PD-L1 (hPD-L1), variants, isoforms, and species homologs of hPD-L1, and analogs having at least one common epitope with hPD-L1. The complete hPD-L1 sequence can be found under GenBank Accession No. Q9NZQ7.

[0049] A "patient" as used herein includes any patient who is afflicted with a cancer (*e.g.*, melanoma). The terms "subject" and "patient" are used interchangeably herein.

[0050] A "therapeutically effective amount" or "therapeutically effective dosage" of a drug or therapeutic agent is any amount of the drug that, when used alone or in

combination with another therapeutic agent, protects a subject against the onset of a disease or promotes disease regression evidenced by a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. The ability of a therapeutic agent to promote disease regression can be evaluated using a variety of methods known to the skilled practitioner, such as in human subjects during clinical trials, in animal model systems predictive of efficacy in humans, or by assaying the activity of the agent in *in vitro* assays.

[0051] "Treatment" or "therapy" of a subject refers to any type of intervention or process performed on, or the administration of an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, slowing down or preventing the onset, progression, development, severity or recurrence of a symptom, complication or condition, or biochemical indicia associated with a disease.

[0052] A "tumor-infiltrating inflammatory cell" is any type of cell that typically participates in an inflammatory response in a subject and which infiltrates tumor tissue. Such cells include tumor-infiltrating lymphocytes (TILs), macrophages, monocytes, eosinophils, histiocytes and dendritic cells.

[0053] The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the indefinite articles "a" or "an" should be understood to refer to "one or more" of any recited or enumerated component.

[0054] The terms "about" or "comprising essentially of" refer to a value or composition that is within an acceptable error range for the particular value or composition as determined by one of ordinary skill in the art, which will depend in part on how the value or composition is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" or "comprising essentially of" can mean within 1 or more than 1 standard deviation per the practice in the art. Alternatively, "about" or "comprising essentially of" can mean a range of up to 10% or 20% (*i.e.*, ±10% or ±20%). For example, about 3mg can include any number between 2.7 mg and 3.3 mg (for 10%) or between 2.4 mg and 3.6 mg (for 20%). Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to

5-fold of a value. When particular values or compositions are provided in the application and claims, unless otherwise stated, the meaning of "about" or "comprising essentially of" should be assumed to be within an acceptable error range for that particular value or composition.

[0055] The term "about once a week" as used herein means approximate number, and "about once a week" can include every seven days ± two days, *i.e.,* every five days to every nine days. The dosing frequency of "once a week" thus can be every five days, every six days, every seven days, every eight days, or every nine days.

[0056] As described herein, any concentration range, percentage range, ratio range or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one-tenth and one-hundredth of an integer), unless otherwise indicated.

[0057] Various aspects of the invention are described in further detail in the following subsections.

Methods of the Invention

[0058] The present invention is directed to a method for treating a PD-L1-negative melanoma in a subject in need thereof. The present invention shows that for PD-L1 negative tumors, a combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody is more suitable than a monotherapy of either an anti-PD-1 antibody or an anti-CTLA-4 antibody.

[0059] Not bound to any theory, the present invention identifies that in patients having a PD-L1-negative tumor progression-free survival and overall response rate are higher following treatment with a combination therapy with an anti-PD-1 antibody and an anti-CTLA-4 antibody than following treatment with either an anti-PD-1 antibody or an anti-CTLA-4 antibody alone. Therefore, in order to increase the response of a patient having a PD-L1-negative tumor, the present invention provides identifying a patient suitable for the combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody.

[0060] In one embodiment, the invention includes a method of treating a PD-L1 negative melanoma comprising administering to a patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4

antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration. In another embodiment, the invention includes a method for treating a melanoma in a patient in need thereof comprising: (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

[0061] In certain embodiments, the invention includes a method for extending a progression-free survival period for over 9 months in a patient afflicted with a PD-L1 negative melanoma tumor comprising administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the patient demonstrates progression-free survival for over 9 months. In other embodiments, the invention provides a method for extending a progression-free survival period for over 9 months in a patient afflicted with a melanoma tumor comprising: (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient demonstrates progression-free survival for over 9 months. According to the invention, the progression-free survival of the patient can be extended, after the administration, over about 10 months, over about 11 months, over about 12 months, over about 13 months, about 14 months, about 15 months, about 16 months, about 17 months, about 18 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, or about 10 years. In a particular embodiment, the progression-free survival of the patient is extended for over 10 months.

[0062] In still other embodiments, the invention is directed to a method for reducing a tumor size at least by 10% in a patient afflicted with a PD-L1 negative melanoma tumor

comprising administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration. In yet other embodiments, the method comprises (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration. The tumor size, after the administration, can be reduced at least about 60%, 70%, 80%, 90% or 100%. The tumor can be completely eliminated from the patient's body after the administration.

[0063] The invention can also include a method of preventing a relapse and/or induce a remission to a patient having a PD-L1 negative melanoma tumor comprising administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration. In some embodiments, the method of the invention comprises (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

[0064] In certain embodiments, the invention includes a method for increasing an objective response rate to be higher than 40% in a patient population, wherein each patient of the patient population is afflicted with a melanoma tumor, in a cancer treatment comprising administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody

or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein each patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the objective response rate is higher than 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%. The methods can further comprise identifying each patient as having a PD-L1-negative melanoma tumor prior to the administration. In other embodiments, each patient in the methods can further be characterized by (i) extended progression-free survival for over 11 months, (ii) tumor size reduction at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration, or (iii) both. In some embodiments, the patient population can be at least 100 patients having a PD-L1-negative melanoma tumor. In some embodiments, the patient population can be at least 200, 300, 400, 500, 600, 700, 800, 900, or 1000 patients having a PD-L1-negative melanoma tumor.

[0065] In further embodiments, the invention provides a method for selecting a suitable cancer therapy course in a patient having a PD-L1 negative melanoma tumor comprising: (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) instructing a healthcare provider to administer to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4. The method further comprises administering the antibodies to the patient.

[0066] The methods of the invention as a result of the combination therapy can treat the melanoma tumor, reduce the tumor size, prevent growth of the tumor, eliminate the tumor from the patient, prevent a relapse of a tumor, induce a remission in a patient, or any combination thereof. In certain embodiments, the combination therapy induces a complete response. In other embodiments, the combination therapy induces a partial response.

Melanoma

[0067] Melanoma (MEL) is a malignant tumor of melanocytes, the melanin-producing cells found predominantly in skin. Though less common than other skin cancers, it is the most dangerous of skin cancers if not diagnosed early and causes the majority (75%) of skin cancer deaths. The incidence of MEL is increasing worldwide in Caucasian populations, especially where peoples with low amounts of skin pigmentation receive

excessive ultraviolet light exposure from the sun. In Europe, the incidence rate is < 10-20 per 100,000 population; in the USA 20-30 per 100,000; and in Australia, where the highest incidence is observed, 50-60 per 100,000 (Garbe et al., 2012). MEL accounts for about 5% of all new cases of cancer in the United States (U.S.), and the incidence continues to rise by almost 3% per year. This translates to an estimated 76,690 new cases in the U.S. in 2013 with 9,480 associated deaths (Siegel et al. (2013)).

[0068] For in situ (stage 0) or early-stage MEL (Stages I-II), surgical excision is the primary treatment. In general, the prognosis is excellent for patients with localized disease and tumors 1.0 mm or less in thickness, with 5-year survival rates of more than 90% (NCCN GUIDELINES®, 2013- Melanoma). Where surgical excision is not feasible for in situ melanoma due to comorbidity or cosmetically sensitive tumor location, topical imiquimod (INN) and radiotherapy are emerging as treatments, especially for *lentigo maligna*. Chemotherapeutic agents for treating MEL include dacarbazine, temozolomide and imatinib for melanoma with a c-KIT mutation, high-dose interleukin-2, and paclitaxel with or without carboplatin. However, these treatments have modest success, with response rates below 20% in first-line (1L) and second-line (2L) settings.

[0069] For patients with localized melanomas more than 1.0 mm in thickness, survival rates range from 50-90%. The likelihood of regional nodal involvement increases with increasing tumor thickness. With Stage III MEL (clinically positive nodes and/or in-transit disease), 5-year survival rates range from 20-70%. By far the most lethal is Stage IV MEL where long-term survival in patients with distant metastatic melanoma is less than 10% (NCCN GUIDELINES®, 2013 - melanoma).

[0070] The types of melanoma that can be treated with the present methods include, but are not limited to, lentigo maligna, lentigo maligna melanoma, superficial spreading melanoma, acral lentiginous melanoma, nucosal melanoma, nodular melanoma, polypoid melanoma, desmoplastic melanoma, amelanotic melanoma, soft-tissue melanoma, melanoma with small nevus-like cells, melanoma with features of a Spitz nevus, or uveal melanoma. The stages of melanoma that can be treated with the present methods include, but are not limited to, (i) Stage I / II (invasive melanoma): T1a characterized by less than 1.0 mm primary tumor thickness, without ulceration, and mitosis < $1/mm^2$; T1b characterized by less than 1.0 mm primary tumor thickness, with ulceration or mitoses ≥

$1/mm^2$; T2a characterized by 1.01–2.0 mm primary tumor thickness, without ulceration; (ii) Stage II (high risk melanoma): T2b characterized by 1.01–2.0 mm primary tumor thickness, with ulceration; T3a characterized by 2.01–4.0 mm primary tumor thickness, without ulceration; T3b characterized by 2.01–4.0 mm primary tumor thickness, with ulceration; T4a characterized by greater than 4.0 mm primary tumor thickness, without ulceration; or T4b characterized by greater than 4.0 mm primary tumor thickness, with ulceration; (iii) Stage III (regional metastasis): N1 characterized by single positive lymph node; N2 characterized by two to three positive lymph nodes or regional skin/in-transit metastasis; or N3 characterized by four positive lymph nodes or one lymph node and regional skin/in-transit metastases; and (iv) Stage IV (distant metastasis): M1a characterized by distant skin metastasis, normal LDH; M1b characterized by Lung metastasis, normal LDH; or M1c characterized by other distant metastasis or any distant metastasis with elevated LDH. PD-L1-negative tumors that are treatable by the present methods can lack PD-L1 expression on the surface of tumor cells and/or tumor infiltrating inflammatory cells.

Measurement of PD-L1 expression

[0071] In certain embodiments, identifying a patient suitable for a combination therapy for the present methods includes measuring or assessing a PD-L1 expression on the surface of the melanoma tumor cells or tumor infiltrating inflammatory cells. The methods of measuring or assessing the PD-L1 expression can be achieved by any methods applicable.

[0072] In order to assess the PD-L1 expression, in one embodiment, a test tissue sample is obtained from the patient who is in need of the therapy. In another embodiment, the assessment of PD-L1 expression can be achieved without obtaining a test tissue sample. In some embodiments, selecting a suitable patient includes (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and/or tumor-infiltrating inflammatory cells; and (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the surface of the cells based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is lower than a predetermined threshold level. A test tissue sample can be considered to be PD-L1-negative if less than about 5% of the cells in the

test tissue express PD-L1 on the cell surface. In other embodiments, a test tissue sample is considered to be PD-L1-negative if less than about 4%, less than about 3%, less than about 2%, less than about 1%, less than about 0.5%, less than about 0.1%, less than about 0.01% or 0% of the cells in the test tissue express PD-L1 on the cell surface. In one particular example, the test tissue sample can be considered to be PD-L1-negative if less than about 5% of the cells in the test tissue express PD-L1 on the cell surface.

[0073] In any of the methods comprising the measurement of PD-L1 expression in a test tissue sample, however, it should be understood that the step comprising the provision of a test tissue sample obtained from a patient is an optional step. That is, in certain embodiments the method includes this step, and in other embodiments, this step is not included in the method. It should also be understood that in certain embodiments the "measuring" or "assessing" step to identify, or determine the number or proportion of, cells in the test tissue sample that express PD-L1 on the cell surface is performed by a transformative method of assaying for PD-L1 expression, for example by performing a reverse transcriptase-polymerase chain reaction (RT-PCR) assay or an IHC assay. In certain other embodiments, no transformative step is involved and PD-L1 expression is assessed by, for example, reviewing a report of test results from a laboratory. In certain embodiments, the steps of the methods up to, and including, assessing PD-L1 expression provides an intermediate result that may be provided to a physician or other healthcare provider for use in selecting a suitable candidate for the combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody. In certain embodiments, the steps that provide the intermediate result is performed by a medical practitioner or someone acting under the direction of a medical practitioner. In other embodiments, these steps are performed by an independent laboratory or by an independent person such as a laboratory technician.

[0074] In certain embodiments of any of the present methods, the proportion of cells that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 RNA. In further embodiments, the presence of PD-L1 RNA is determined by RT-PCR, *in situ* hybridization or RNase protection. In other embodiments, the proportion of cells that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 polypeptide. In further embodiments, the presence of PD-L1 polypeptide is determined

by immunohistochemistry (IHC), enzyme-linked immunosorbent assay (ELISA), *in vivo* imaging, or flow cytometry. In some embodiments, PD-L1 expression is assayed by IHC. In other embodiments of all of these methods, cell surface expression of PD-L1 is assayed using, *e.g.*, IHC or *in vivo* imaging.

[0075] Imaging techniques have provided important tools in cancer research and treatment. Recent developments in molecular imaging systems, including positron emission tomography (PET), single-photon emission computed tomography (SPECT), fluorescence reflectance imaging (FRI), fluorescence-mediated tomography (FMT), bioluminescence imaging (BLI), laser-scanning confocal microscopy (LSCM) and multiphoton microscopy (MPM), will likely herald even greater use of these techniques in cancer research. Some of these molecular imaging systems allow clinicians to not only see where a tumor is located in the body, but also to visualize the expression and activity of specific molecules, cells, and biological processes that influence tumor behavior and/or responsiveness to therapeutic drugs (Condeelis and Weissleder, 2010). Ab specificity, coupled with the sensitivity and resolution of PET, makes immunoPET imaging particularly attractive for monitoring and assaying expression of antigens in tissue samples (McCabe and Wu, 2010; Olafsen *et al.*, 2010). In certain embodiments of any of the present methods, PD-L1 expression is assayed by immunoPET imaging. In certain embodiments of any of the present methods, the proportion of cells in a test tissue sample that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 polypeptide on the surface of cells in the test tissue sample. In certain embodiments, the test tissue sample is a FFPE tissue sample. In other embodiments, the presence of PD-L1 polypeptide is determined by IHC assay. In further embodiments, the IHC assay is performed using an automated process. In some embodiments, the IHC assay is performed using an anti-PD-L1 mAb to bind to the PD-L1 polypeptide, if present.

Assaying Cell-Surface PD-L1 Expression by Automated IHC

[0076] In one embodiment of the present methods, an automated IHC method is used to assay the expression of PD-L1 on the surface of cells in FFPE tissue specimens. This disclosure provides methods for detecting the presence of human PD-L1 antigen in a test tissue sample, or quantifying the level of human PD-L1 antigen or the proportion of cells

in the sample that express the antigen, which methods comprise contacting the test sample, and a negative control sample, with a mAb that specifically binds to human PD-L1, under conditions that allow for formation of a complex between the Ab or portion thereof and human PD-L1. In certain embodiments, the test and control tissue samples are FFPE samples. The formation of a complex can then be detected, wherein a difference in complex formation between the test sample and the negative control sample is indicative of the presence of human PD-L1 antigen in the sample. Various methods are used to quantify PD-L1 expression.

[0077] In a particular embodiment, the automated IHC method comprises: (a) deparaffinizing and rehydrating mounted tissue sections in an autostainer; (b) retrieving antigen using a decloaking chamber and pH 6 buffer, heated to 110°C for 10 min; (c) setting up reagents on an autostainer; and (d) running the autostainer to include steps of neutralizing endogenous peroxidase in the tissue specimen; blocking non-specific protein-binding sites on the slides; incubating the slides with primary Ab; incubating with a postprimary blocking agent; incubating with NovoLink Polymer; adding a chromogen substrate and developing; and counterstaining with hematoxylin.

[0078] For assessing PD-L1 expression in tumor tissue samples, a pathologist examines the number of membrane PD-L1$^+$ tumor cells in each field under a microscope and mentally estimates the percentage of cells that are positive, then averages them to come to the final percentage. The different staining intensities are defined as 0/negative, 1+/weak, 2+/moderate, and 3+/strong. Typically, percentage values are first assigned to the 0 and 3+ buckets, and then the intermediate 1+ and 2+ intensities are considered. For highly heterogeneous tissues, the specimen is divided into zones, and each zone is scored separately and then combined into a single set of percentage values. The percentages of negative and positive cells for the different staining intensities are determined from each area and a median value is given to each zone. A final percentage value is given to the tissue for each staining intensity category: negative, 1+, 2+, and 3+. The sum of all staining intensities needs to be 100%.

[0079] Staining is also assessed in tumor-infiltrating inflammatory cells such as macrophages and lymphocytes. In most cases macrophages serve as an internal positive control since staining is observed in a large proportion of macrophages. While not

required to stain with 3+ intensity, an absence of staining of macrophages should be taken into account to rule out any technical failure. Macrophages and lymphocytes are assessed for plasma membrane staining and only recorded for all samples as being positive or negative for each cell category. Staining is also characterized according to an outside/inside tumor immune cell designation. "Inside" means the immune cell is within the tumor tissue and/or on the boundaries of the tumor region without being physically intercalated among the tumor cells. "Outside" means that there is no physical association with the tumor, the immune cells being found in the periphery associated with connective or any associated adjacent tissue.

[0080] In certain embodiments of these scoring methods, the samples are scored by two pathologists operating independently, and the scores are subsequently consolidated. In certain other embodiments, the identification of positive and negative cells is scored using appropriate software.

[0081] A histoscore is used as a more quantitative measure of the IHC data. The histoscore is calculated as follows:

Histoscore = [(% tumor x 1 (low intensity)) + (% tumor x 2 (medium intensity)) + (% tumor x 3 (high intensity)]

[0082] To determine the histoscore, the pathologist estimates the percentage of stained cells in each intensity category within a specimen. Because expression of most biomarkers is heterogeneous the histoscore is a truer representation of the overall expression. The final histoscore range is 0 (no expression) to 300 (maximum expression). A test sample can be designated as PD-L1-negative even though some level of PD-L1 expression is detected. For example, a designation of PD-L1-negative can be assigned to a specimen having a final histoscore of about 15 or less, of about 10 or less, about 9 or less, about 8 or less, about 7 or less, about 6 or less, about 5 or less, about 4 or less, about 3 or less, about 2 or less, about 1 or less, or of 0.

[0083] An alternative means of quantifying PD-L1 expression in a test tissue sample IHC is to determine the adjusted inflammation score (AIS) score defined as the density of inflammation multiplied by the percent PD-L1 expression by tumor-infiltrating inflammatory cells (Taube et al., 2012).

Anti-PD-L1 Antibodies

[0084] Anti-PD-L1 Abs useful for the invention include Abs engineered starting from Abs having one or more of the V_H and/or V_L sequences disclosed herein, which engineered Abs can have altered properties from the starting Abs. An anti-PD-L1 Ab can be engineered by a variety of modifications as described above for the engineering of modified anti-PD-1 Abs of the invention.

[0085] Anti-PD-L1 Abs of the invention also include isolated Abs selected for their ability to bind to PD-L1 in formalin-fixed, paraffin-embedded (FFPE) tissue specimens. The use of FFPE samples is essential for the long-term follow-up analysis of the correlation between PD-L1 expression in tumors and disease prognosis or progression. The use of different Abs to stain PD-L1 in frozen versus FFPE tissues, and the ability of certain Abs to distinguish membranous and/or cytoplasmic forms of PD-L1, may account for some of the disparate data reported in the literature correlating PD-L1 expression with disease prognosis (Hamanishi *et al.*, 2007; Gadiot *et al.*, 2011). This disclosure provides several rabbit mAbs that bind with high affinity specifically to membranous human PD-L1 in FFPE tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells.

[0086] In some embodiments, an anti-PD-L1 antibody useful for the present methods includes mAb 28-8 set forth in SEQ ID NOs. 1 and 2, respectively. The sequences of the heavy and light chain CDR domains of mAb 28-8, as delineated using the Kabat system, are set forth in SEQ ID NOs. 3-8. In other embodiments, an anti-PD-L1 antibody useful for the invention comprises mAbs 28-1, 28-12, 29-8 and 20-12 or an antigen-binding portion thereof, for example, including Fab, $F(ab')_2$ Fd, Fv, and scFv, di-scFv or bi-scFv, and scFv-Fc fragments, diabodies, triabodies, tetrabodies, and isolated CDRs.

Anti-PD-1 antibodies

[0087] PD-1 is a key immune checkpoint receptor expressed by activated T and B cells and mediates immunosuppression. PD-1 is a member of the CD28 family of receptors, which includes CD28, CTLA-4, ICOS, PD-1, and BTLA. Two cell surface glycoprotein ligands for PD-1 have been identified, Programmed Death Ligand-1 (PD-L1) and Programmed Death Ligand-2 (PD-L2), that are expressed on antigen-presenting cells as

well as many human cancers and have been shown to down regulate T cell activation and cytokine secretion upon binding to PD-1. Inhibition of the PD-1/PD-L1 interaction mediates potent antitumor activity in preclinical models.

[0088] HuMAbs that bind specifically to PD-1 with high affinity have been disclosed in U.S. Patent Nos. 8,008,449 and 8,779,105. Other anti-PD-1 mAbs have been described in, for example, U.S. Patent Nos. 6,808,710, 7,488,802, 8,168,757 and 8,354,509, and PCT Publication No. WO 2012/145493. Each of the anti-PD-1 HuMAbs disclosed in U.S. Patent No. 8,008,449 has been demonstrated to exhibit one or more of the following characteristics: (a) binds to human PD-1 with a K_D of 1 x 10^{-7} M or less, as determined by surface plasmon resonance using a Biacore biosensor system; (b) does not substantially bind to human CD28, CTLA-4 or ICOS; (c) increases T-cell proliferation in a Mixed Lymphocyte Reaction (MLR) assay; (d) increases interferon-γ production in an MLR assay; (e) increases IL-2 secretion in an MLR assay; (f) binds to human PD-1 and cynomolgus monkey PD-1; (g) inhibits the binding of PD-L1 and/or PD-L2 to PD-1; (h) stimulates antigen-specific memory responses; (i) stimulates Ab responses; and (j) inhibits tumor cell growth *in vivo*. Anti-PD-1 Abs useful for the present invention include mAbs that bind specifically to human PD-1 and exhibit at least one, preferably at least five, of the preceding characteristics.

[0089] In one embodiment, the anti-PD-1 Ab is nivolumab. Nivolumab (also known as "OPDIVO®"; formerly designated 5C4, BMS-936558, MDX-1106, or ONO-4538) is a fully human IgG4 (S228P) PD-1 immune checkpoint inhibitor Ab that selectively prevents interaction with PD-1 ligands (PD-L1 and PD-L2), thereby blocking the down-regulation of antitumor T-cell functions (U.S. Patent No. 8,008,449; Wang *et al.*, 2014 *Cancer Immunol Res.* 2(9):846-56).

[0090] In another embodiment, the anti-PD-1 Ab is pembrolizumab. Pembrolizumab (also known as "KEYTRUDA®", lambrolizumab, and MK-3475) is a humanized monoclonal IgG4 antibody directed against human cell surface receptor PD-1 (programmed death-1 or programmed cell death-1). Pembrolizumab is described, for example, in U.S. Patent Nos. 8,354,509 and 8,900,587; *see also* http://www.cancer.gov/drugdictionary?cdrid=695789 (last accessed: December 14,

2014). Pembrolizumab has been approved by the FDA for the treatment of relapsed or refractory melanoma.

[0091] In other embodiments, the anti-PD-1 Ab is MEDI0608 (formerly AMP-514), which is a monoclonal antibody. MEDI0608 is described, for example, in US Pat. No. 8,609,089B2 or in http://www.cancer.gov/drugdictionary?cdrid=756047 (last accessed December 14, 2014).

[0092] In some embodiments, the anti-PD-1 antibody is Pidilizumab (CT-011), which is a humanized monoclonal antibody. Pidilizumab is described in US Pat. No. 8,686,119 B2 or WO 2013/014668 A1.

[0093] Anti-PD-1 Abs useful for the disclosed compositions also include isolated Abs that bind specifically to human PD-1 and cross-compete for binding to human PD-1 with nivolumab (*see, e.g.*, U.S. Patent Nos. 8,008,449 and 8,779,105; WO 2013/173223). The ability of Abs to cross-compete for binding to an antigen indicates that these Abs bind to the same epitope region of the antigen and sterically hinder the binding of other cross-competing Abs to that particular epitope region. These cross-competing Abs are expected to have functional properties very similar to those of nivolumab by virtue of their binding to the same epitope region of PD-1. Cross-competing Abs can be readily identified based on their ability to cross-compete with nivolumab in standard PD-1 binding assays such as Biacore analysis, ELISA assays or flow cytometry (*see, e.g.*, WO 2013/173223).

[0094] In certain embodiments, the Abs that cross-compete for binding to human PD-1 with, or bind to the same epitope region of human PD-1 as, nivolumab are mAbs. For administration to human subjects, these cross-competing Abs can be chimeric Abs, or humanized or human Abs. Such chimeric, humanized or human mAbs can be prepared and isolated by methods well known in the art.

[0095] Anti-PD-1 Abs useful for the compositions of the disclosed invention also include antigen-binding portions of the above Abs. It has been amply demonstrated that the antigen-binding function of an Ab can be performed by fragments of a full-length Ab. Examples of binding fragments encompassed within the term "antigen-binding portion" of an Ab include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_{H1} domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting

of the V_H and C_{H1} domains; and (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an Ab.

[0096] Anti-PD-1 Abs suitable for use in the disclosed compositions are Abs that bind to PD-1 with high specificity and affinity, block the binding of PD-L1 and or PD-L2, and inhibit the immunosuppressive effect of the PD-1 signaling pathway. In any of the compositions or methods disclosed herein, an anti-PD-1 "antibody" includes an antigen-binding portion or fragment that binds to the PD-1 receptor and exhibits the functional properties similar to those of whole Abs in inhibiting ligand binding and upregulating the immune system. In certain embodiments, the anti-PD-1 Ab or antigen-binding portion thereof cross-competes with nivolumab for binding to human PD-1. In other embodiments, the anti-PD-1 Ab or antigen-binding portion thereof is a chimeric, humanized or human monoclonal Ab or a portion thereof. In certain embodiments, the Ab is a humanized Ab. In other embodiments, the Ab is a human Ab. Abs of an IgG1, IgG2, IgG3 or IgG4 isotype can be used.

[0097] In certain embodiments, the anti-PD-1 Ab or antigen-binding portion thereof comprises a heavy chain constant region which is of a human IgG1 or IgG4 isotype. In certain other embodiments, the sequence of the IgG4 heavy chain constant region of the anti-PD-1 Ab or antigen-binding portion thereof contains an S228P mutation which replaces a serine residue in the hinge region with the proline residue normally found at the corresponding position in IgG1 isotype antibodies. This mutation, which is present in nivolumab, prevents Fab arm exchange with endogenous IgG4 antibodies, while retaining the low affinity for activating Fc receptors associated with wild-type IgG4 antibodies (Wang *et al.*, 2014). In yet other embodiments, the Ab comprises a light chain constant region which is a human kappa or lambda constant region. In other embodiments, the anti-PD-1 Ab or antigen-binding portion thereof is a mAb or an antigen-binding portion thereof. In certain embodiments of any of the therapeutic methods described herein comprising administration of an anti-PD-1 Ab, the anti-PD-1 Ab is nivolumab. In other embodiments, the anti-PD-1 Ab is pembrolizumab. In other embodiments, the anti-PD-1 Ab is chosen from the human antibodies 17D8, 2D3, 4H1, 4A11, 7D3 and 5F4 described in U.S. Patent No. 8,008,449. In still other embodiments, the anti-PD-1 Ab is MEDI0608 (formerly AMP-514), AMP-224, or Pidilizumab (CT-011).

Anti-CTLA-4 antibodies

[0098] Anti-CTLA-4 antibodies of the instant invention bind to human CTLA-4 so as to disrupt the interaction of CTLA-4 with a human B7 receptor. Because the interaction of CTLA-4 with B7 transduces a signal leading to inactivation of T-cells bearing the CTLA-4 receptor, disruption of the interaction effectively induces, enhances or prolongs the activation of such T cells, thereby inducing, enhancing or prolonging an immune response.

[0099] HuMAbs that bind specifically to CTLA-4 with high affinity have been disclosed in U.S. Patent Nos. 6,984,720 and 7,605,238. Other anti-PD-1 mAbs have been described in, for example, U.S. Patent Nos. 5,977,318, 6,051,227, 6,682,736, and 7,034,121. The anti-PD-1 HuMAbs disclosed in U.S. Patent No. Nos. 6,984,720 and 7,605,238 have been demonstrated to exhibit one or more of the following characteristics: (a) binds specifically to human CTLA-4 with a binding affinity reflected by an equilibrium association constant (K_a) of at least about 10^7 M^{-1}, or about 10^9 M^{-1}, or about 10^{10} M^{-1} to 10^{11} M^{-1} or higher, as determined by Biacore analysis; (b) a kinetic association constant (k_a) of at least about 10^3, about 10^4, or about 10^5 m^{-1} s^{-1}; (c) a kinetic disassociation constant (k_d) of at least about 10^3, about 10^4, or about 10^5 m^{-1} s^{-1}; and (d) inhibits the binding of CTLA-4 to B7-1 (CD80) and B7-2 (CD86). Anti-CTLA-4 Abs useful for the present invention include mAbs that bind specifically to human CTLA-4 and exhibit at least one, at least two, or at least three of the preceding characteristics.

[0100] An exemplary clinical anti-CTLA-4 Ab is the human mAb 10D1 (now known as ipilimumab and marketed as YERVOY®) as disclosed in U.S. Patent No. 6,984,720. Ipilimumab is an anti-CTLA-4 Ab for use in the methods disclosed herein. Ipilimumab is a fully human, IgG1 monoclonal Ab that blocks the binding of CTLA-4 to its B7 ligands, thereby stimulating T cell activation and improving overall survival (OS) in patients with advanced melanoma.

[0101] Another anti-CTLA-4 Ab useful for the present methods is tremelimumab (also known as CP-675,206). Tremelimumab is human IgG2 monoclonal anti-CTLA-4 antibody. Tremelimumab is described in WO/2012/122444, U.S. Publ. No. 2012/263677, or WO Publ. No. 2007/113648 A2.

[0102] Anti-CTLA-4 Abs useful for the disclosed composition also include isolated Abs that bind specifically to human CTLA-4 and cross-compete for binding to human CTLA-4 with ipilimumab or tremelimumab or bind to the same epitope region of human CTLA-4 as ipilimumab or tremelimumab. In certain embodiments, the Abs that cross-compete for binding to human CTLA-4 with, or bind to the same epitope region of human CTLA-4 as does ipilimumab or tremelimumab, are Abs comprising a heavy chain of the human IgG1 isotype. For administration to human subjects, these cross-competing Abs are chimeric Abs, or humanized or human Abs. Useful anti-CTLA-4 Abs also include antigen-binding portions of the above Abs such as Fab, $F(ab')_2$, Fd or Fv fragments.

Dosages

[0103] The combination of an anti-PD-1 antibody and an anti-CTLA-4 antibody can be administered to a suitable patient in therapeutically effective amounts. For example, each Ab can be administered at a dosage ranging from about 0.1 to about 20.0 mg/kg body weight. In certain embodiments, each of the anti-PD-1 and anti-CTLA-4 Abs is individually administered at a dosage of about 0.1, 0.3, 0.5, 1, 3, 5, 10 or 20 mg/kg, *e.g.*, about 1 to about 10 mg/kg, *e.g.*, about 1 to about 3 mg/kg, *e.g.*, about 3 mg/kg, *e.g.*, about 1 mg/kg. Each of the anti-PD-1 Ab and anti-CTLA-4 Ab can be administered at a dosing frequency of at least once every week, at least once every 2, 3 or 4 weeks, or at least once a month, for up to 6 to up to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In some embodiments, the anti-PD-1 Ab is administered at a dosage of 1 or 3 mg/kg. In certain embodiments, the sequenced regimen comprises administering the anti-PD-1 Ab to the subject at a dosing frequency of once every week, once every 2, 3 or 4 weeks, or once a month for 6 to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In other embodiments, the anti-PD-1 is administered at a dosage of 1 mg/kg at a dosing frequency of once every 3 weeks for up to 48 doses. In some embodiments, the anti-CTLA-4 Ab is administered at a dosage of 1 or 3 mg/kg. In certain embodiments, the sequenced regimen comprises administering the anti-CTLA-4 Ab to the subject at a dosing frequency of once every week, once every 2, 3 or 4 weeks, or once a month for 6 to 72 doses, or for as long as clinical benefit is observed, or until

unmanageable toxicity or disease progression occurs. In other embodiments, the anti-CTLA-4 Ab is administered at a dosage of 3 mg/kg at a dosing frequency of once every 3 weeks for up to 48 doses.

[0104] In other embodiments, the anti-PD-1 and anti-CTLA-4 Abs are administered at the following dosages: (a) 0.1 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab; (b) 0.3 mg/kg anti-PD-1 Ab and 3 mg/kg of anti- CTLA-4 Ab; (c) 1 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab; (d) 3 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab; (e) 5 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab; (f) 10 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab; (g) 0.1 mg/kg anti-PD-1 Ab and 1 mg/kg of anti-CTLA-4 Ab; (h) 0.3 mg/kg anti-PD-1 Ab and 1 mg/kg of anti-CTLA-4 Ab; (i) 1 mg/kg anti-PD-1 Ab and 1 mg/kg of anti-CTLA-4 Ab; (j) 3 mg/kg anti-PD-1 Ab and 1 mg/kg of anti-CTLA-4 Ab; (k) 5 mg/kg anti-PD-1 Ab and 1 mg/kg of anti-CTLA-4 Ab; or (l) 10 mg/kg anti-PD-1 Ab and 1 mg/kg of anti-CTLA-4 Ab. In a particular embodiment, the methods include administration of 1 mg/kg of an anti-PD-1 antibody and 3 mg/kg of an anti-CTLA-4 antibody.

[0105] In some embodiments, the dosage of each of the anti-PD-1 and anti-CTLA-4 Abs is kept constant during the induction dosing schedule and the maintenance dosing schedule. In certain embodiments, a regimen comprises: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 Abs at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 10 doses, followed by administration of the anti-PD-1 Ab alone at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 12 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 Abs at a dosing frequency of at least once every 8, 12 or 16 weeks, or at least once a quarter, for at least 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs.

[0106] In certain embodiments of this method, the maintenance dosing schedule comprises combined administration of up to 4, 6, 8, 10, 12 or 16 doses of the anti-PD-1 and anti-CTLA-4 Abs. In other embodiments, the concurrent regimen comprises: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-

CTLA-4 Abs at a dosing frequency of once every 2, 3 or 4 weeks, or once a month, for 2, 4, 6 or 8 doses, followed by administration of the anti-PD-1 Ab alone at a dosing frequency of once every 2, 3 or 4 weeks, or once a month, for 2, 4, 6, 8 or 12 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 Abs at a dosing frequency of once every 8, 12 or 16 weeks, or once a quarter, for 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs.

[0107] In a particular embodiment, the anti-PD-1 and anti-CTLA-4 Abs are administered at dosages of 1 mg/kg anti-PD-1 Ab every three weeks and 3 mg/kg of anti-CTLA-4 Ab every three weeks.

[0108] In certain embodiments of the present methods, the anti-PD-1 and anti-CTLA-4 Abs are formulated for parenteral administration, *e.g.*, intravenous administration. In certain other embodiments, when the anti-PD-1 and anti-CTLA-4 Abs are administered in combination, they are administered within 30 minutes of each other. Either Ab may be administered first, that is, in certain embodiments, the anti-PD-1 Ab is administered before the anti-CTLA-4 Ab, whereas in other embodiments, the anti-CTLA-4 Ab is administered before the anti-PD-1 Ab. Typically, each Ab is administered intravenously over a period of 60 minutes. In further embodiments, the anti-PD-1 and anti-CTLA-4 Abs are administered concurrently, either admixed as a single composition in a pharmaceutically acceptable formulation for concurrent administration, or concurrently as separate compositions with each Ab in a pharmaceutically acceptable formulation.

[0109] In some embodiments, the anti-PD-1 Ab and the anti-CTLA-4 Ab are formulated in a single composition. The ratio of the amount of the anti-PD-1 Ab and the amount of the anti-CTLA-4 Ab in the single composition can be 10:1 to 1:10. In other embodiments, the ratio of the amount of the anti-PD-1 Ab and the amount of the anti-CTLA-4 Ab in the single composition is 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, or 1:5. In a particular embodiment, the ratio of the amount of the anti-PD-1 Ab and the amount of the anti-CTLA-4 Ab in the single composition is 1:3.

[0110] In some embodiments, the composition is administered at a flat dose regardless of the weight of the patient. For example, each of the anti-PD-1 Ab with the anti-CTLA-4 Ab may be administered at a flat dose of 20, 50, 75, 80, 160, 200, 240, 300, 400, 500, 750

or 1500 mg, without regard to the patient's weight. In some embodiments the composition is administered at a weight-based dose at any dose disclosed herein. In some embodiments, the amount of the anti-PD-1 Ab and the amount of the anti-CTLA-4 Ab administered to the patient at a single dose are identical.

[0111] In certain embodiments of the present methods, the therapeutically effective dosage of the anti-PD-1 Ab or antigen-binding portion thereof comprises about 60 mg, about 80 mg, about 100 mg, about 120 mg, about 140 mg, about 160 mg, about 180 mg, about 200 mg, about 220 mg, about 240 mg, about 260 mg, about 280 mg, or about 300 mg. In some embodiments, the dose of the anti-PD-1 antibody in the composition is between about 60 mg and about 300 mg, between about 60 mg and about 100 mg, between about 100 mg and about 200 mg, or between about 200 mg and about 300 mg. In some embodiments, the amount of the anti-PD-1 antibody in the composition is at least about 80 mg, about 160 mg, or about 240mg. In some embodiments, the dose of the anti-PD-1 antibody in the composition is at least about 240 mg or at least about 80 mg.

[0112] In other embodiments, the therapeutically effective dosage of the anti-CTLA-4 Ab or antigen-binding portion thereof comprises about 60 mg, about 80 mg, about 100 mg, about 120 mg, about 140 mg, about 160 mg, about 180 mg, about 200 mg, about 220 mg, about 240 mg, about 260 mg, about 280 mg, or about 300 mg. In some embodiments, the dose of the anti-CTLA-4 antibody in the composition is between about 60 mg and about 300 mg, between about 60 mg and about 100 mg, between about 100 mg and about 200 mg, or between about 200 mg and about 300 mg. In some embodiments, the amount of the anti-CTLA-4 antibody in the composition is at least about 80 mg, about 160 mg, or about 240 mg. In some embodiments, the dose of the anti-CTLA-4 antibody in the composition is at least about 240 mg or at least about 80 mg.

[0113] In some embodiments, a flat dose of an anti-PD-1 Ab is 80 mg, and a flat dose of an anti-CTLA-4 antibody is 240 mg.

[0114] In some embodiments, the anti-PD-1 Ab is administered at a subtherapeutic dose, *i.e.*, a dose of the therapeutic agent that is significantly lower than the usual or FDA-approved dose when administered as monotherapy for the treatment of the cancer. The quantity of the second antibody in the composition is calculated based on the desired ratio.

[0115] In some embodiments, the composition is administered by intravenous infusion about once per week, about once every 2 weeks, about once every 3 weeks, or about once a month. In certain embodiments, the composition is administered once every 3 weeks. In some embodiments, the infusion occurs over at least about 10 minutes, about 20 minutes, about 30 minutes, about 45 minutes, about 60 minutes, about 90 minutes, about 2 hours, about 3 hours, about 4 hours or about 5 hours.

[0116] Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention can be flat or varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being unduly toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts. A composition of the present invention can be administered via one or more routes of administration using one or more of a variety of methods well known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

Kits

[0117] Also within the scope of the present invention are kits, including pharmaceutical kits, comprising a combination of an anti-PD-1 Ab and an anti-CTLA-4 Ab, for therapeutic uses, and diagnostic kits comprising an anti-PD-L1 Ab for assaying membranous PD-L1 expression as a biomarker for screening patients for the combination therapy or for predicting the efficacy of the combination therapy. Kits typically include a label indicating the intended use of the contents of the kit and instructions for use. The term "label" includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit. In certain embodiments of a pharmaceutical kit, the

anti-PD-1 Ab is co-packaged with an anti-CTLA-4 Ab in unit dosage form. In certain embodiments of a diagnostic kit, the anti-PD-L1 Ab is co-packaged with an anti-PD-1 Ab and an anti-CTLA-4 Ab for performing an assay to detect and/or quantify PD-L1 expression.

[0118] In certain embodiments, the pharmaceutical kit comprises the anti-human PD-1 HuMAb, nivolumab. In other embodiments, the pharmaceutical kit comprises the anti-human PD-L1 HuMAb, BMS-936559. In yet other embodiments, the pharmaceutical kit comprises the anti-human CTLA-4 HuMAb, ipilimumab. In certain embodiments, the diagnostic kit comprises the rabbit anti-human PD-L1 mAb, 28-8, comprising the V_H and V_L regions whose amino acid sequences are set forth in SEQ ID NOs. 1 and 2, respectively. In other embodiments, the diagnostic kit comprises the murine anti-human PD-L1 mAb, 5H1 (Dong et al., 2002).

[0119] In some embodiments, the invention provides a kit for treating a patient afflicted with a melanoma tumor, the kit comprising:

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

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

(c) instructions for using the anti-PD-1 antibody or the antigen-binding portion thereof and the anti-CTLA-4 antibody or the antigen-binding portion thereof in the methods disclosed herein.

[0120] The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of all references cited throughout this application are expressly incorporated herein by reference.

EXAMPLES

EXAMPLE 1

[0121] A randomized, double-blind, multicenter, phase 3 trial was conducted to evaluate the safety and efficacy of nivolumab alone or nivolumab combined with ipilimumab in comparison with ipilimumab alone in previously untreated metastatic melanoma.

Patients

[0122] Eligible patients had histologically confirmed stage III (unresectable) or stage IV melanoma, and no prior systemic treatment for unresectable or metastatic melanoma. Other eligibility criteria included an age of at least 18 years, an Eastern Cooperative Oncology Group (ECOG) performance status score of 0 (indicating no symptoms) or 1 (indicating mild symptoms), measurable disease by computed tomography or magnetic resonance imaging per RECIST v1.1, availability of tissue collected from metastatic or unresectable tumors for the assessment of PD-L1 status, and known BRAF V600 mutation status (or consent to BRAF V600 mutation testing per local standards). Key exclusion criteria were presence of active brain metastases, ocular melanoma, or autoimmune disease, and any prior treatment with an anti-PD-1, anti-PD-L1, anti-PD-L2, or anti-CTLA-4 antibody. Patients who required systemic corticosteroid treatment or other immunosuppressive medications within 14 days of study drug administration were excluded.

Study Design and Treatment

[0123] In the double-blind, phase 3 study, enrolled patients were randomly assigned in a 1:1:1 ratio to receive 3 mg of nivolumab per kilogram of body weight every 2 weeks (plus ipilimumab-matched placebo), or 1 mg of nivolumab per kilogram every 3 weeks plus 3 mg of ipilimumab per kilogram every 3 weeks for 4 doses (plus nivolumab-matched placebo), followed by 3 mg of nivolumab per kilogram every 2 weeks for cycle 3 and beyond, or 3 mg of ipilimumab per kilogram every 3 weeks for 4 doses (plus nivolumab-matched placebo).

[0124] Both nivolumab and ipilimumab were administered by intravenous infusion. Randomization was stratified according to tumor PD-L1 status (positive vs. negative or indeterminate), BRAF mutation status (V600 mutation positive vs. wild-type), and American Joint Committee on Cancer metastasis stage (M0, M1a, or M1b vs. M1c). Treatment continued until RECIST v1.1-defined disease progression, unacceptable toxicity, or withdrawal of consent. Patients could be treated beyond progression provided they had a clinical benefit without clinical deterioration, and did not have substantial adverse effects, as assessed by the investigator.

[0125] Progression-free survival and overall survival were co-primary end points. Secondary end points included objective response rate, tumor PD-L1 expression as a predictive biomarker for progression-free and overall survival, and health-related quality of life. Exploratory end points include duration of objective response and safety/tolerability of study drug therapy.

Assessments

[0126] Patients were assessed for tumor response, according to RECIST v1.1 at 12 weeks after randomization and continuing every 6 weeks for 49 weeks, and then every 12 weeks until progression or treatment discontinuation, whichever occurred later. Progression-free survival was defined as the time between the date of randomization and the first date of documented progression or death, whichever occurred first. Patients treated beyond progression were considered to have progressive disease at the time of the initial progression event, as assessed by the investigator, regardless of subsequent tumor responses. Expression of PD-L1 on the surface of the tumor cells was assessed in a central laboratory by immunohistochemistry in formalin-fixed, paraffin-embedded tumor specimens using a rabbit monoclonal anti-human PD-L1 antibody (clone 28-8) and an analytically validated automated assay developed by Dako (Carpinteria, CA). PD-L1 positivity was defined as at least 5% of tumor cells showing cell surface PD-L1 staining of any intensity in a section containing at least 100 tumor cells that could be evaluated. Indeterminate status was attributed to samples for which tumor cell–surface expression could not be discerned because of melanin content or strong cytoplasmic staining.

[0127] Any patient who received at least one dose of study drug in each of the 3 treatment groups was included in the assessment of safety. The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.18. Safety assessments were made continuously during the treatment phase, and up to 100 days after the last dose of study drug.

Statistical Analysis

[0128] A study sample size of approximately 915 patients, randomized to the 3 treatment arms in a 1:1:1 ratio, was planned. For the comparison of progression-free survival, the number of events projected to be observed at a follow-up of at least 9 months provided

approximately 83% power to detect an average hazard ratio of 0.71 with a type I error of 0.005 (two-sided). Progression-free survival was compared between nivolumab plus ipilimumab and ipilimumab alone, and between nivolumab alone and ipilimumab alone with the use of a two-sided log-rank test stratified according to PD-L1 status, BRAF mutation status, and metastasis stage (as described above). The study was not designed for a formal statistical comparison between the nivolumab alone and nivolumab plus ipilimumab groups. Hazard ratios and corresponding two-sided 99.5% confidence intervals (CIs) were estimated using a Cox proportional hazards model, with treatment group as a single covariate, stratified by the above factors. Progression-free survival curves, medians with 95% CIs, and progression-free survival rates at 6, 12, and 18 months with 95% CIs were estimated using Kaplan-Meier methodology. Overall survival will be analyzed when all patients have a minimum follow-up of 22 months.

Results

Patients and Treatment

[0129] From July 2013 through March 2014, a total of 1296 patients were enrolled at 137 centers in Australia, Europe, Israel, New Zealand, and North America. A total of 945 patients underwent randomization: 316 patients were assigned to the nivolumab group, 314 to the nivolumab plus ipilimumab group, and 315 to the ipilimumab group (Figure 1). Baseline characteristics were balanced across the three groups. A total of 58.0% had stage M1c disease, 36.1% had an elevated lactate dehydrogenase level, 31.5% had a BRAF mutation, and 73.5% had negative PD-L1 status (Table 1).

Table 1. Baseline Characteristics of the Patients.

Characteristic	Nivolumab alone (N=316)	Nivolumab plus Ipilimumab (N=314)	Ipilimumab alone (N=315)	Total (N=945)
Age — yr				
Mean	58.7	59.3	60.8	59.6
Range	25–90	18–88	18–89	18–90
Age groups — no. (%)				
<65 yr	198 (62.7)	185 (58.9)	182 (57.8)	565 (59.8)
≥ 65, < 75 yr	79 (25.0)	94 (29.9)	89 (28.3)	262 (27.7)
≥ 75 yr	39 (12.3)	35 (11.1)	44 (14.0)	118 (12.5)
Sex — no. (%)				
Male	202 (63.9)	206 (65.6)	202 (64.1)	610 (64.6)
Female	114 (36.1)	108 (34.4)	113 (35.9)	335 (35.4)
ECOG performance status — no. (%)				
0	238 (75.3)	230 (73.2)	224 (71.1)	692 (73.2)
1	77 (24.4)	83 (26.4)	91 (28.9)	251 (26.6)
2	1 (0.3)	0	0	1 (0.1)
Not reported	0	1 (0.3)	0	1 (0.1)
M stage — no. (%)				
M1c	184 (58.2)	181 (57.6)	183 (58.1)	548 (58.0)
M0, M1a, or M1b	132 (41.8)	133 (42.4)	132 (41.9)	397 (42.0)
Lactate dehydrogenase — no. (%)*				
≤ ULN	196 (62.0)	199 (63.4)	194 (61.6)	589 (62.3)
>ULN	112 (35.4)	114 (36.3)	115 (36.5)	341 (36.1)
≤2x ULN	271 (85.8)	276 (87.9)	279 (88.6)	826 (87.4)

>2x ULN	37 (11.7)	37 (11.8)	30 (9.5)	104 (11.0)
Unknown	8 (2.5)	1 (0.3)	6 (1.9)	15 (1.6)
Brain metastases at baseline — no. (%)				
Yes	8 (2.5)	11 (3.5)	15 (4.8)	34 (3.6)
No	308 (37.5)	303 (96.5)	300 (95.2)	911 (96.4)
PD-L1 status — no. (%)				
Positive	80 (27.8)	68 (24.5)	75 (27.1)	223(26.4)
Negative	208 (72.2)	210 (75.5)	202 (72.9)	620 (73.5)
BRAF status — no. (%)				
Mutation	100 (31.6)	101 (32.2)	97 (30.8)	298 (31.5)
No mutation	216 (68.4)	213 (67.8)	218 (69.2)	647 (68.5)

* ULN denotes upper limit of normal.

[0130] All randomized patients had been followed for a minimum of 9 months at the time of database lock (February 17, 2015); 117 of 313 patients (37.4%) in the nivolumab group, 93 of 313 patients (29.7%) in the nivolumab plus ipilimumab group, and 50 of 311 patients (16.1%) in the ipilimumab group were continuing study treatment (Table 2). The most frequent reason for discontinuation was disease progression in the nivolumab and ipilimumab monotherapy groups (154 of 313 patients [49.2%] and 202 of 311 patients [65.0%], respectively), versus study drug toxicity in the nivolumab plus ipilimumab group (120 of 313 patients [38.3%]). The number of patients who had died was 85 (27.2%) in the nivolumab group, 86 (27.5%) in the nivolumab plus ipilimumab group, and 114 (36.7%) in the ipilimumab group.

[0131] The median number of doses in patients who received nivolumab alone or ipilimumab alone was 15 (range 1-38) and 4 (1-4), respectively. In the combination group, the median number of doses was 4 (range 1–39) for nivolumab and 4 (range 1–4) for ipilimumab; 147 of 313 patients (47%) received four or more doses of nivolumab monotherapy after combination treatment.

Efficacy

[0132] The median progression-free survival was 6.5 months (95% confidence interval [CI], 4.3 to 9.5) in the nivolumab group, 11.5 months (95% CI, 8.9 to 16.5) in the nivolumab plus ipilimumab group, and 2.9 months (95% CI, 2.8 to 3.4) in the ipilimumab group (Figure 2A). A significant improvement in progression-free survival was observed in the nivolumab plus ipilimumab group as compared with the ipilimumab group (hazard ratio, 0.42; 95% CI, 0.31 to 0.57; $P<0.0001$) (Figure 2A). A significant improvement in progression-free survival was also observed in the nivolumab group as compared with the ipilimumab group (hazard ratio, 0.57; 95% CI, 0.43 to 0.76; $P<0.00001$) (Figure 2A). The hazard ratio for the comparison between nivolumab plus ipilimumab and nivolumab groups was 0.74 (95% CI, 0.60 to 0.92).

[0133] Analyses of progression-free survival among prespecified patient subgroups showed a consistent improvement with nivolumab or nivolumab plus ipilimumab as compared with ipilimumab, including subgroups defined by PD-L1 status, BRAF mutation status, and metastasis stage (Figure 3). In the combination group, median PFS

was 11.7 months (95% CI, 8.0 to not reached) among patients with a BRAF mutation and was 11.2 months (95% CI, 8.3 to not reached) in patients with wild-type BRAF (Figure 3B). For patients with a positive PD-L1 tumor status, median progression-free survival in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups was 14.0 months (95% CI, 9.1 to not reached), 14.0 months (95% CI, 9.7 to not reached), and 3.9 months (95% CI, 2.8 to 4.2), respectively (Figure 2B). For patients with a negative PD-L1 tumor status, median progression-free survival in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups was 5.3 months (95% CI, 2.8 to 7.1 months), 11.2 months (95% CI, 8.0 to not reached) and 2.8 months (95% CI, 2.8 to 3.1), respectively (Fig. 2C).

[0134] Investigator-assessed objective response rates were 43.7% (95% CI, 38.1 to 49.3%), 57.6% (95% CI, 52.0 to 63.2), and 19.0% (95% CI, 14.9 to 23.8) in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively (Table 3). The percentage of patients with a complete response was higher in the nivolumab plus ipilimumab group than in either the nivolumab or ipilimumab alone groups (11.5% vs. 8.9% and 2.2%) (Table 3). Time to objective response was similar in each group (Table 3), and the median duration of response was not reached in any of the groups.

[0135] Median reduction in the sum of the longest diameters of tumor target lesions was -34.5% (interquartile range: -75.4 to 15.4), -51.1% (-75.8 to -10.2), and 5.8% (-28.0 to 33.3) in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively (Figure 4). Among patients with PD-L1-positive tumors, the objective response rates were 57.5% (95% CI, 45.9 to 68.5), 72.1% (95% CI, 59.9 to 82.3), and 21.3% (95% CI, 12.7 to 32.3) for the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively; in patients with PD-L1-negative tumors, the objective response rates were 41.3% (95% CI, 34.6 to 48.4), 54.8% (95% CI, 47.8 to 61.6), and 17.8% (95% CI, 12.8 to 23.8) (Table 4).

Table 2. Patient Disposition.*

	Nivolumab alone (N=313)	Nivolumab plus Ipilimumab (N=313)	Ipilimumab alone (N=311)
Patients in the treatment period — no. (%)			
Continuing	117 (37.4)	93 (29.7)	50 (16.1)
Not continuing	196 (62.6)	220 (70.3)	261 (83.9)
Reason for not continuing the treatment — no. (%)			
Disease progression	154 (49.2)	69 (22.0)	202 (65.0)
Study drug toxicity	27 (8.6)	120 (38.3)	47 (15.1)
Adverse event unrelated to study drug	5 (1.6)	12 (3.8)	4 (1.3)
Patient request to discontinue treatment	5 (1.6)	5 (1.6)	4 (1.3)
Death	5 (1.6)	5 (1.6)	4 (1.3)
Maximum clinical benefit	2 (0.6)	2 (0.6)	0
Poor/non-compliance	1 (0.3)	1 (0.3)	1 (0.3)
Patient withdrew consent	0	3 (1.0)	0
Lost to follow-up	1 (0.3)	0	0
Patient no longer meets study criteria	0	1 (0.3)	0
Other	0	3 (1.0)	2 (0.6)
Patients in the study — no. (%)			
Continuing	223 (71.2)	221 (70.6)	189 (60.8)
Died	85 (27.2)	86 (27.5)	114 (36.7)
Not continuing	5 (1.6)	6 (1.9)	8 (2.6)

At the time of database lock on February 17, 2015.

Table 3. Response to Treatment.

Response	Nivolumab alone (N=316)	Nivolumab plus Ipilimumab (N=314)	Ipilimumab alone (N=315)
Best overall response — no. (%)[†]			
Complete response	28 (8.9)	36 (11.5)	7 (2.2)
Partial response	110 (34.8)	145 (46.2)	53 (16.8)
Stable disease	34 (10.8)	41 (13.1)	69 (21.9)
Progressive disease	119 (37.7)	71 (22.6)	154 (48.9)
Could not be determined	25 (7.9)	21 (6.7)	32 (10.2)
Objective response[‡]			
No. of patients (% [95% CI])	138 (43.7 [38.1–49.3])	181 (57.6 [52.0–63.2])	60 (19.0 [14.9–23.8])
Estimated odds ratio (95% CI)[§]	3.40 (2.02–5.72)	6.11 (3.59–10.38)	---
Two-sided P value	<0.00001	<0.00001	---
Time to objective response — mo			
No. of responders	138	181	60
Median	2.78	2.76	2.79
Range	2.3–12.5	1.1–11.6	2.5–12.4

[†] ***Best overall response were assessed by the investigators with the use of RECIST v1.1.***

[‡] ***Data include patients with a complete response and those with a partial response.***

[§] ***Relative to ipilimumab alone.***

Table 4. Objective Response Rate by PD-L1 Status.

PD-L1 Positive			
	Nivolumab alone (N=80)	**Nivolumab plus Ipilimumab (N=68)**	**Ipilimumab alone (N=75)**
Objective response[†]			
No. of patients (% [95% CI])	46 (57.5 [45.9–68.5])	49 (72.1 [59.9–82.3])	16 (21.3 [12.7–32.3])
Estimated odds ratio (95% CI)[§]	5.03 (2.44–10.37)	10.41 (4.63–23.40)	---
PD-L1 Negative			
	Nivolumab alone (N=208)	**Nivolumab plus Ipilimumab (N=210)**	**Ipilimumab alone (N=202)**
Objective response[†]			
No. of patients (% [95% CI])	86 (41.3 [34.6–48.4])	115 (54.8 [47.8–61.6])	36 (17.8 [12.8–23.8])
Estimated odds ratio (95% CI)[§]	3.25 (2.05–5.13)	5.90 (3.71–9.38)	---

[†] ***Best overall response was assessed by the investigators with the use of RECIST v1.1.***

[§] Relative to ipilimumab alone.

Adverse Events

[0136] Treatment-related adverse events of any grade occurred in 82.1%, 95.5%, and 86.2% of patients in the nivolumab, nivolumab plus ipilimumab groups, and ipilimumab groups, respectively (Table 5). The most common adverse events in the nivolumab group were fatigue (in 34.2% of patients), rash (in 21.7%), and diarrhea (in 19.2%). In the nivolumab plus ipilimumab and ipilimumab groups, diarrhea (in 44.1% and 33.1% of patients, respectively), fatigue (in 35.1% and 28.0%), and pruritus (in 33.2% and 35.4%) were most common (Table 5). The incidence of treatment-related adverse events of grade 3 or 4 was also higher in the nivolumab plus ipilimumab group than in either the nivolumab or ipilimumab groups (55.0% vs. 16.3% and 27.3%), with diarrhea being the most common (2.2% , 9.3%, and 6.1% in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively) (Table 5). Treatment-related adverse events of any grade leading to discontinuation occurred in 7.7%, 36.4%, and 14.8% of patients in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively, with the most common being diarrhea (in 1.9%, 8.3%, and 4.5%, respectively) and colitis (in 0.6%, 8.3%, and 7.7%, respectively) (Table 5). One death due to study-drug toxicity was reported in the nivolumab group (neutropenia) and one in the ipilimumab group (cardiac arrest), although such adverse events have not been associated with these drugs in prior studies. No treatment-related deaths were reported in the combination group.

[0137] The most frequent grade 3 or 4 treatment-related select adverse events were diarrhea (2.2%, 9.3%, and 6.1% of patients in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively), colitis (in 0.6%, 7.7%, and 8.7%, respectively), increased alanine aminotransferase (in 1.3%, 8.3%, and 1.6%, respectively), and increased aspartate aminotransferase (in 1.0%, 6.1%, and 0.6%, respectively) (Table 6). With the use of immune modulatory agents, resolution rates for grade 3 or 4 select adverse events were generally similar across treatment groups, and were between 85-100% across organ categories in the nivolumab plus ipilimumab group. As observed in prior studies, most endocrine events in all treatment groups did not resolve (Table 7).

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Table 5. Adverse Events (Safety Population).*

Event	Nivolumab alone (N=313)		Nivolumab plus Ipilimumab (N=313)		Ipilimumab alone (N=311)	
	Total	Grade 3 or 4	Total	Grade 3 or 4	Total	Grade 3 or 4
	no. of patients with event (%)					
Any adverse event	311 (99.4)	136 (43.5)	312 (99.7)	215 (68.7)	308 (99.0)	173 (55.6)
Treatment-related adverse event†	257 (82.1)	51 (16.3)	299 (95.5)	172 (55.0)	268 (86.2)	85 (27.3)
Diarrhea	60 (19.2)	7 (2.2)	138 (44.1)	29 (9.3)	103 (33.1)	19 (6.1)
Fatigue	107 (34.2)	4 (1.3)	110 (35.1)	13 (4.2)	87 (28.0)	3 (1.0)
Pruritus	59 (18.8)	0	104 (33.2)	6 (1.9)	110 (35.4)	1 (0.3)
Rash	81 (25.9)	2 (0.6)	126 (40.3)	15 (4.8)	102 (32.8)	6 (1.9)
Nausea	41 (13.1)	0	81 (25.9)	7 (2.2)	50 (16.1)	2 (0.6)
Pyrexia	18 (5.8)	0	58 (18.5)	2 (0.6)	21 (6.8)	1 (0.3)
Decreased appetite	34 (10.9)	0	56 (17.9)	4 (1.3)	39 (12.5)	1 (0.3)
Increase in alanine aminotransferase	12 (3.8)	4 (1.3)	55 (17.6)	26 (8.3)	12 (3.9)	5 (1.6)
Vomiting	20 (6.4)	1 (0.3)	48 (15.3)	8 (2.6)	23 (7.4)	1 (0.3)
Increase in aspartate aminotransferase	12 (3.8)	3 (1.0)	48 (15.3)	19 (6.1)	11 (3.5)	2 (0.6)
Hypothyroidism	27 (8.6)	0	47 (15.0)	1 (0.3)	13 (4.2)	0
Colitis	4 (1.3)	2 (0.6)	37 (11.8)	24 (7.7)	36 (11.6)	27 (8.7)
Arthralgia	24 (7.7)	0	33 (10.5)	1 (0.3)	19 (6.1)	0
Headache	23 (7.3)	0	32 (10.2)	1 (0.3)	24 (7.7)	1 (0.3)
Dyspnea	14 (4.5)	1 (0.3)	32 (10.2)	2 (0.6)	13 (4.2)	0
Treatment-related adverse event leading to discontinuation	24 (7.7)	16 (5.1)	114 (36.4)	92 (29.4)	46 (14.8)	41 (13.2)

* The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.
† The treatment-related adverse events listed here were reported in at least 10% of the patients in any of the three study groups.

Table 6. Treatment-related Select Adverse Events (Safety Population).*

Event	Nivolumab alone (N=313)		Nivolumab plus Ipilimumab (N=313)		Ipilimumab alone (N=311)	
	Total	Grade 3 or 4	Total	Grade 3 or 4	Total	Grade 3 or 4
	no. of patients with event (%)					
Any select adverse event	194 (62.0)	24 (7.7)	275 (87.9)	124 (39.6)	229 (73.6)	58 (18.6)
Treatment-related select adverse event†						
Skin	131 (41.9)	5 (1.6)	185 (59.1)	18 (5.8)	168 (54.0)	9 (2.9)
Pruritus	59 (18.8)	0	104 (33.2)	6 (1.9)	110 (35.4)	1 (0.3)
Rash	68 (21.7)	1 (0.3)	89 (28.4)	9 (2.9)	65 (20.9)	5 (1.6)
Rash maculo-papular	13 (4.2)	1 (0.3)	37 (11.8)	6 (1.9)	37 (11.9)	1 (0.3)
Vitiligo	23 (7.3)	1 (0.3)	21 (6.7)	0	12 (3.9)	0
Gastrointestinal	61 (19.5)	7 (2.2)	145 (46.3)	46 (14.7)	114 (36.7)	36 (11.6)
Diarrhea	60 (19.2)	7 (2.2)	138 (44.1)	29 (9.3)	103 (33.1)	19 (6.1)
Colitis	4 (1.3)	2 (0.6)	37 (11.8)	24 (7.7)	36 (11.6)	27 (8.7)
Hepatic	20 (6.4)	8 (2.6)	94 (30.0)	59 (18.8)	22 (7.1)	5 (1.6)
Increase in alanine aminotransferase	12 (3.8)	4 (1.3)	55 (17.6)	26 (8.3)	12 (3.9)	5 (1.6)
Increase in aspartate aminotransferase	12 (3.8)	3 (1.0)	48 (15.3)	19 (6.1)	11 (3.5)	2 (0.6)
Endocrine	45 (14.4)	2 (0.6)	94 (30.0)	15 (4.8)	34 (10.9)	7 (2.3)
Hypothyroidism	27 (8.6)	0	47 (15.0)	1 (0.3)	13 (4.2)	0
Hyperthyroidism	13 (4.2)	0	31 (9.9)	3 (1.0)	3 (1.0)	0
Hypophysitis	2 (0.6)	1 (0.3)	24 (7.7)	5 (1.6)	12 (3.9)	6 (1.9)
Pulmonary	5 (1.6)	1 (0.3)	22 (7.0)	3 (1.0)	6 (1.9)	1 (0.3)
Pneumonitis	4 (1.3)	1 (0.3)	20 (6.4)	3 (1.0)	5 (1.6)	1 (0.3)

* The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.
† The treatment-related select adverse events listed here were reported in at least 5% of the patients in any of the three study groups.

Table 7. Management of Treatment-related Select Adverse Events With Immune Modulatory Medication (IMM).

	Nivolumab			Nivolumab plus Ipilimumab			Ipilimumab		
Select Adverse Event Organ Category	**Patients managed with IMM, n (%)**	**Patients with resolution of AE after treatment with IMM, n (%)**	**Median time to resolution, weeks (95% CI)**	**Patients managed with IMM, n (%)**	**Patients with resolution of AE after treatment with IMM, n (%)**	**Median time to resolution, weeks (95% CI)**	**Patients managed with IMM, n (%)**	**Patients with resolution of AE after treatment with IMM, n (%)**	**Median time to resolution, weeks (95% CI)**
Skin Grade 3-4	33/131 (25.2) 3/5 (60.0)	17 (54.8) 3 (75.0)	34.9 (18.0, NE) 2.1 (0.9, NE)	80/185 (43.2) 12/18 (66.7)	55 (75.3) 12 (85.7)	8.6 (7.0, 12.6) 3.4 (1.1, 12.4)	58/168 (34.5) 5/9 (55.6)	41 (74.5) 5 (83.3)	12.4 (8.9, 19.1) 6.1 (4.4, NE)
Gastrointestinal Grade 3-4	9/61 (14.8) 5/7 (71.4)	5 (71.4) 3 (50.0)	4.0 (0.8, NE) NE (0.8, NE)	71/145 (49.0) 41/46 (89.1)	62 (93.9) 41 (97.6)	4.5 (3.5, 5.7) 3.0 (1.9, 4.3)	54/114 (47.4) 33/36 (91.7)	44 (88.0) 31 (88.6)	4.9 (2.9, 7.6) 4.7 (1.9, 6.1)
Endocrine Grade 3-4	5/45 (11.1) 2/2 (100)	2 (40.0) 0	24.3 (7.1, NE) NE (NE, NE)	36/94 (38.3) 10/15 (66.7)	14 (41.2) 5 (45.5)	NE (10.4, NE) NE (4.5, NE)	15/34 (44.1) 7/7 (100.0)	4 (28.6) 3 (42.9)	NE (0.71, NE) NE (0.43, NE)
Hepatic Grade 3-4	8/22 (36.4) 7/8 (87.5)	6 (100.0) 6 (100.0)	7.0 (2.0, 27.1) 7.0 (2.0, 27.1)	46/95 (48.4) 37/60 (61.7)	43 (95.6) 38 (100.0)	5.9 (3.3, 6.6) 4.1 (3.0, 6.1)	3/22 (13.6) 2/5 (40.0)	3 (100.0) 2 (100.0)	4.1 (4.0, 7.7) 5.9 (4.0, 7.7)
Pulmonary Grade 3-4	4/5 (80.0) 1/1 (100)	4 (100.0) 1 (100.0)	3.3 (2.3, 9.1) 2.3 (NE, NE)	17/22 (77.3) 2/3 (66.7)	16 (94.1) 2 (100.0)	6.1 (1.1, 8.3) 4.2 (1.1, 7.3)	3/6 (50.0) 1/1 (100)	2 (66.7) 1 (100.0)	6.1 (6.0, 6.3) 4.7 (NE, NE)
Renal Grade 3-4	1/3 (33.3) 0/1	1 (100.0) 0	0.3 (NE, NE) -	4/17 (23.5) 3/6 (50.0)	3 (100.0) 3 (100.0)	1.7 (0.4, 3.7) 1.7 (0.4, 3.6)	3/8 (37.5) 1/1 (100)	3 (100.0) 1 (100.0)	4.6 (0.6, 16.1) 4.6 (NE, NE)

Discussion

[0138] In this randomized, double-blind, phase 3 study, both nivolumab alone and the combination of nivolumab and ipilimumab significantly increased progression-free survival and objective response rates, as compared with ipilimumab alone, in previously untreated advanced melanoma. These results were observed independently of PD-L1 tumor status, BRAF mutation status, or metastasis stage. Baseline characteristics of study participants were typical of patients with advanced melanoma, although the BRAF mutation rate (31.5%) was lower than the 40-50% generally reported for advanced disease. While not a primary end point of the study, the combination of nivolumab and ipilimumab resulted in numerically longer progression-free survival and a higher response rate as compared with nivolumab alone in the overall study population. While time to response was similar between groups, the first tumor assessment was done at week 12 and thus the possibility that responses may have occurred earlier with the combination remains unknown.

[0139] The median progression-free survival reported for the combination of nivolumab and ipilimumab in this study (11.7 months in BRAF-mutant patients) is similar to that recently reported for the combination of BRAF and MEK inhibition in BRAF-mutant metastatic melanoma (9.9 months for vemurafenib and cobimetinib; 9.3 to 11.4 months for dabrafenib and trametinib). Resistance to such targeted therapies is almost inevitable when used as monotherapy and in many cases is very rapid. The confirmed rate of objective response for combined nivolumab and ipilimumab (57.6%) is numerically higher than observed with PD-1 blockade alone in advanced melanoma (nivolumab [40%] in treatment-naïve patients with wild-type BRAF or pembrolizumab [37%] in ipilimumab-naïve patients).

[0140] The results of subgroup analyses suggest that the greatest benefit for the combination of nivolumab and ipilimumab versus nivolumab alone may occur in the setting of negative PD-L1 tumor expression. In the PD-L1-positive group, both nivolumab alone and nivolumab plus ipilimumab resulted in a similar prolongation of progression-free survival as compared with ipilimumab alone, although objective response rates were numerically higher in the combination group versus either nivolumab or ipilimumab alone. Thus, the use of PD-L1 as a biomarker may allow clinicians to make more informed decisions about the risk-benefit of combination therapy versus monotherapy. Nonetheless, the observation of at least additive

activity of the combination of ipilimumab and nivolumab in the setting of negative PD-L1 expression is of interest in melanoma as well as in other tumor types in which PD-1 checkpoint inhibitors are under evaluation.

[0141] The incidence of adverse events in this study was, in general, lowest in the nivolumab group and highest in the combination group. The overall incidence of grade 3 or 4 drug-related adverse events was higher in the combination group as compared with ipilimumab alone (39.6% versus 18.6%), as a result of a slightly higher incidence in most adverse events, particularly hepatic toxicity, where the rates of grade 3 or 4 ALT/AST elevations were 6-8% for the combination and approximately 1% for ipilimumab alone. One drug-related death was reported in each of the nivolumab and ipilimumab groups but none in the combination group. Overall, the safety profile of the combination of nivolumab and ipilimumab was consistent with previous experience with nivolumab or ipilimumab alone. No new safety signals were identified, and adverse events were manageable with established treatment guidelines as most select adverse events resolved with immune modulatory agents. These data suggest that the combination of nivolumab and ipilimumab can be used safely in a broad range of clinical settings.

[0142] In summary, the report shows increased progression-free survival and objective response rates for nivolumab alone and the combination of nivolumab and ipilimumab, as compared with ipilimumab alone, in previously untreated advanced melanoma. Adverse events with the combination were managed with established algorithms, with no study drug-related deaths. The combination of nivolumab and ipilimumab may represent a means to improve outcomes with either agent as monotherapy, particularly for patients having PD-L1-negative tumors. Overall, nivolumab alone and the combination of nivolumab and ipilimumab are promising treatment options for previously untreated advanced melanoma.

SEQUENCES

V_H amino acid sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:1)

Met Glu Thr Gly Leu Arg Trp Leu Leu Leu Val Ala Val Leu Lys Gly Val Gln Cys Leu Ser Val Glu Glu Ser Gly Gly Arg Leu Val Thr Pro Gly Thr Pro Leu Thr Leu Thr Cys Thr Ala Ser Gly Phe Thr Ile Thr Asn Tyr His Met Phe Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile Gly Val Ile Thr Ser Ser Gly Ile Gly Ser Ser Ser Thr Thr Tyr Tyr Ala Thr Trp Ala Lys Gly Arg Phe Thr Ile Ser Lys Thr Ser Thr Thr Val Asn Leu Arg Ile Thr Ser Pro Thr Thr Glu Asp Thr Ala Thr Tyr Phe Cys Ala Arg Asp Tyr Phe Thr Asn Thr Tyr Tyr Ala Leu Asp Ile Trp Gly Pro Gly Thr Leu Val Thr Val Ser Ser

V_L amino acid sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:2)

Met Asp Thr Arg Ala Pro Thr Gln Leu Leu Gly Leu Leu Leu Leu Trp Leu Pro Gly Ala Arg Cys Ala Leu Val Met Thr Gln Thr Pro Ser Ser Thr Ser Thr Ala Val Gly Gly Thr Val Thr Ile Lys Cys Gln Ala Ser Gln Ser Ile Ser Val Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ser Ala Ser Thr Leu Ala Ser Gly Val Pro Ser Arg Phe Lys Gly Ser Arg Ser Gly Thr Glu Tyr Thr Leu Thr Ile Ser Gly Val Gln Arg Glu Asp Ala Ala Thr Tyr Tyr Cys Leu Gly Ser Ala Gly Ser

Heavy Chain CDR1 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:3)

Asn Tyr His Met Phe

Heavy Chain CDR2 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:4)

Val Ile Thr Ser Ser Gly Ile Gly Ser Ser Ser Thr Thr Tyr Tyr Ala Thr Trp Ala Lys Gly

Heavy Chain CDR3 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:5)

Asp Tyr Phe Thr Asn Thr Tyr Tyr Ala Leu Asp Ile

Light Chain CDR1 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:6)

Gln Ala Ser Gln Ser Ile Ser Val Tyr Leu Ala

Light Chain CDR2 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:7)

Ser Ala Ser Thr Leu Ala Ser

Light Chain CDR3 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:8)

Leu Gly Ser Ala Gly Ser Asp Asp Ala Ala

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET – Page 1 of 2

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Express Mail Label No. _____

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Arvin	YANG	Princeton, NJ

Additional inventors are being named on the _____ separately numbered sheets attached hereto.

TITLE OF THE INVENTION (500 characters max):

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

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

[X] The address corresponding to Customer Number: 115018

OR

[ ] Firm or Individual Name

Address

City	State	Zip
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ENCLOSED APPLICATION PARTS (*check all that apply*)

[X] Application Data Sheet. See 37 CFR 1.76. [ ] CD(s), Number of CDs _____

[X] Drawing(s) *Number of Sheets* 6 [X] Other (specify) See 1 in Addendum

[X] Specification (e.g., description of the invention) *Number of Pages* 61

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No.

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SIGNATURE _____ DATE April 28, 2015

TYPED OR PRINTED NAME Ji-Eun Kim REGISTRATION NO. 68,504 (*if appropriate*)

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Addendum

1. Authorization to Treat a Reply As Incorporating An Extension of Time Under 37 C.F.R. § 1.136(a)(3); and An executed Power of Attorney by Applicant (PTO/AIA/82B) and Transmittal for Power of Attorney to One or More Registered Practitioners (PTO/AIA/82A)