following: 1) at least one on-treatment tumor evaluation, 2) clinical progression, or 3) death prior to the first on-treatment tumor evaluation.

‡ Patients who had a PR after one tumor assessment but did not have sufficient follow-up time for confirmation of the initial PR.

Table 12. Duration of Confirmed Objective Responses of Individual Patients

Cohort	Dose	Response	Duration of Response, weeks
1	0.3 mg/kg nivolumab + 3 mg/kg ipilimumab	CR	48.0+
		PR	72.1+, 65.4+
2	1 mg/kg nivolumab + 3 mg/kg ipilimumab	CR	36.1+, 28.9+, 24.7+
		PR	54.4+, 52.6, 23+, 18.4+, 14.0 +, 11.1+
2a	3 mg/kg nivolumab + 1 mg/kg ipilimumab	CR	7.7+
		PR	18.1+, 12.3, 6.6+, 6.4+, 6.1+
3	3 mg/kg nivolumab + 3 mg/kg ipilimumab	PR	38.7+, 31.3+, 12.1+
6	1 mg/kg nivolumab + prior ipilimumab	CR	24.1+
		PR	16.1+, 12.1+, 8.7+, 8.4+, 8.1+
7	3 mg/kg nivolumab + prior ipilimumab	CR	NA*
		PR	NA*

* Not available; as of the date of this analysis, no confirmed objective responses had been reported.

Assessment of tumor PD-L1 expression and absolute lymphocyte count

Tumor PD-L1 expression and alterations in the peripheral blood ALC have been explored as biomarkers for nivolumab and ipilimumab monotherapy, respectively (Topalian *et al.*, 2012b; Berman *et al.*, 2009; Ku *et al.*, 2010; Postow *et al.*, 2012; Delyon *et al.*, 2013). Tumor PD-L1 expression was characterized via IHC staining and pharmacodynamic changes in peripheral blood ALC was analyzed. Using a ≥ 5% cut off to define PD-L1 positivity, tumor specimens from 21 of 56 (38%) patients were PD-L1-positive. ORs were seen in patients with either PD-L1-positive (6/13) or PD-L1-negative (9/22) tumors amongst patients treated with the concurrent regimen (post-hoc P-value > 0.99; Fisher's exact test). In sequenced-regimen cohorts, a numerically higher number of overall responses were seen in patients with PD-L1-positive tumor samples (4/8) compared with patients with PD-L1-negative tumors (1/13), but the numbers are small.

In contrast to observations with ipilimumab monotherapy, a consistent rise in ALC from baseline was not detected in patients treated with the concurrent combination or in patients treated with nivolumab following ipilimumab therapy. In the concurrent-regimen cohorts, patients with a low ALC at weeks 5 to 7 (< 1000 cells/μL) (Ku *et al.*, 2010) had similar OR (43%) compared with patients with a normal ALC at weeks 5 to 7 (40%). Likewise, in the sequenced-regimen cohorts, 17% of patients with low ALC had OR and 23% of patients with normal or high ALC had OR.

Sequence Listing Summary

SEQ ID NO:	Description
1	V_H amino acid sequence of 17D8
2	V_H amino acid sequence of 2D3
3	V_H amino acid sequence of 4H1
4	V_H amino acid sequence of 5C4
5	V_H amino acid sequence of 4A11
6	V_H amino acid sequence of 7D3
7	V_H amino acid sequence of 5F4
8	V_L amino acid sequence of 17D8
9	V_L amino acid sequence of 2D3
10	V_L amino acid sequence of 4H1
11	V_L amino acid sequence of 5C4

SEQ ID NO:	Description
12	V_L amino acid sequence of 4A11
13	V_L amino acid sequence of 7D3
14	V_L amino acid sequence of 5F4
15	V_H amino acid sequence of 3G10
16	V_H amino acid sequence of 12A4
17	V_H amino acid sequence of 10A5
18	V_H amino acid sequence of 5F8
19	V_H amino acid sequence of 10H10
20	V_H amino acid sequence of 1B12
21	V_H amino acid sequence of 7H1
22	V_H amino acid sequence of 11E6
23	V_H amino acid sequence of 12B7
24	V_H amino acid sequence of 13G4
25	V_L amino acid sequence of 3G10
26	V_L amino acid sequence of 12A4
27	V_L amino acid sequence of 10A5
28	V_L amino acid sequence of 5F8
29	V_L amino acid sequence of 10H10
30	V_L amino acid sequence of 1B12
31	V_L amino acid sequence of 7H1
32	V_L amino acid sequence of 11E6
33	V_L amino acid sequence of 12B7
34	V_L amino acid sequence of 13G4
35	V_H amino acid sequence of 28-8
36	V_L amino acid sequence of 28-8
37	Heavy chain CDR1 sequence of 28-8
38	Heavy chain CDR2 sequence of 28-8
39	Heavy chain CDR3 sequence of 28-8
40	Light chain CDR1 sequence of 28-8
41	Light chain CDR2 sequence of 28-8
42	Light chain CDR1 sequence of 28-8

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CLAIMS

What is claimed is:

1. A method of treating a subject afflicted with a cancer comprising administering to the subject a therapeutically effective amount of an antibody or an antigen-binding portion thereof that disrupts the interaction between Programmed Death-1 (PD-1) and Programmed Death Ligand-1 (PD-L1, wherein the antibody or antigen-binding portion thereof binds specifically to PD-1 or to PD-L1.

2. The method of claim 1, wherein the cancer is selected from the group consisting of melanoma, renal cell carcinoma, squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, colorectal cancer, castration-resistant prostate cancer, ovarian cancer, gastric cancer, hepatocellular carcinoma, pancreatic carcinoma, squamous cell carcinoma of the head and neck, carcinomas of the esophagus, gastrointestinal tract and breast, and a hematological malignancy.

3. The method of claim 1, wherein the therapeutically effective amount of the antibody or antigen-binding portion thereof comprises a dose ranging from 0.1 to 10.0 mg/kg body weight which is administered at a dosing schedule of once per week, once every two weeks, or once a month.

4. A method for immunotherapy of a subject afflicted with cancer, which method comprises:

(a) selecting a subject that is a suitable candidate for immunotherapy, the selecting comprising:

(i) providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells;

(ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; and

(iii) selecting the subject as a suitable candidate based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and

(b) administering a composition comprising a therapeutically effective amount of an anti-PD-1 antibody to the selected subject.

5. The method of claim 4, wherein the proportion of cells that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 polypeptide on the surface of cells in the test tissue sample.

6. The method of claim 5, wherein the test tissue sample is a formalin-fixed paraffin-embedded (FFPE) tissue sample.

7. The method of claim 6, wherein the presence of PD-L1 polypeptide is determined using an automated IHC assay.

8. The method of claim 12, wherein the IHC assay is performed using an anti-PD-L1 monoclonal antibody to bind to the PD-L1 polypeptide, wherein the anti-PD-L1 monoclonal antibody is selected from 28-8, 28-1, 28-12, 29-8, and 5H1.

9. The method of claim 4, wherein the predetermined threshold level is 1% of tumor cells or a single tumor-infiltrating inflammatory cell expressing cell surface PD-L1 as determined by automated IHC using mAb 28-8.

10. A method for treatment of a subject afflicted with cancer, which method comprises:

(a) selecting a subject that is not suitable for anti-PD-1 antibody immunotherapy, the selecting comprising:

(i) providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells;

(ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; and

(iii) selecting the subject as not suitable for anti-PD-1 antibody immunotherapy based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is less than a predetermined threshold level; and

(b) administering a standard-of-care therapeutic other than an anti-PD-1 antibody to the selected subject.

11. A monoclonal antibody or an antigen-binding portion thereof that binds specifically to a cell surface-expressed PD-L1 polypeptide in a formalin-fixed, paraffin-embedded (FFPE) tissue sample.

12. The monoclonal antibody or antigen-binding portion thereof of claim 16, which antibody or portion thereof comprises the CDR1, CDR2 and CDR3 regions in a heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 35, and the CDR1, CDR2 and CDR3 regions in a light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 36.

13. The monoclonal antibody or antigen-binding portion thereof of claim 16, which antibody or portion thereof comprises a heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 35, and a light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 36.

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

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

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

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

(i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 10 doses, followed by administration of the anti-PD-1 alone at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 12 doses; followed by

(ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a

dosing frequency of at least once every 8, 12 or 16 weeks, or at least once a quarter, for at least 4, 6, 8, 10, 12 or 16 doses.

15. The method of claim 14, wherein the anti-PD-1 and anti-CTLA-4 antibodies are administered at the following dosages:

(a) 0.1 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody;
(b) 0.3 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody;
(c) 1 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody;
(d) 3 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody;
(e) 5 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody;
(f) 10 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody;
(g) 0.1 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;
(h) 0.3 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;
(i) 1 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;
(j) 3 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;
(k) 5 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; or
(l) 10 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody.

16. A method of treating a subject afflicted with a cancer, the subject having previously been treated with an anti-CTLA-4 antibody, which method comprises administering to the subject in a sequenced regimen an antibody or an antigen-binding portion thereof that specifically binds to and inhibits PD-1 at a dosage ranging from 0.1 to 20.0 mg/kg body weight and at a dosing frequency of at least once every week, at least once every 2, 3 or 4 weeks, or at least once a month, for up to 6 to up to 72 doses.

17. The method of claim 15 or 16, wherein the anti-PD-1 antibody is nivolumab.

18. The method of 15 or 16, wherein the anti-CTLA-4 antibody is ipilimumab.

19. An anti-PD-1 antibody or an antigen-binding portion thereof for use in treating a subject afflicted with a cancer in a concurrent regimen comprising combined administration with an anti-CTLA-4 antibody or an antigen-binding portion thereof, wherein the anti-PD-1 and anti-CTLA-4 antibodies are each administered

at a dosage ranging from 0.1 to 20.0 mg/kg body weight, and further wherein the concurrent regimen comprises:

(i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 12 doses, followed by administration of the anti-PD-1 alone at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 10 doses; followed by

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

20. A kit for treating a subject afflicted with a cancer, the kit comprising:

(a) a dosage ranging from 0.1 to 20.0 mg/kg body weight of an antibody or an antigen-binding portion thereof that specifically binds to and inhibits PD-1;

(b) a dosage ranging from 0.1 to 20.0 mg/kg body weight of an antibody or an antigen-binding portion thereof that specifically binds to and inhibits CTLA-4; and

(c) instructions for using the anti-PD-1 and anti-CTLA-4 antibodies the concurrent regimen method of claim 14.

21. A kit for treating a subject afflicted with a cancer, the kit comprising:

(a) a dosage ranging from 0.1 to 20.0 mg/kg body weight of an antibody or an antigen-binding portion thereof that specifically binds to and inhibits PD-1; and

(b) instructions for using the anti-PD-1 antibody in the sequenced regimen method of claim 16.

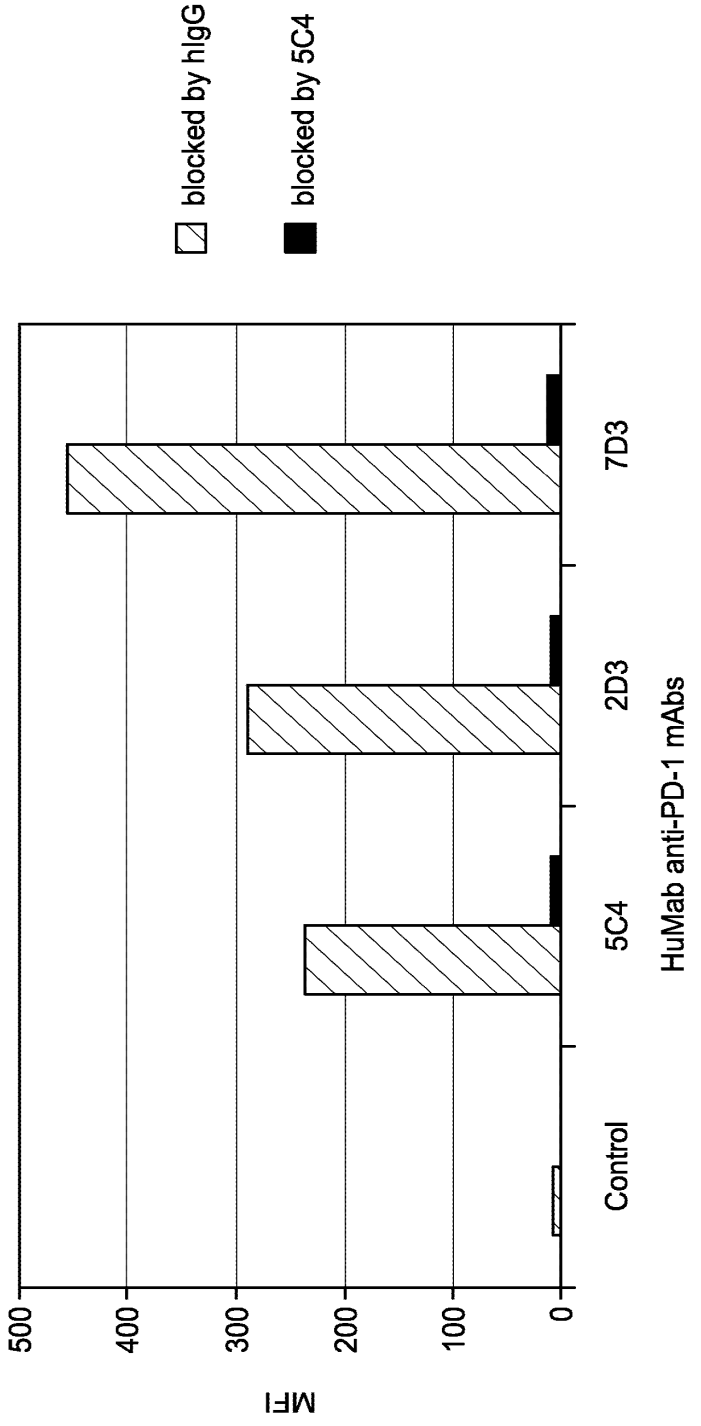

blocked by hIgG
blocked by 5C4
MFI
500
400
300
200
100
0
Control
5C4
2D3
7D3
HuMab anti-PD-1 mAbs

FIG. 1A

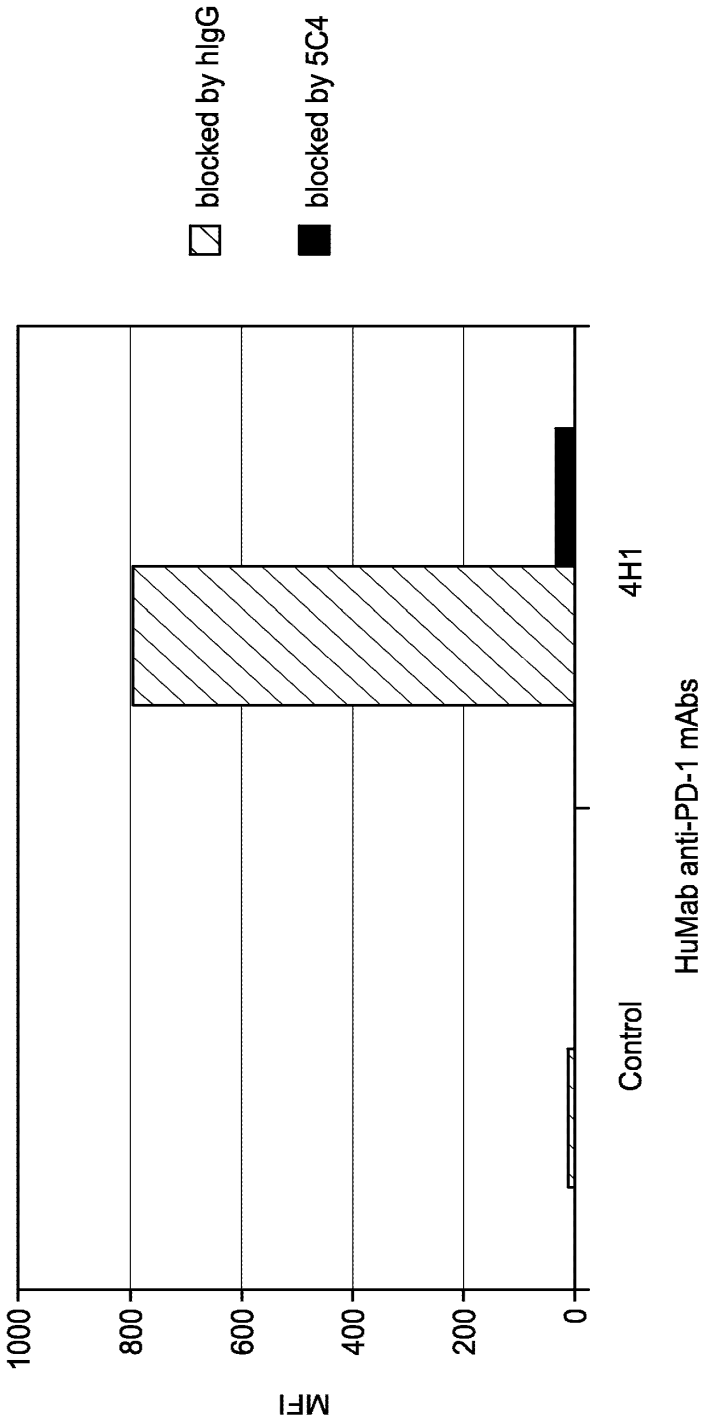

blocked by hIgG
blocked by 5C4
MFI
1000
800
600
400
200
0
Control
4H1
HuMab anti-PD-1 mAbs

FIG. 1B

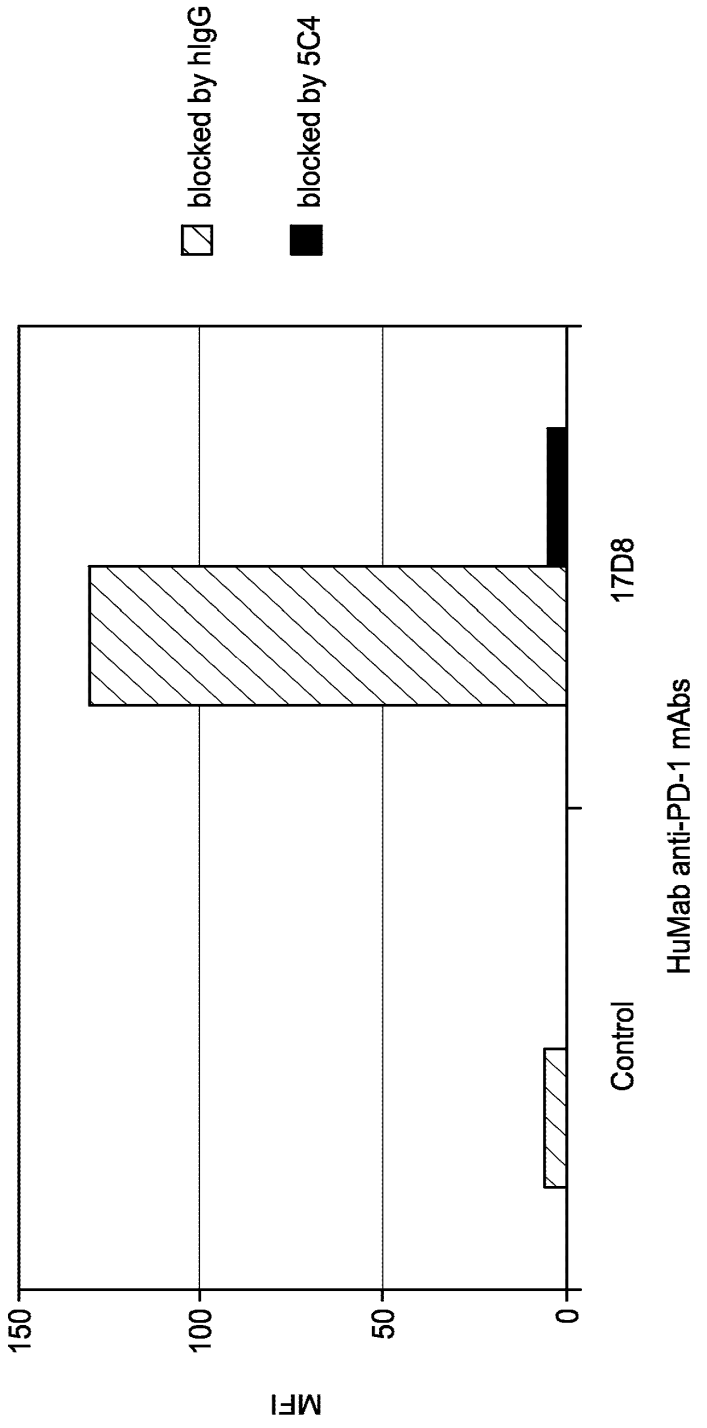

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blocked by 5C4
MFI
150
100
50
0
Control
17D8
HuMab anti-PD-1 mAbs

FIG. 1C

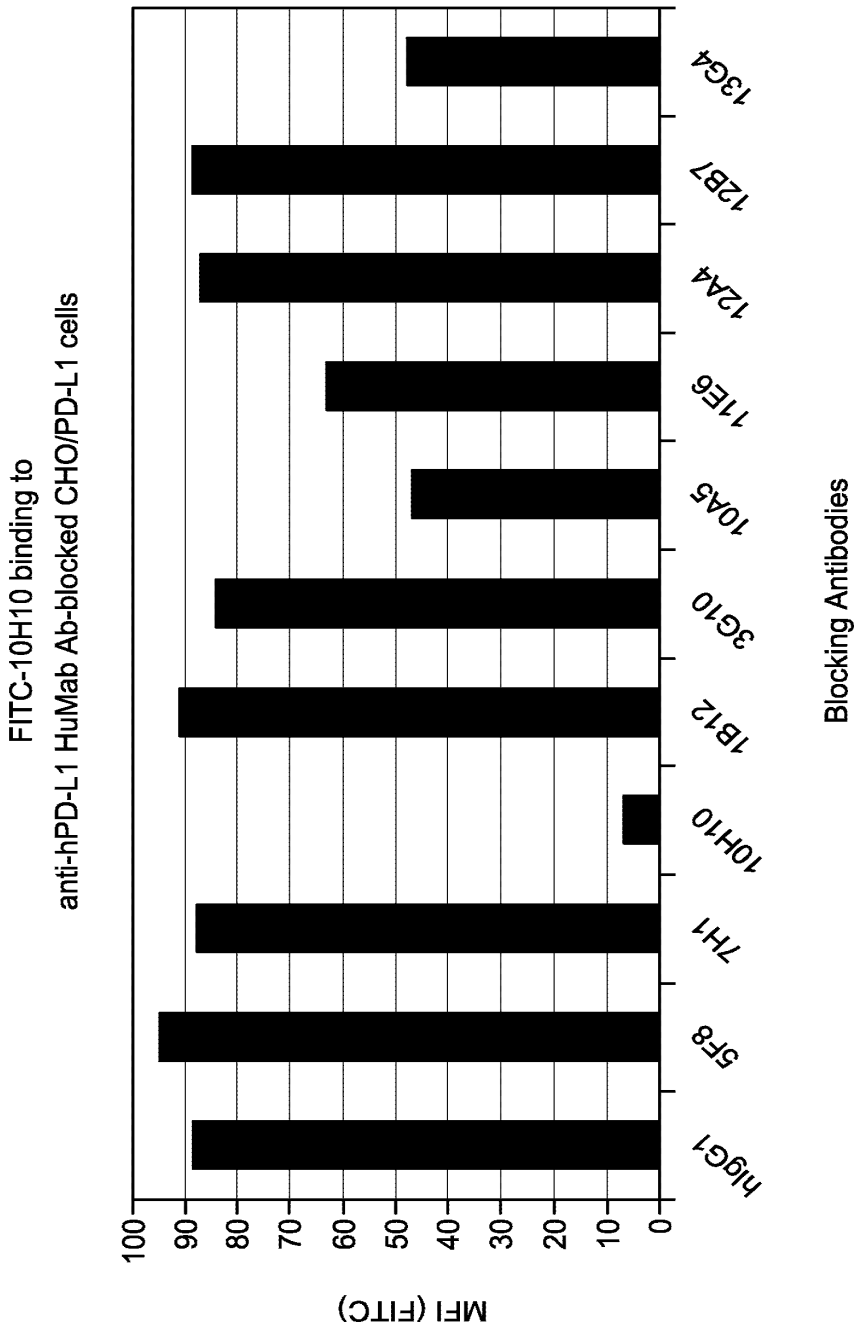

FITC-10H10 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
0
10
20
30
40
50
60
70
80
90
100
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2A

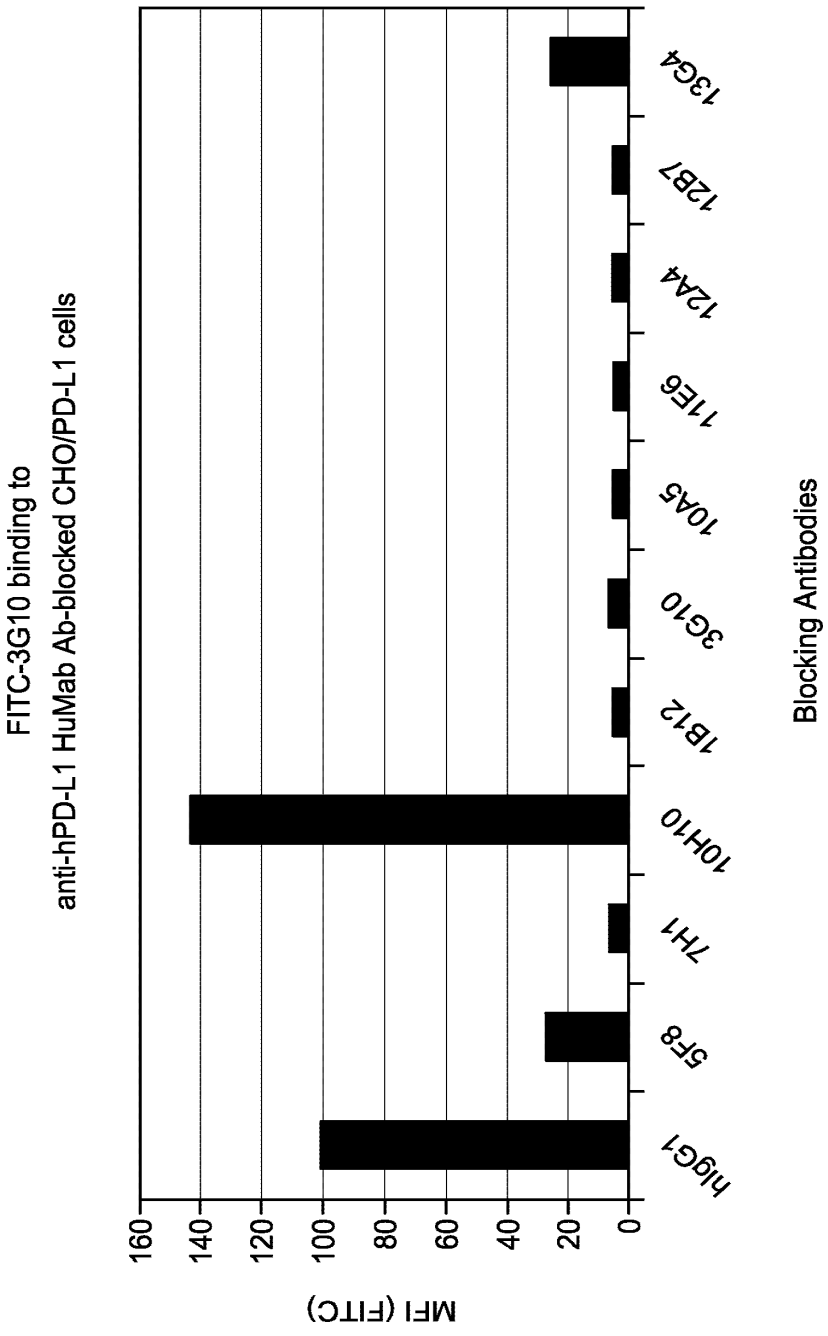

FITC-3G10 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
0
20
40
60
80
100
120
140
160
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2B

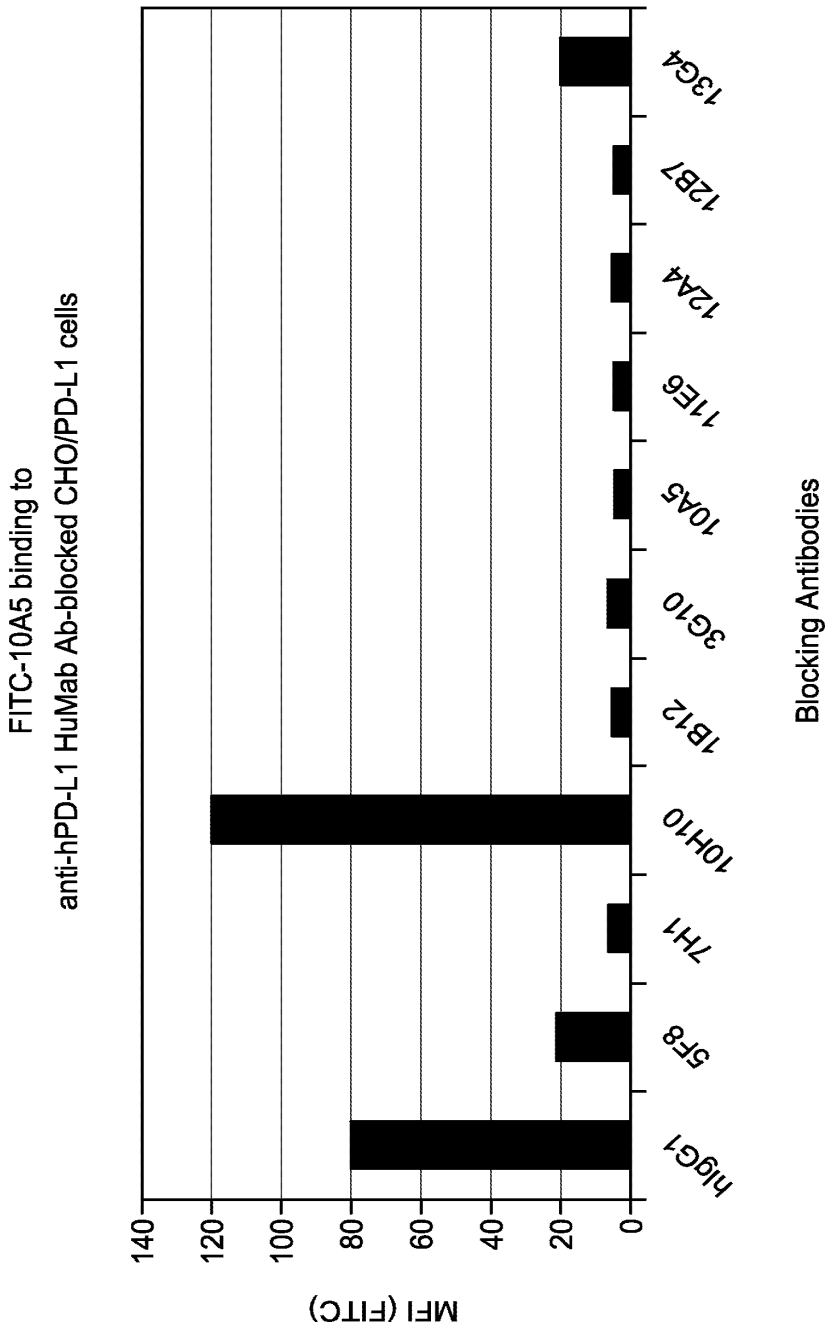

FITC-10A5 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
0
20
40
60
80
100
120
140
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2C

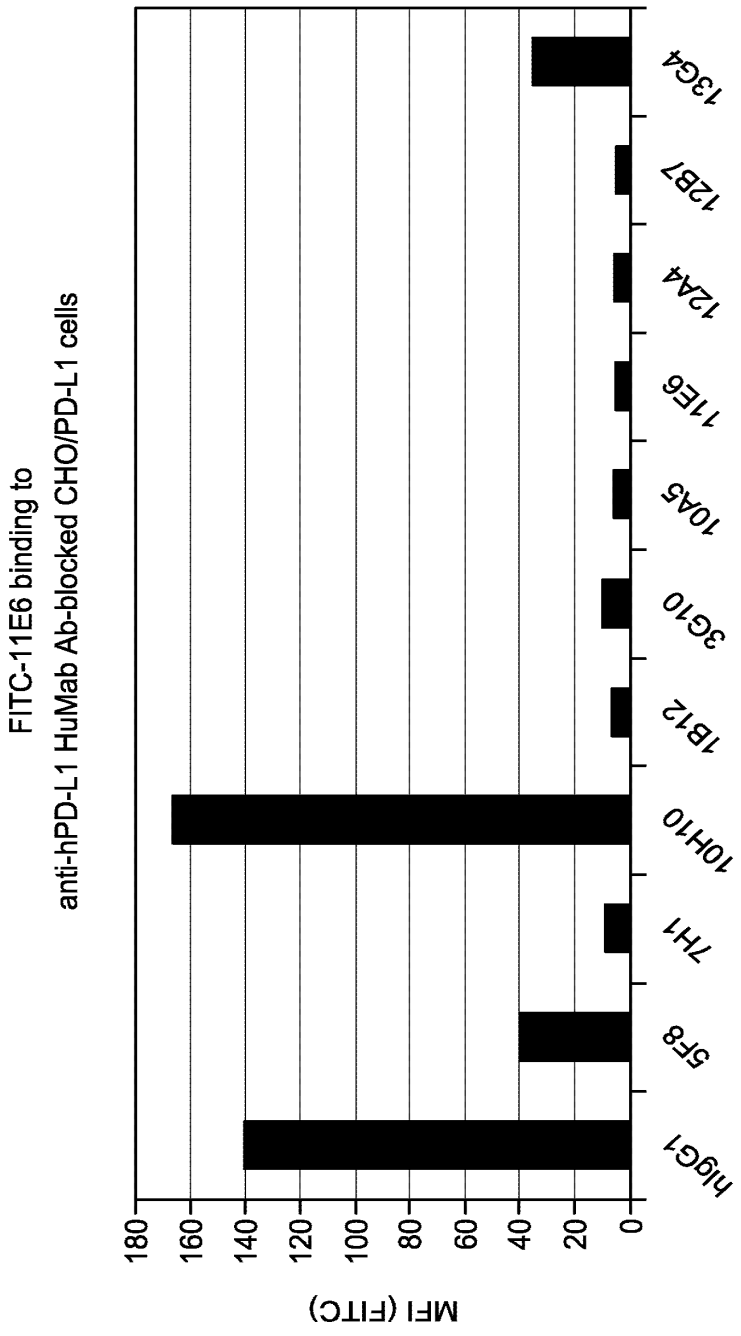

FITC-11E6 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
0
20
40
60
80
100
120
140
160
180
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2D

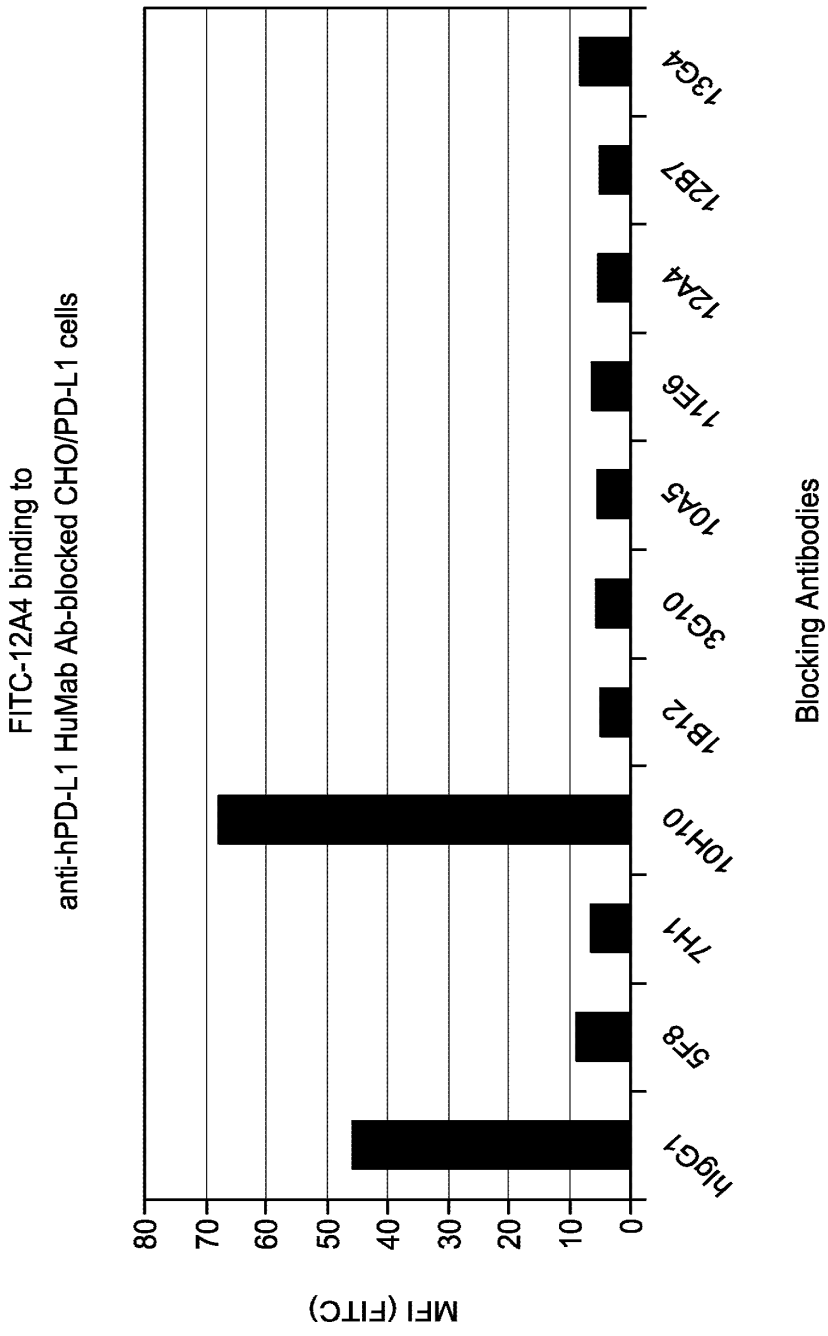

FITC-12A4 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
0
10
20
30
40
50
60
70
80
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2E

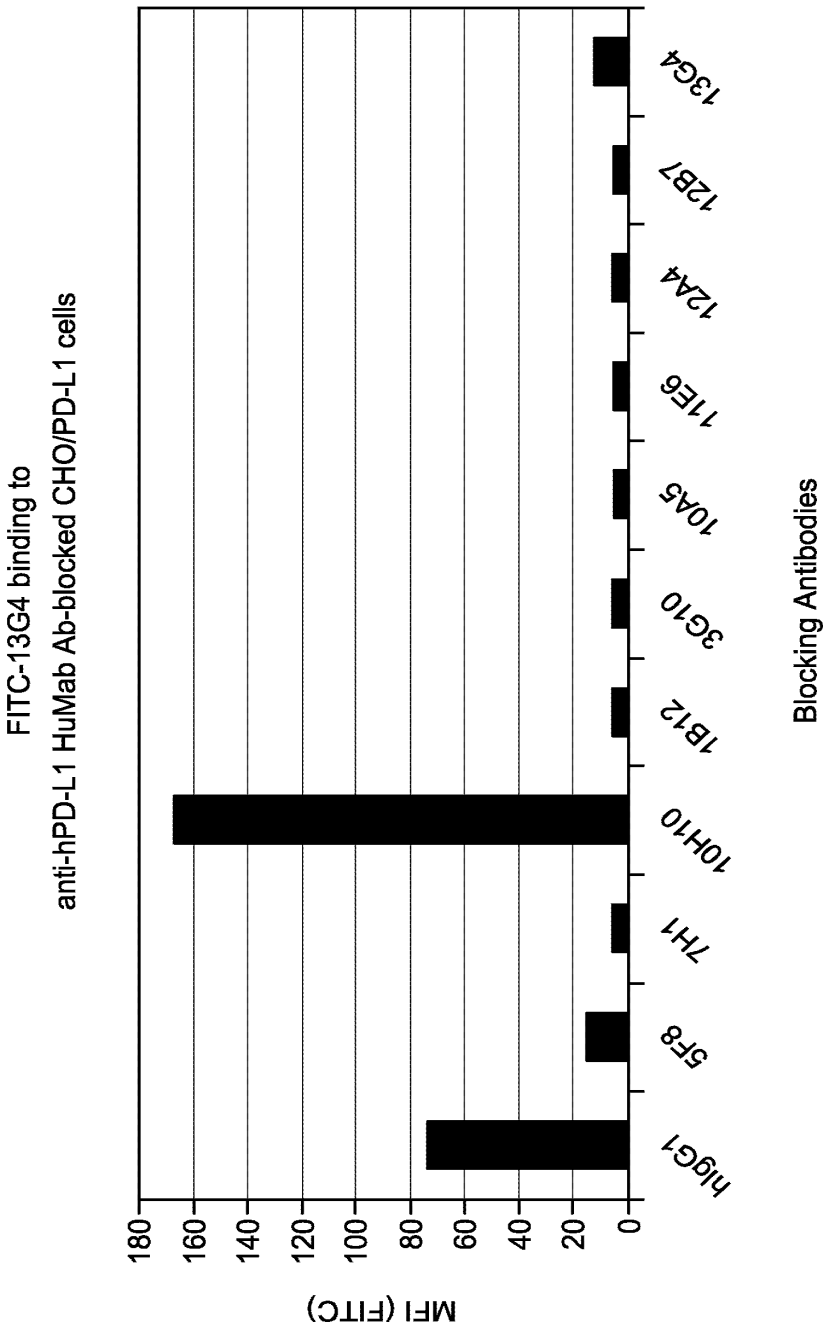

FITC-13G4 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
0
20
40
60
80
100
120
140
160
180
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2F

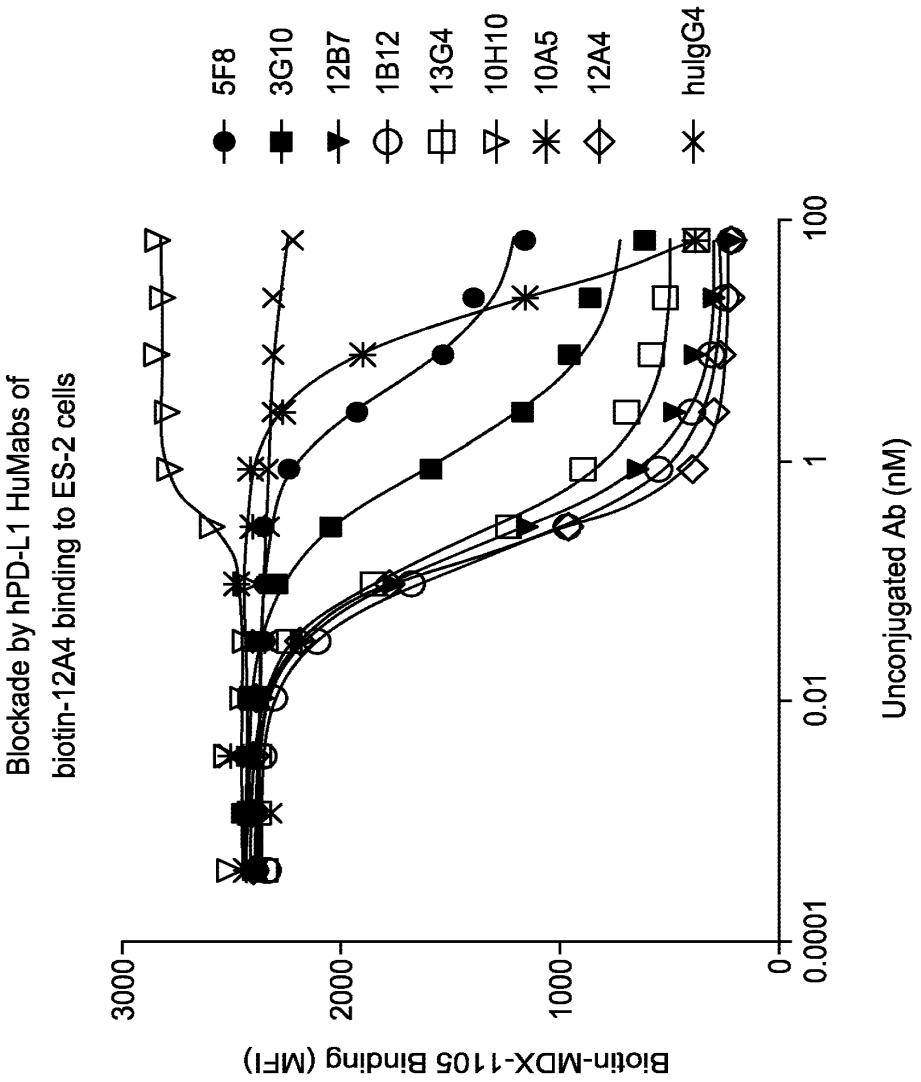

Blockade by hPD-L1 HuMabs of
biotin-12A4 binding to ES-2 cells
Biotin-MDX-1105 Binding (MFI)
3000
2000
1000
0
0.0001
0.01
1
100
Unconjugated Ab (nM)
5F8
3G10
12B7
1B12
13G4
10H10
10A5
12A4
hulgG4

FIG. 3

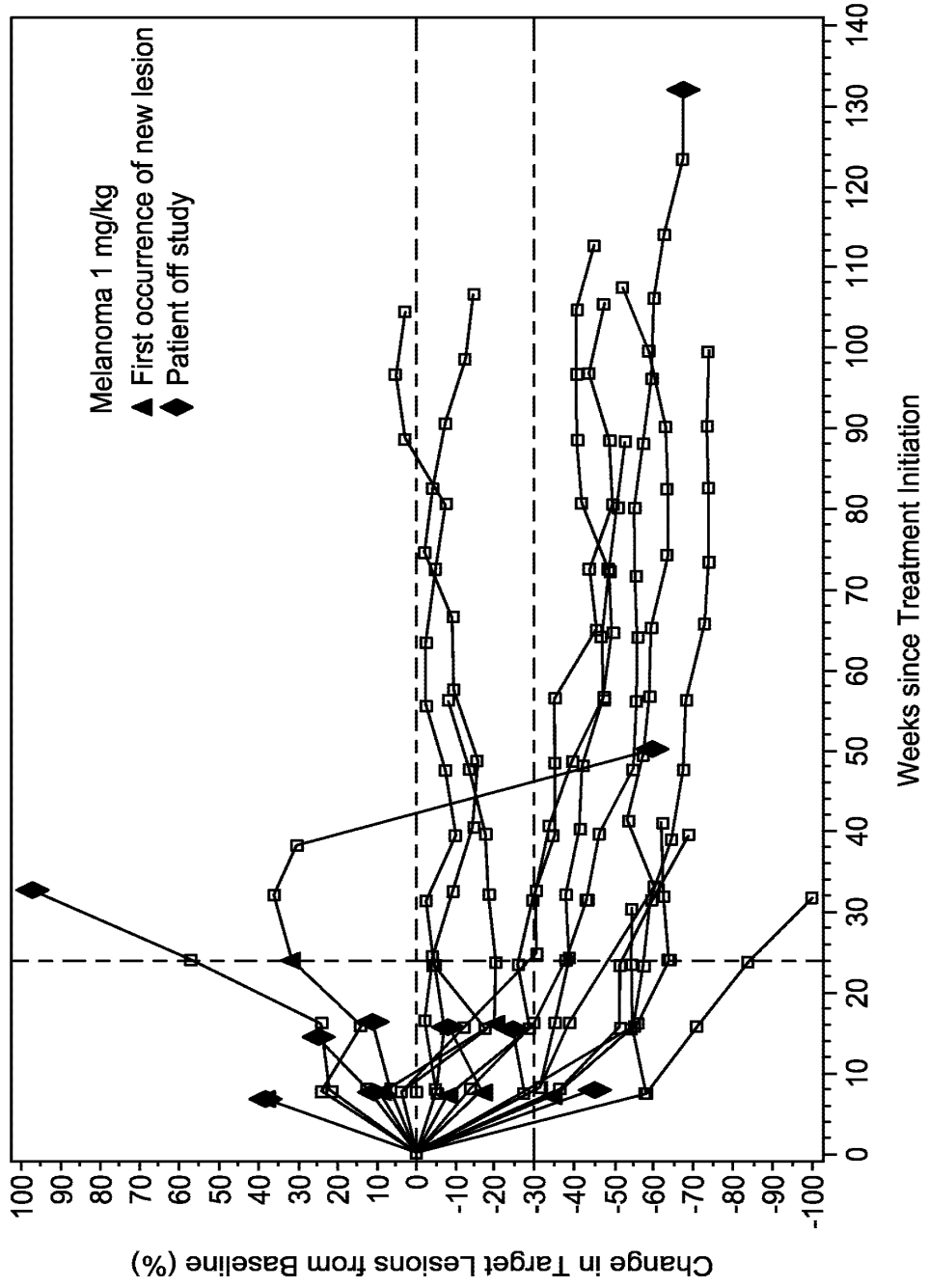

Melanoma 1 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
Weeks since Treatment Initiation
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140

FIG. 4



FIG. 5

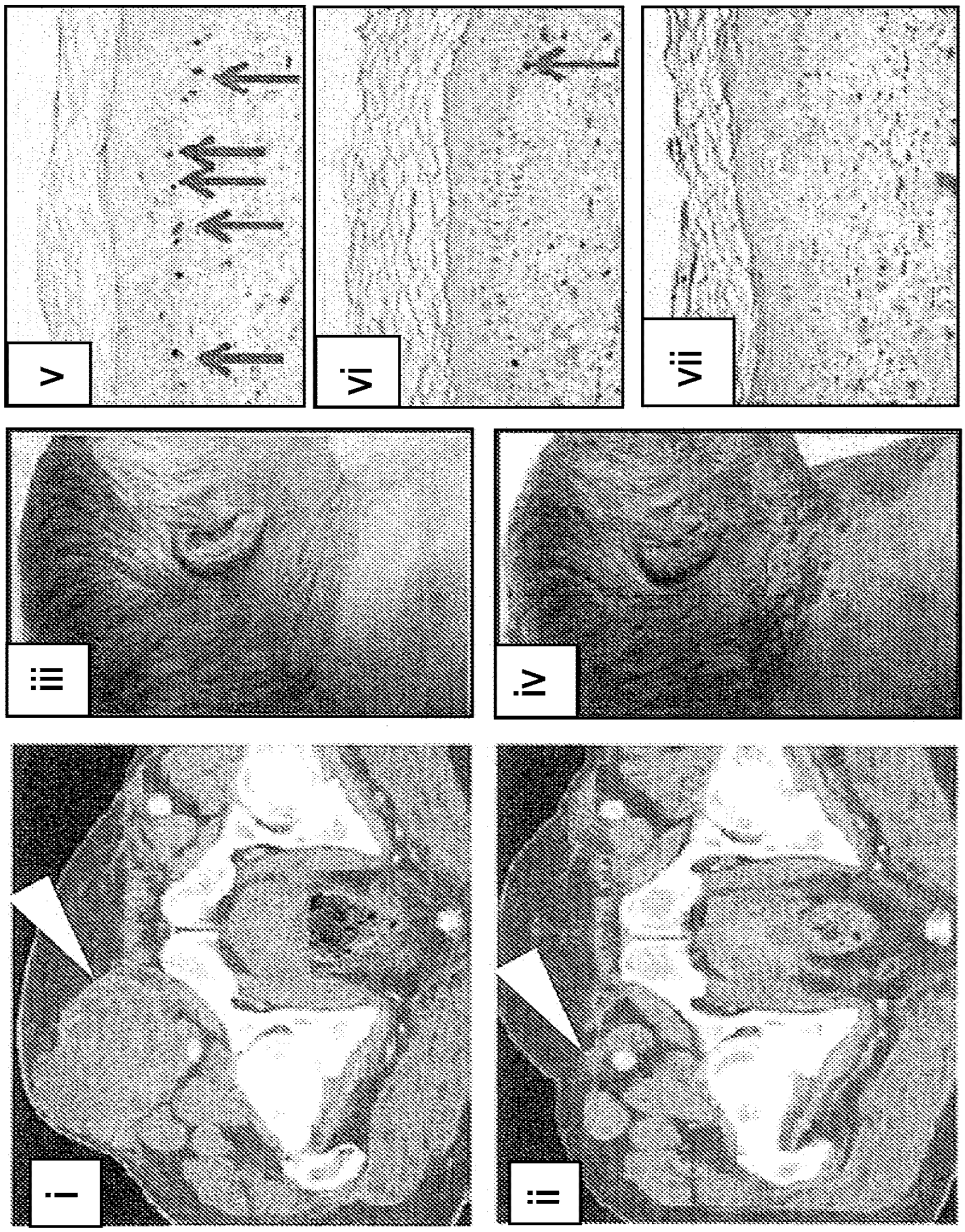


FIG. 6

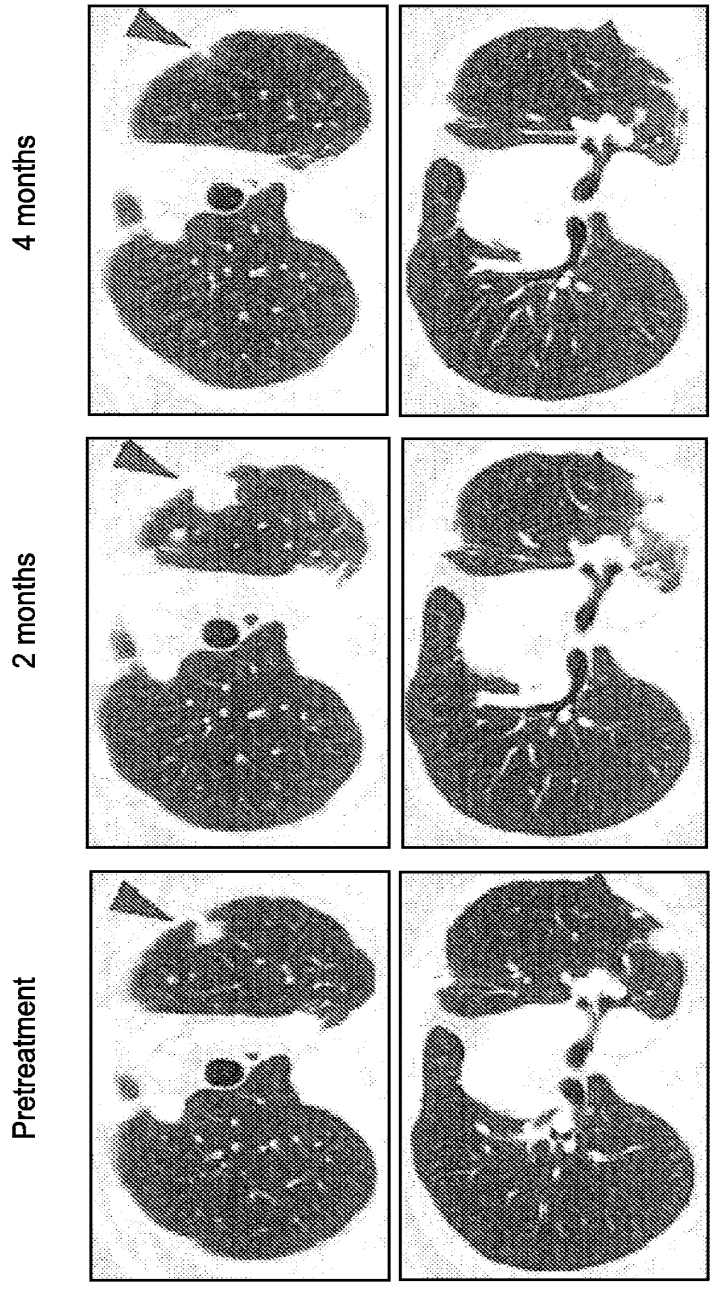
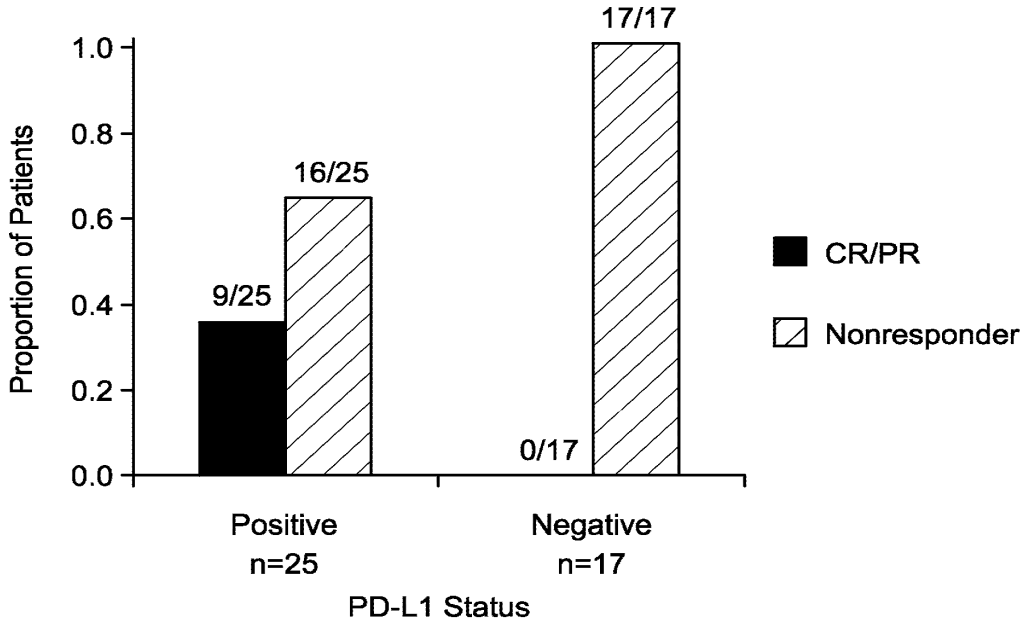


FIG. 7



Association Between Pretreatment Tumor PD-L1 Expression and Clinical Response			
Response Status	PD-L1-Positive no. (%)	PD-L1-Negative no. (%)	Total no. (%)
CR/PR	9(36)	0	9(21)
Nonresponder	16*(64)	17(100)	33(79)
All Patients	25	17	42

Fisher's exact test for association P=0.006.

*Two patients categorized as nonresponders at the time of data analysis are still under evaluation.

FIG. 8A

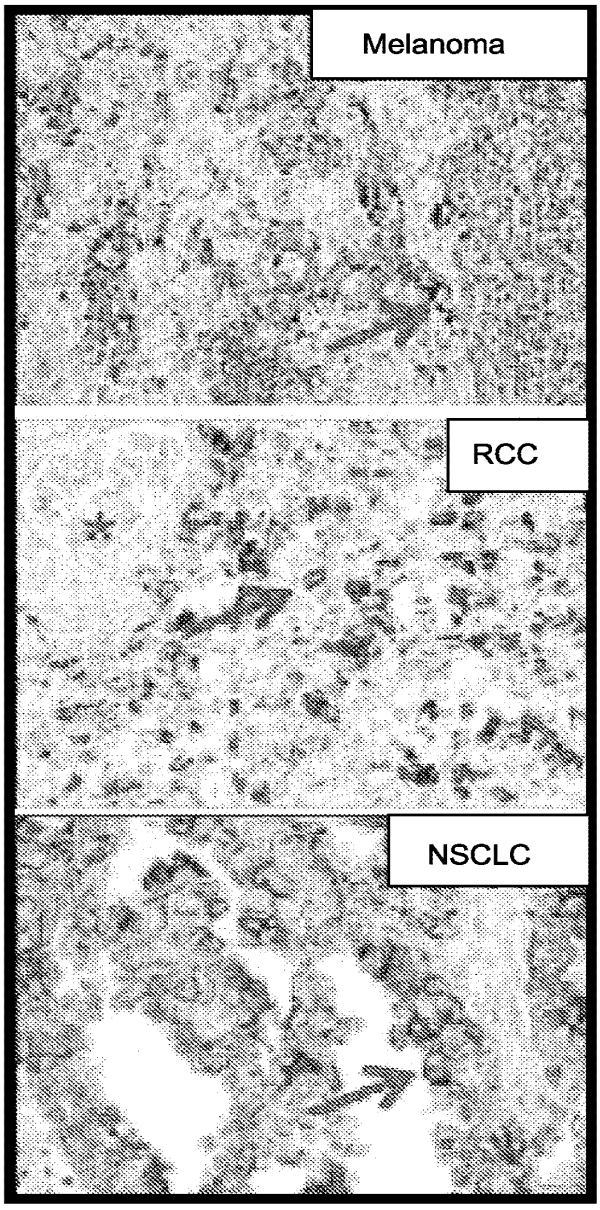


FIG. 8B

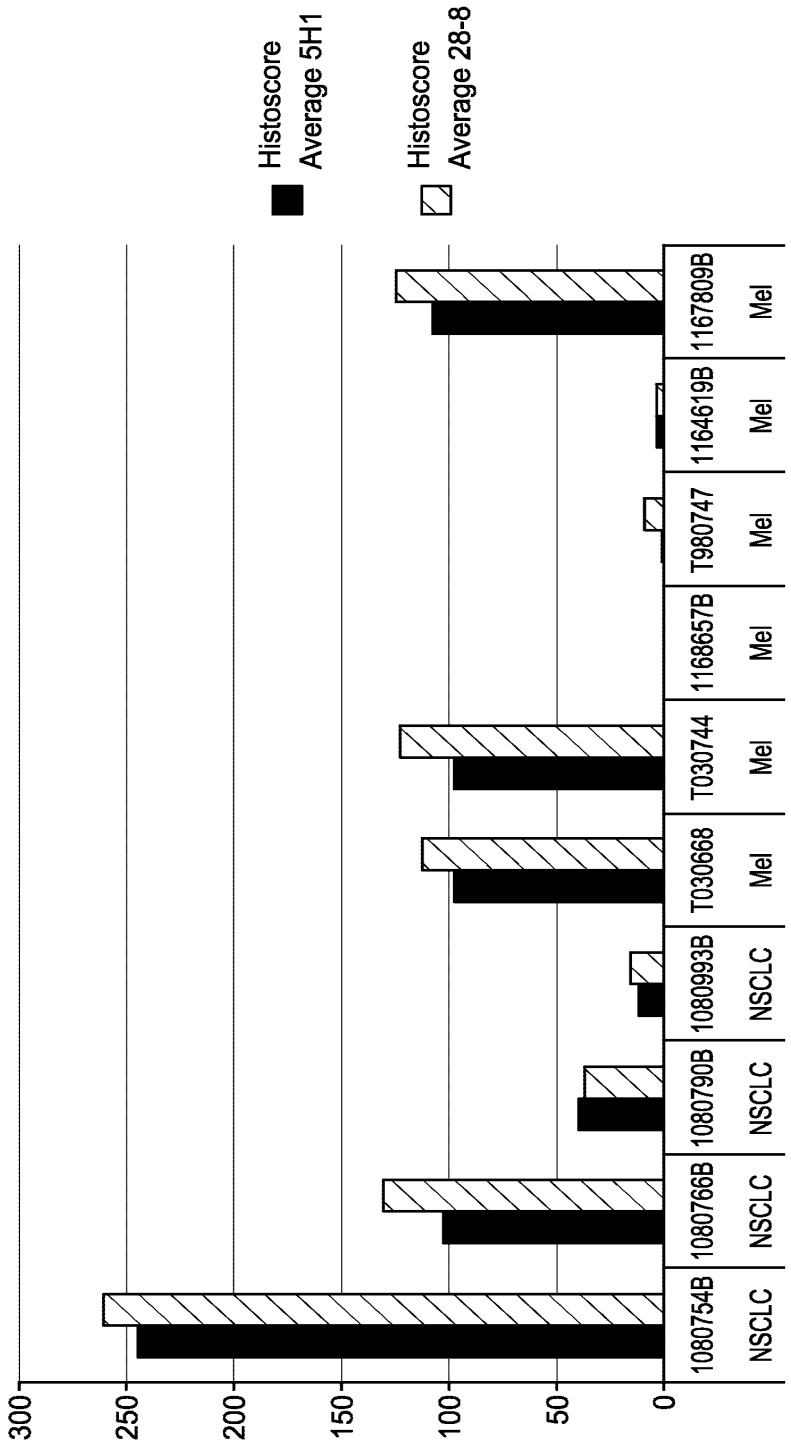


FIG. 9

EX1002 - Page 685

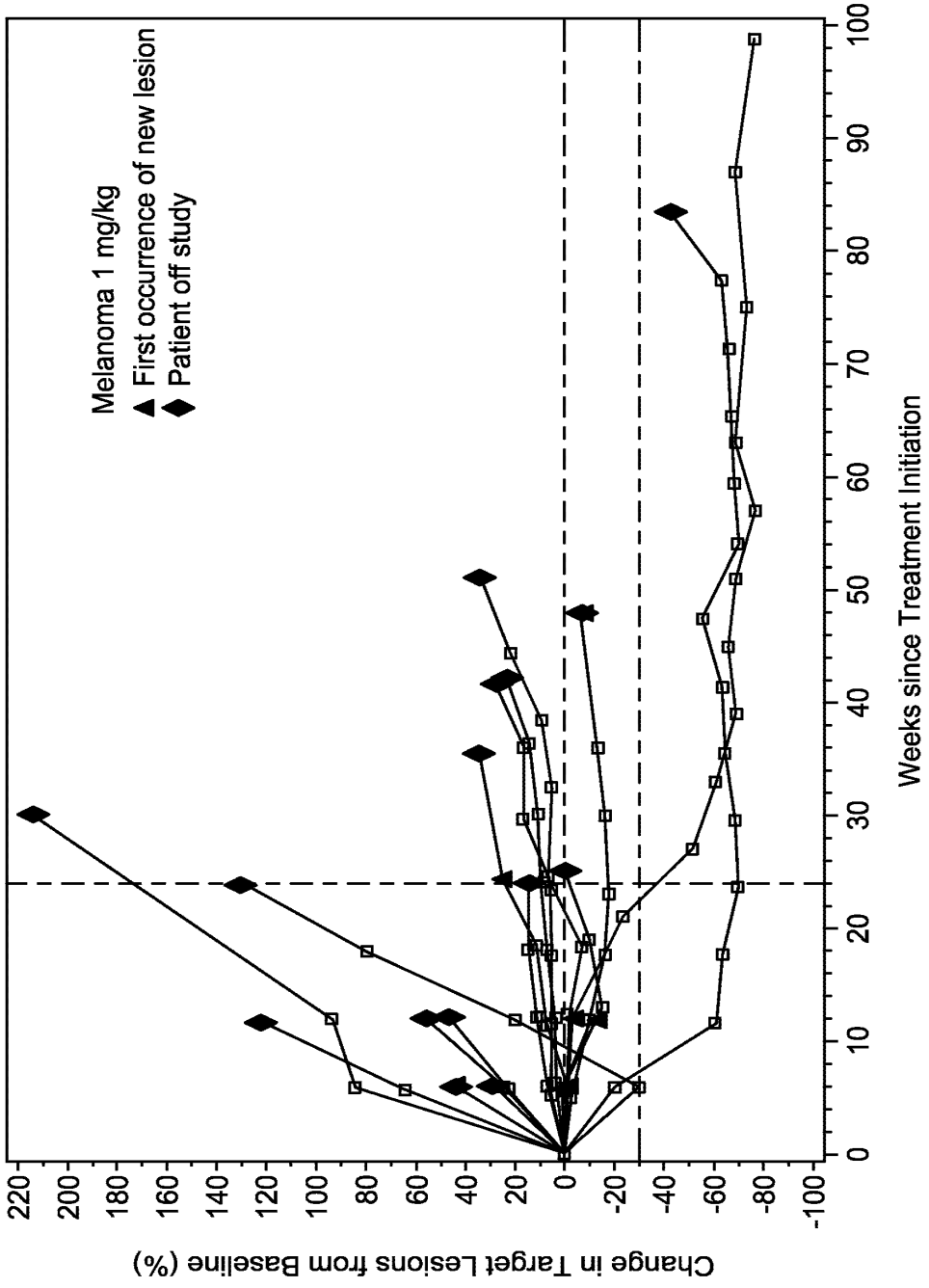

Melanoma 1 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
220
200
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
Weeks since Treatment Initiation

FIG. 10A

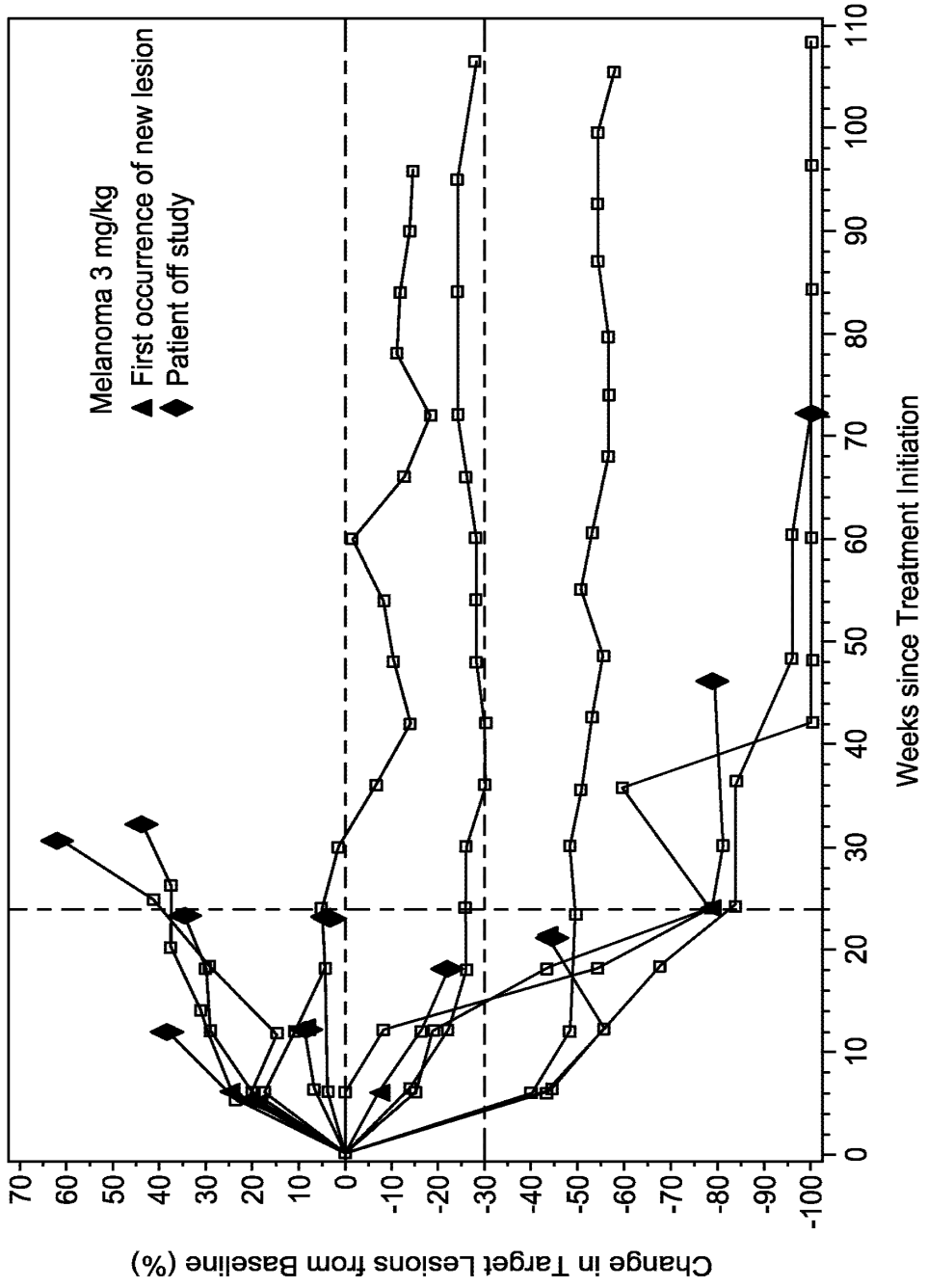

Melanoma 3 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
Weeks since Treatment Initiation
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
10
20
30
40
50
60
70
80
90
100
110

FIG. 10B

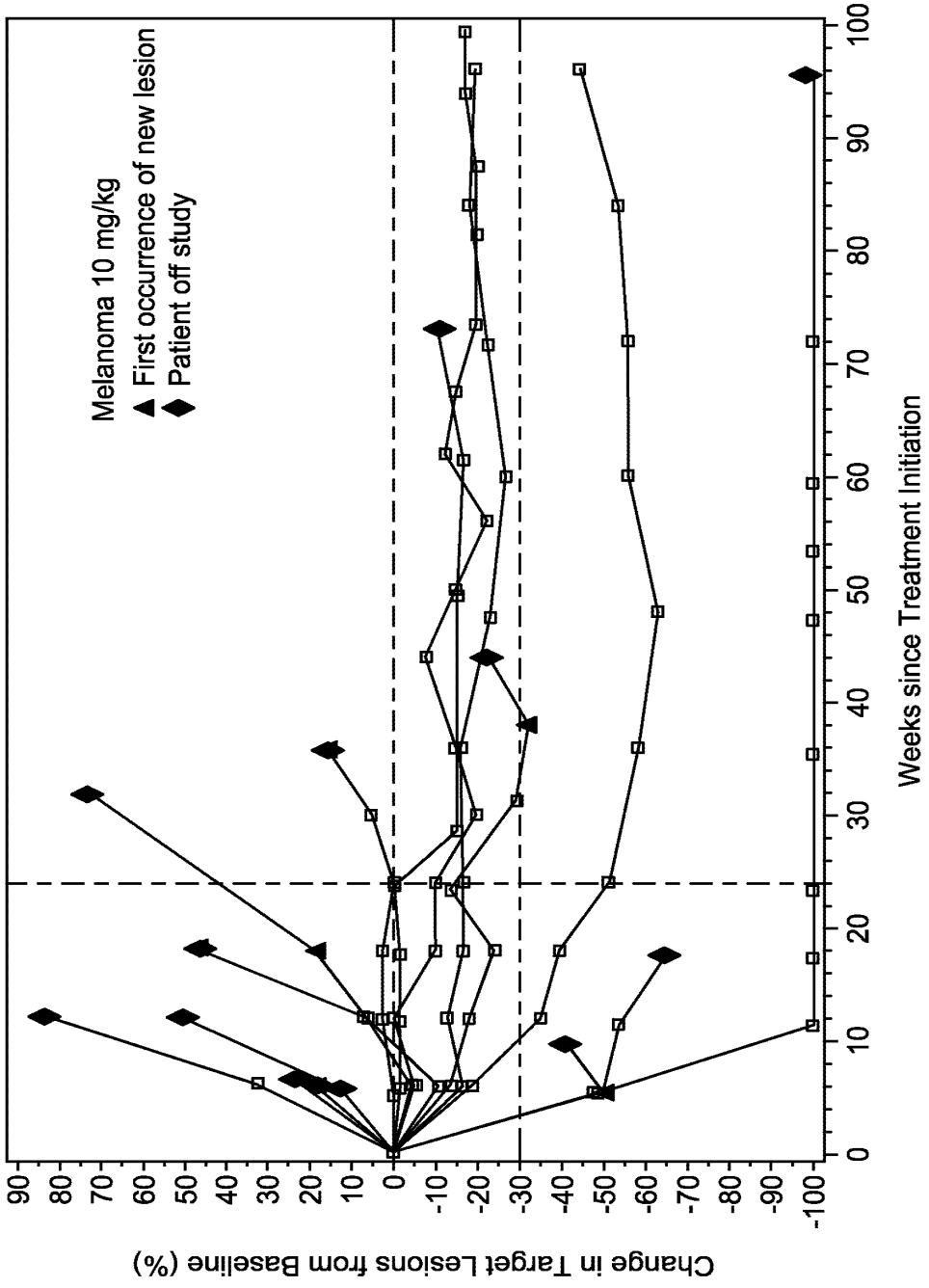

Melanoma 10 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
Weeks since Treatment Initiation
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
10
20
30
40
50
60
70
80
90
100

FIG. 10C

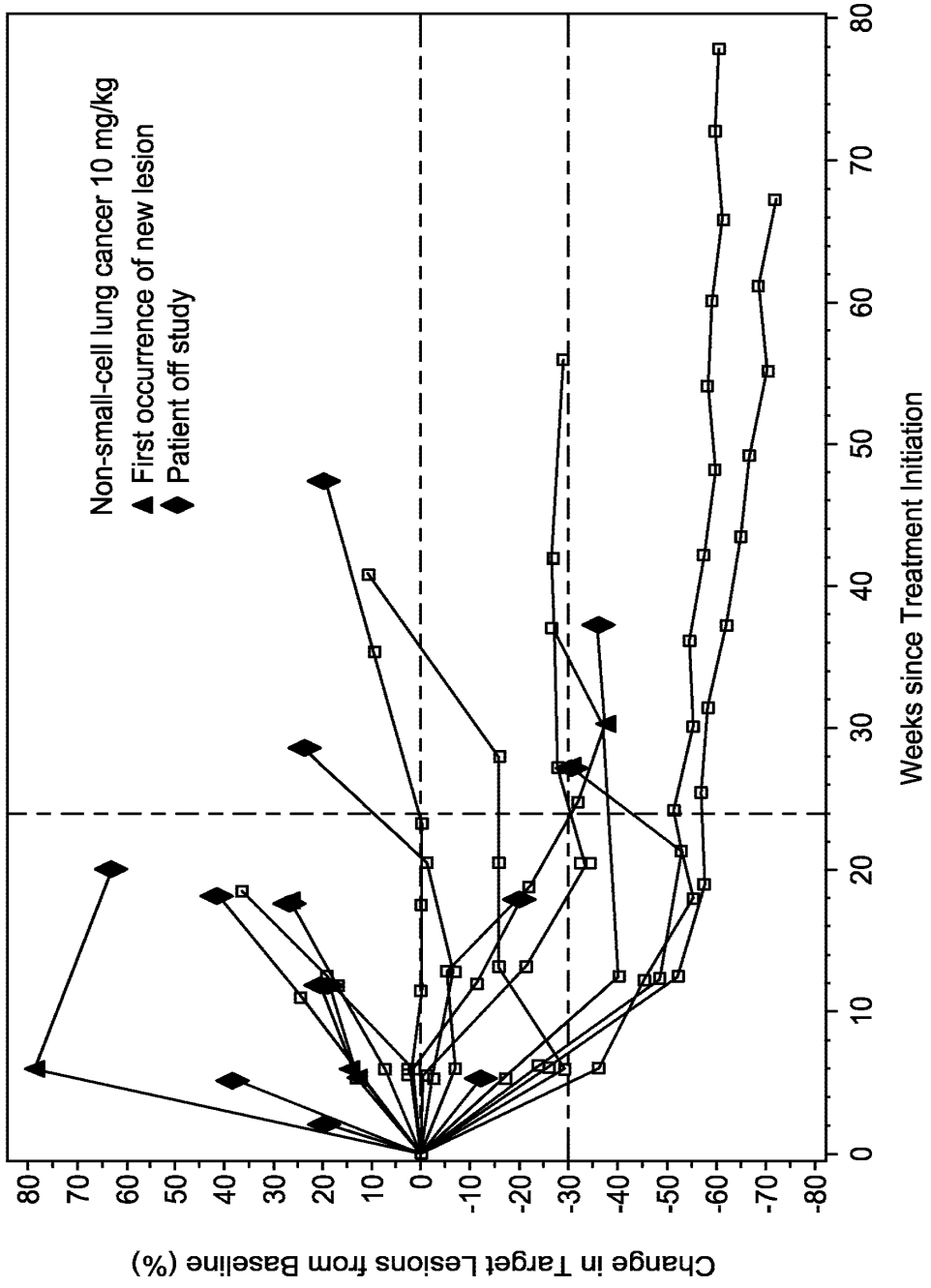
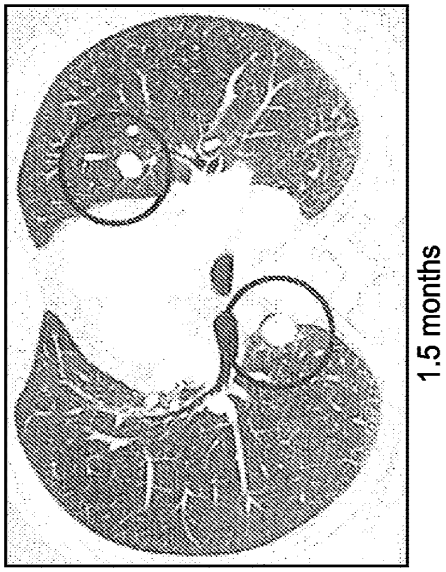
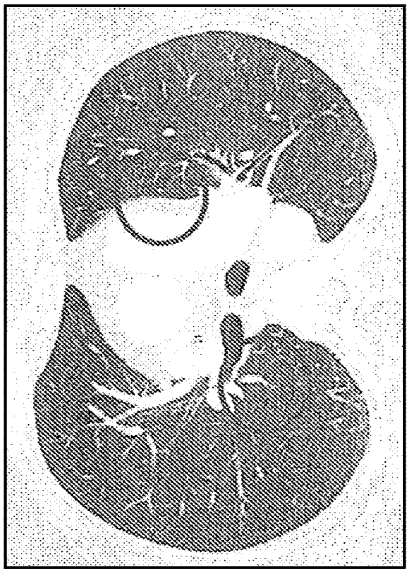

Non-small-cell lung cancer 10 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
0
10
20
30
40
50
60
70
80
Weeks since Treatment Initiation

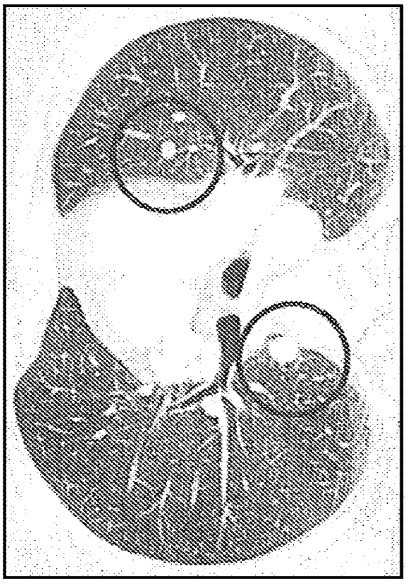
FIG. 10D



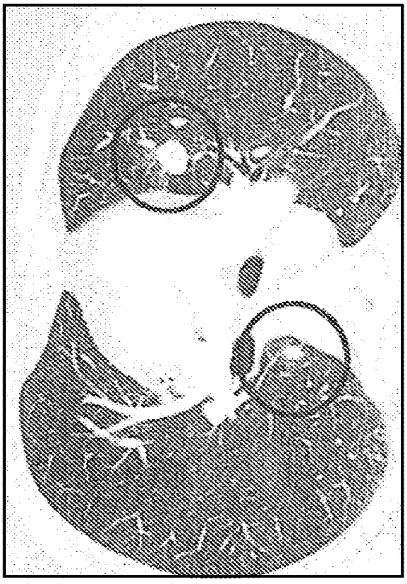
1.5 months



10 months



Pretreatment



3 months

FIG. 11

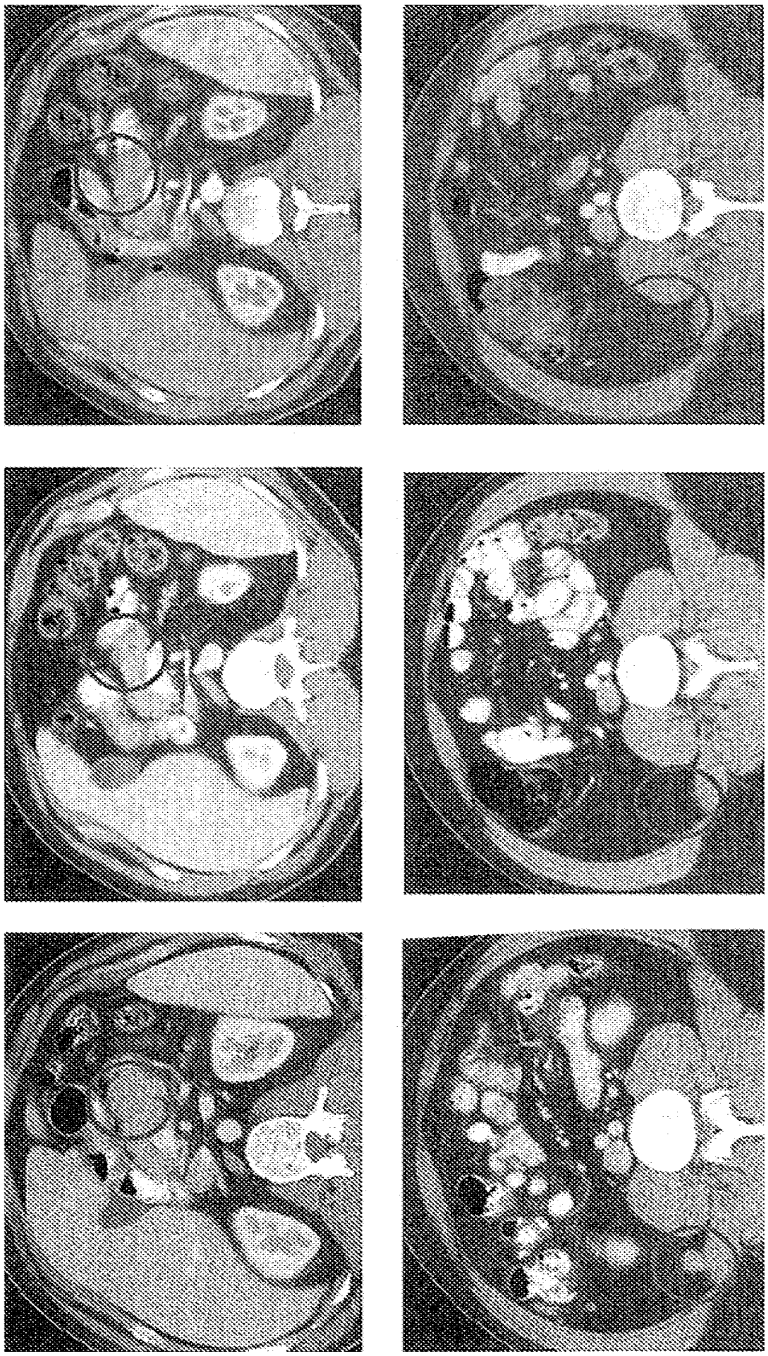


FIG. 12

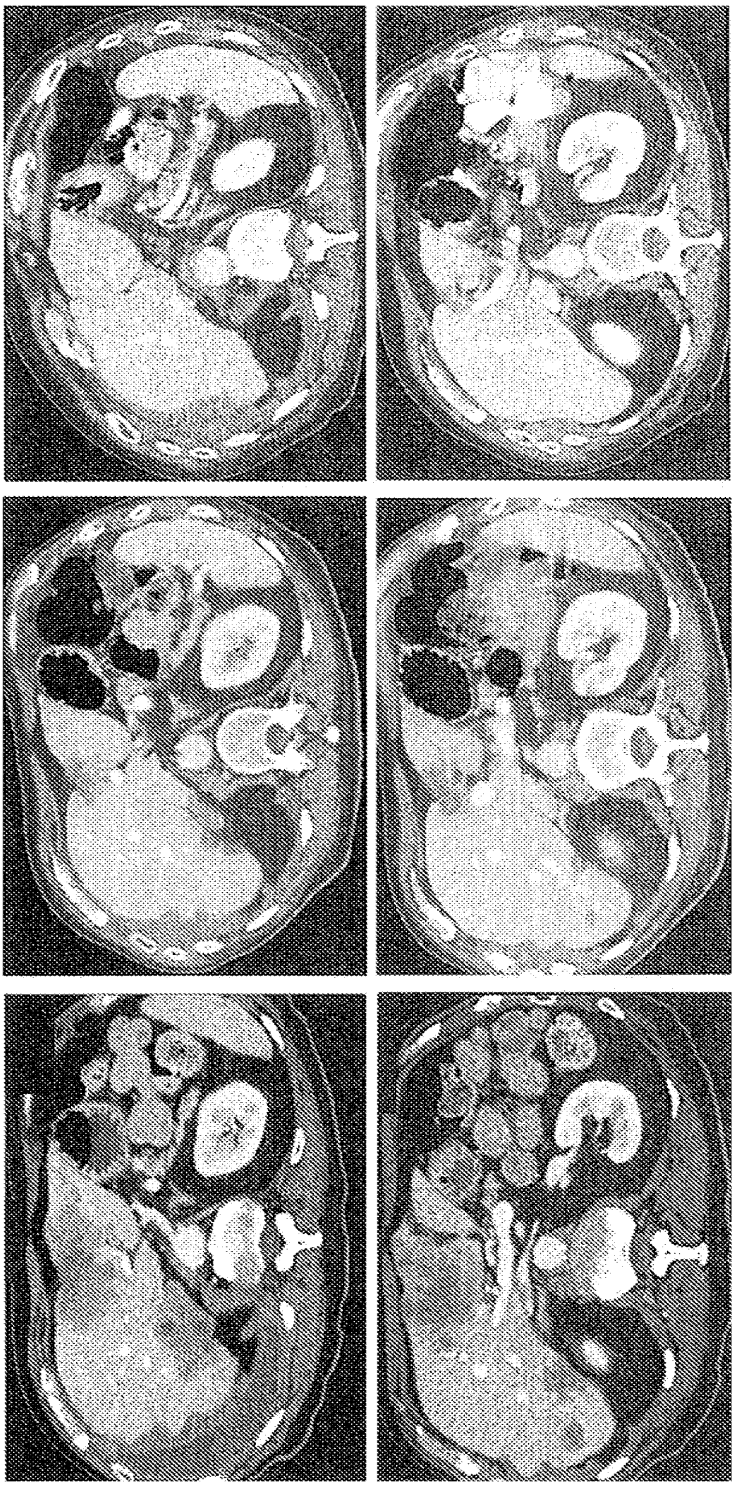


FIG. 13

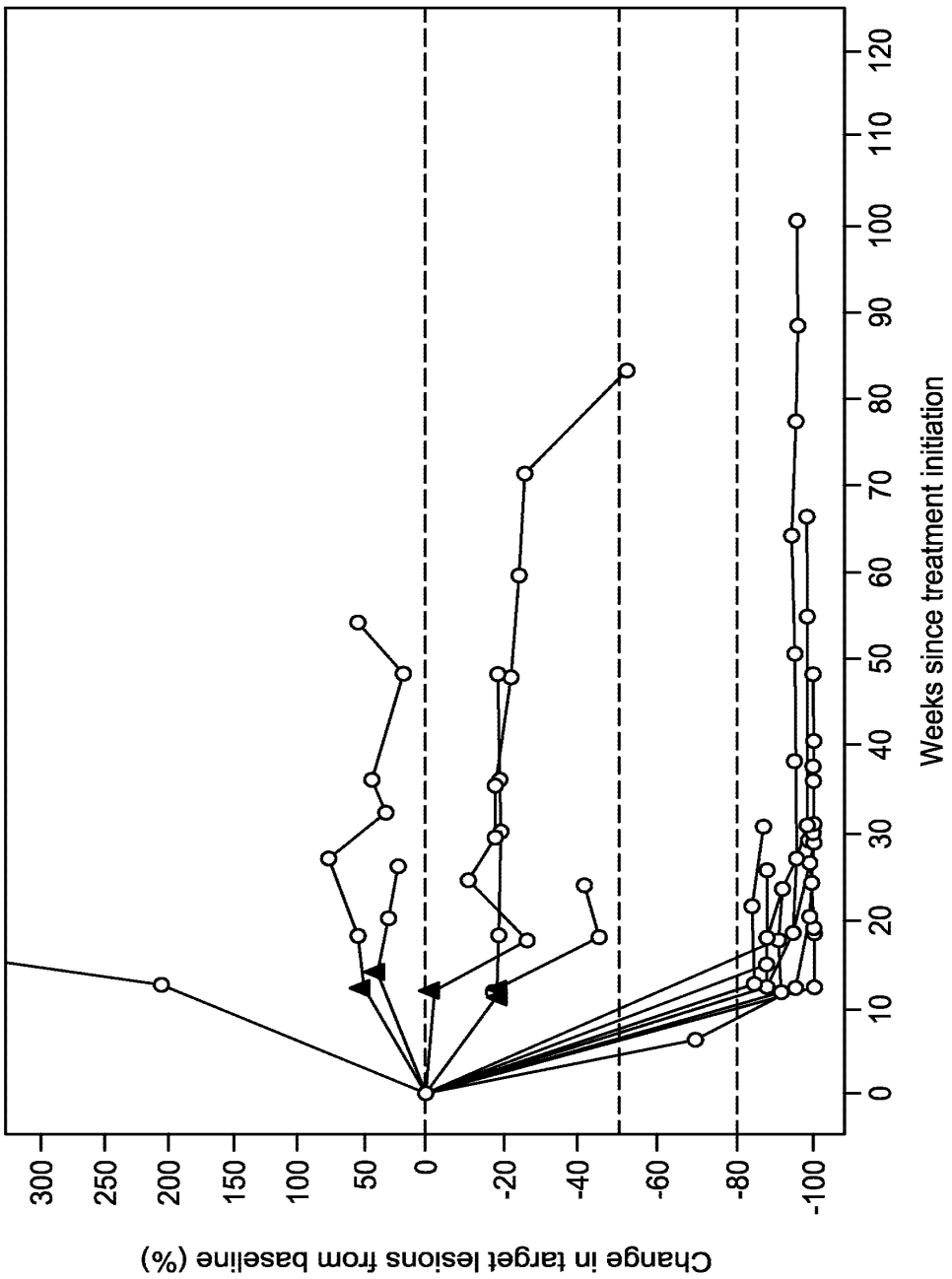

Change in target lesions from baseline (%)
300
250
200
150
100
50
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
Weeks since treatment initiation

FIG. 14A

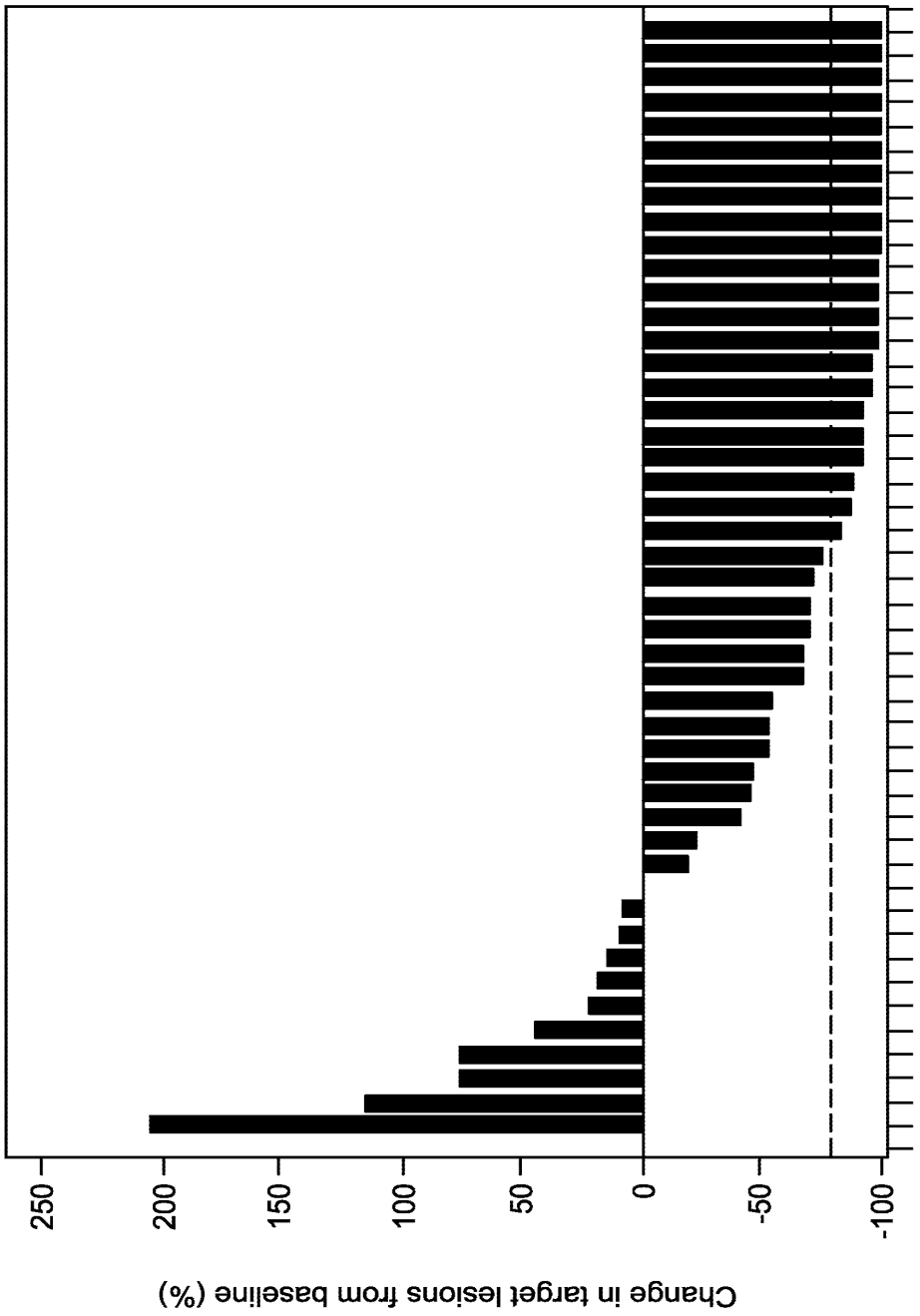

250
200
150
100
50
0
-50
-100
Change in target lesions from baseline (%)
Patients

FIG. 14B

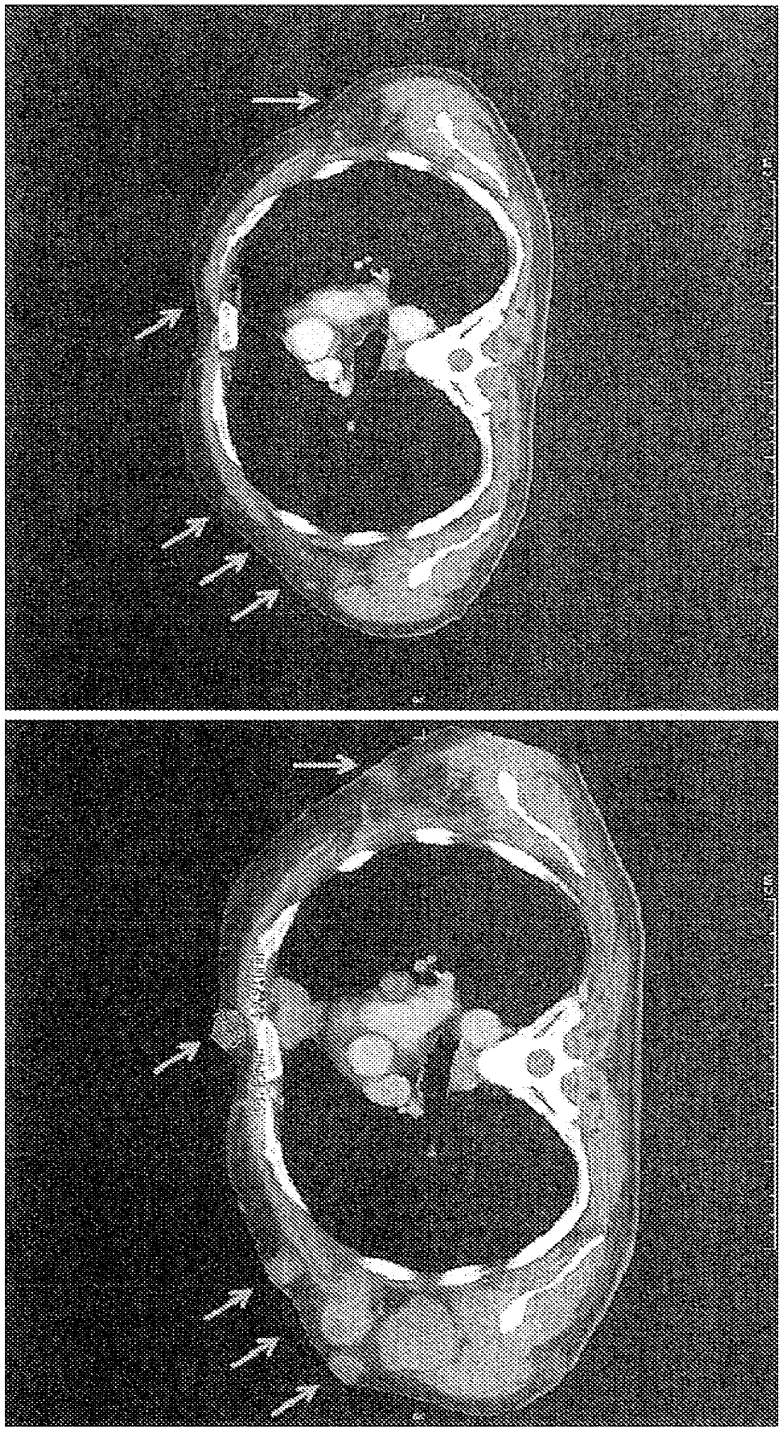


FIG. 15

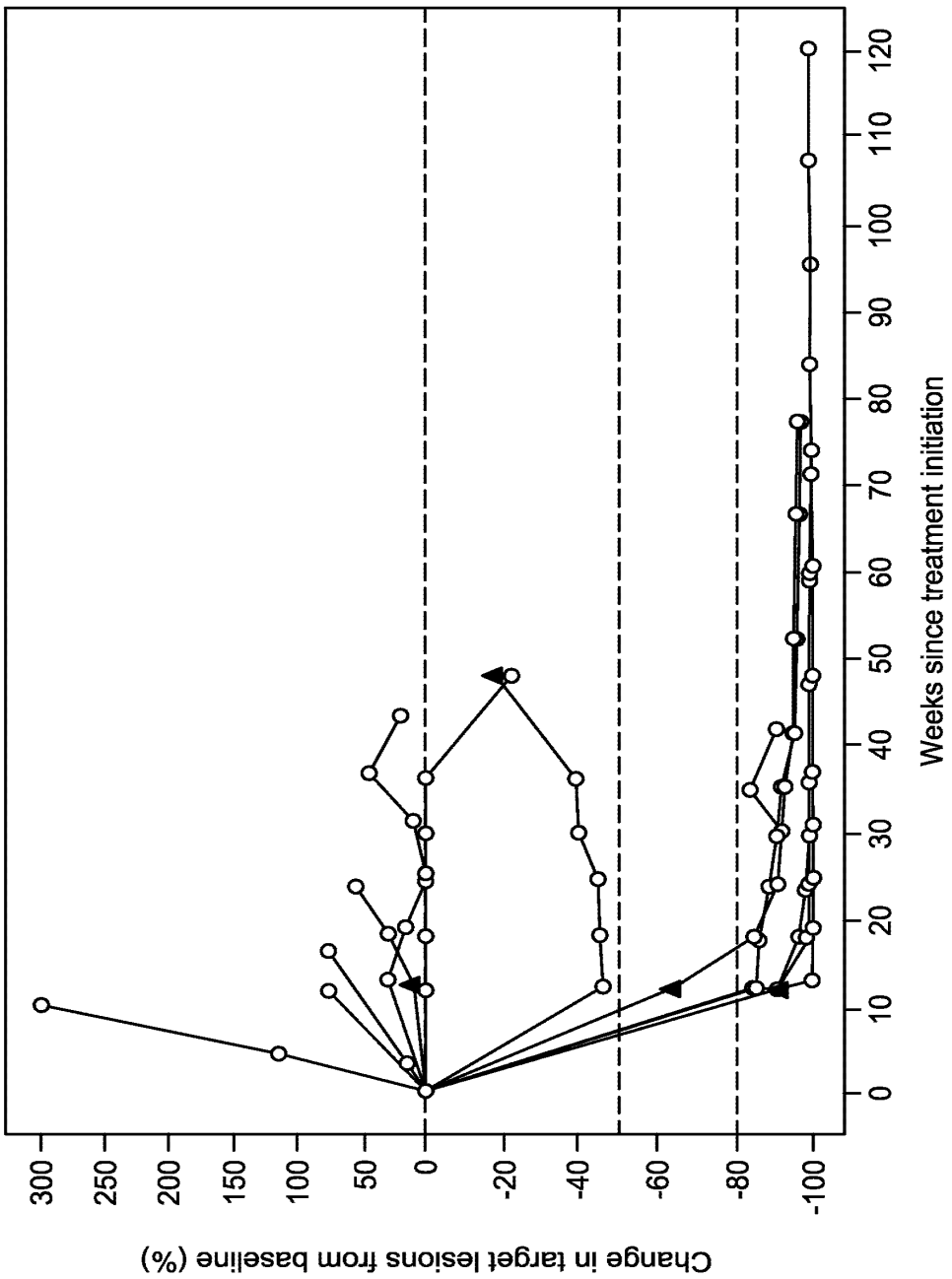

Change in target lesions from baseline (%)
300
250
200
150
100
50
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
Weeks since treatment initiation

FIG. 16A

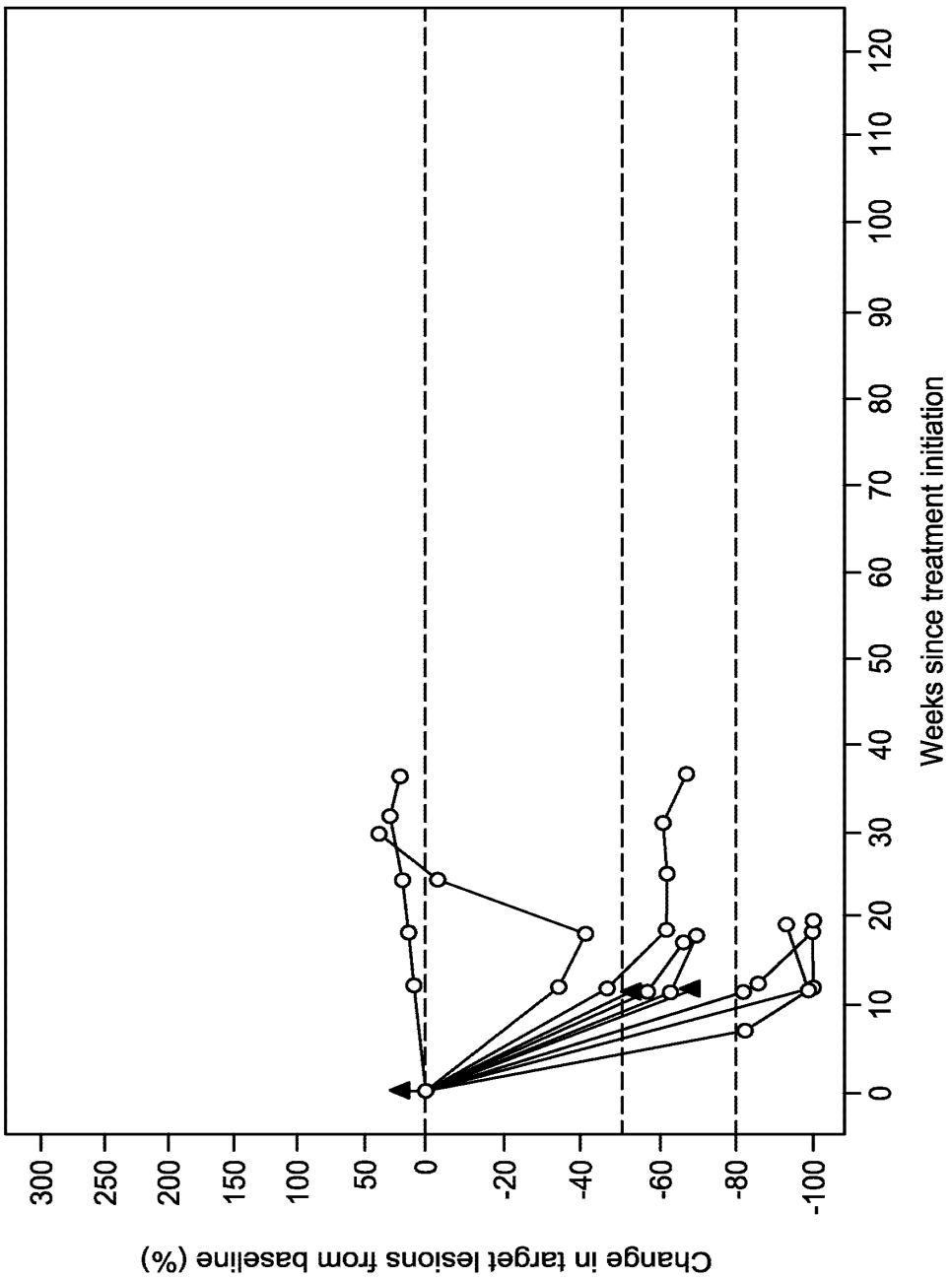

Change in target lesions from baseline (%)
300
250
200
150
100
50
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
Weeks since treatment initiation

FIG. 16B

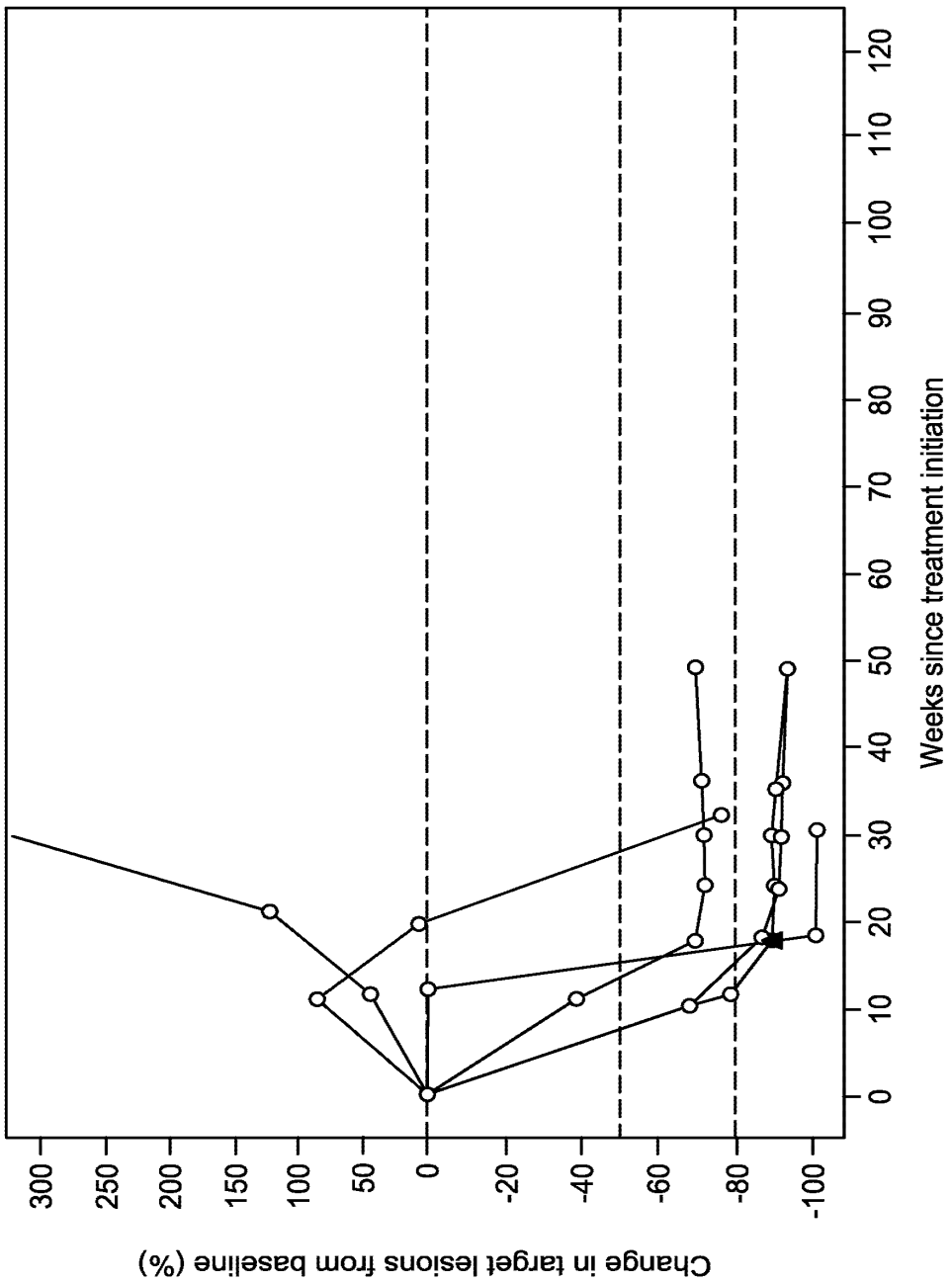

Change in target lesions from baseline (%)
300
250
200
150
100
50
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
Weeks since treatment initiation

FIG. 16C

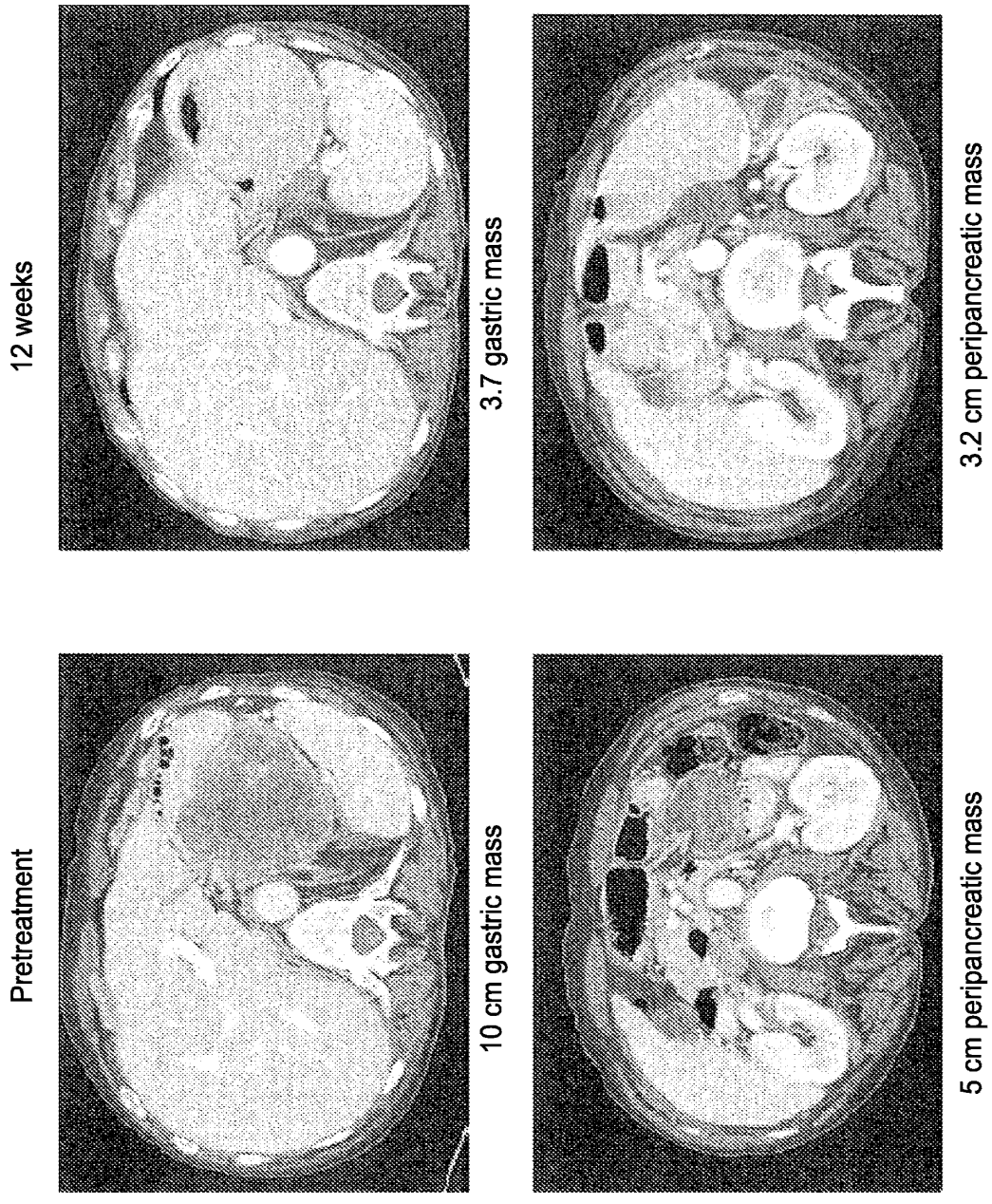

12 weeks
3.7 gastric mass
3.2 cm peripancreatic mass
Pretreatment
10 cm gastric mass
5 cm peripancreatic mass

FIG. 17

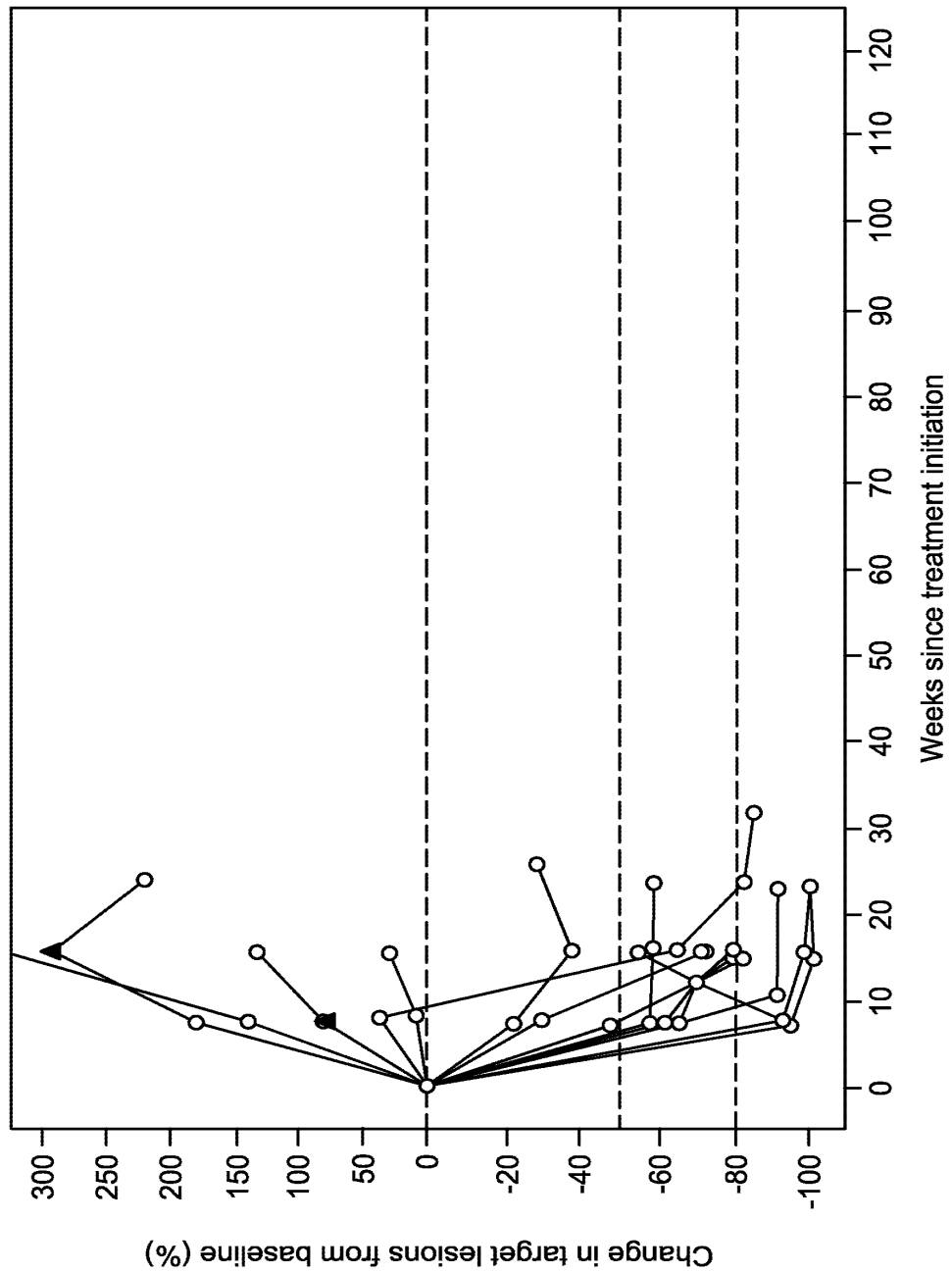

Change in target lesions from baseline (%)
300
250
200
150
100
50
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
Weeks since treatment initiation

FIG. 18A

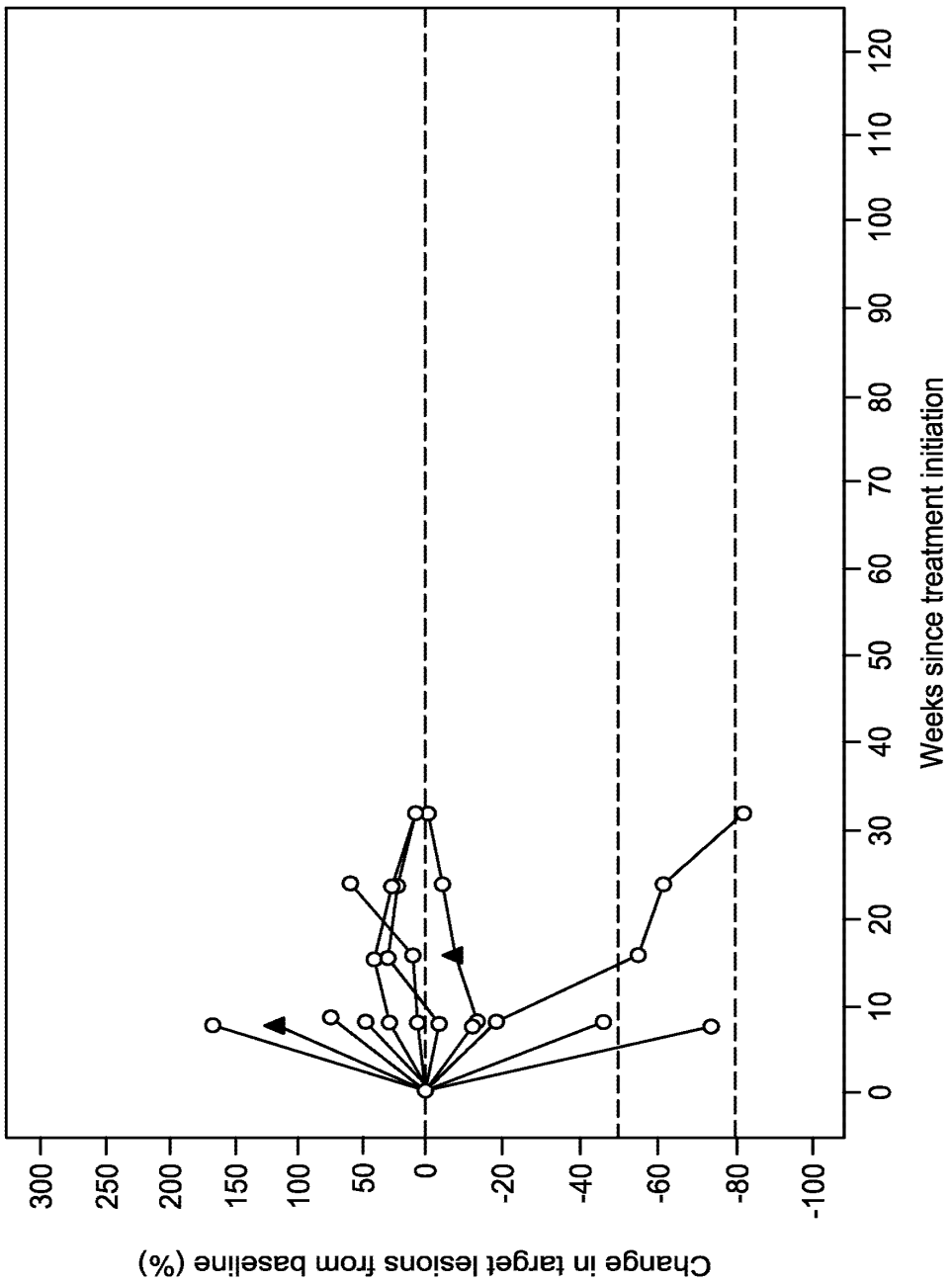

Change in target lesions from baseline (%)
300
250
200
150
100
50
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
Weeks since treatment initiation

FIG. 18B

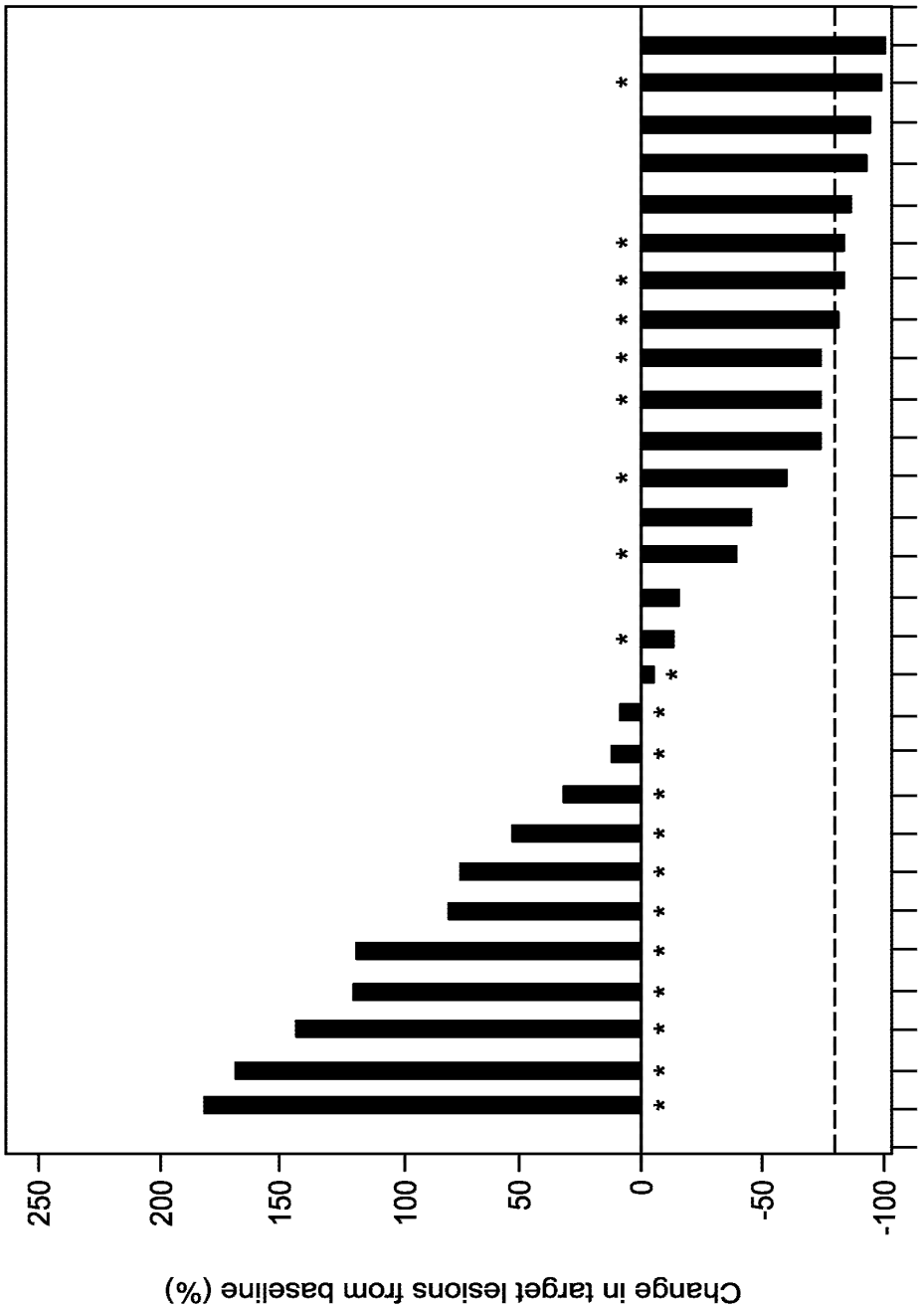

Change in target lesions from baseline (%)
250
200
150
100
50
0
-50
-100
Patients

FIG. 18C

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/040764

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/005874 A2 (MEDAREX INC [US]; KORMAN ALAN J [US]; SELBY MARK J [US]; WANG CHANGYU) 11 January 2007 (2007-01-11) pages 79,80,75; example all	1-3
X	WO 2006/121168 A1 (ONO PHARMACEUTICAL CO [JP]; MEDAREX INC [US]; KORMAN ALAN J; SRINIVASA) 16 November 2006 (2006-11-16) pages 58,59,55; example all	1-3
X	WO 2004/056875 A1 (WYETH CORP [US]; CAMBRIDGE ANTIBODY TECH [GB]; COLLINS MARY [US]; WOOD) 8 July 2004 (2004-07-08) pages 4,25,42; example all	1-3

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 July 2013	31/10/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Fellows, Edward

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HIRANO FUMIYA ET AL: "Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 65, no. 3, 1 February 2005 (2005-02-01), pages 1089-1096, XP002419626, ISSN: 0008-5472 the whole document	1-3
A	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; April 2011 (2011-04), DODSON LINDZY F ET AL: "Potential targets for pancreatic cancer immunotherapeutics", XP002703660, Database accession no. PREV201100567322 the whole document & IMMUNOTHERAPY, vol. 3, no. 4, April 2011 (2011-04), pages 517-537, ISSN: 1750-743X, DOI: 10.2217/IMT.11.10	1-3
A	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; July 2011 (2011-07), ANDORSKY DAVID J ET AL: "Programmed Death Ligand 1 Is Expressed by Non-Hodgkin Lymphomas and Inhibits the Activity of Tumor-Associated T Cells", XP002703661, Database accession no. PREV201100493026 the whole document & CLINICAL CANCER RESEARCH, vol. 17, no. 13, July 2011 (2011-07), pages 4232-4244, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-10-2660	1-3

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2013/040764

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/040764

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

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

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

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

see additional sheet

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

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

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

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

1-3

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-3

   A method of treating a subject with cancer comprising administering to the subject a therapeutically effective amount of an antibody, or an antigen-binding portion thereof, that disrupts the interaction between PD-1 and PD-L1 wherein the antibody or antigen-binding portion thereof binds specifically to PD-1 or PD-L1 and related subject-matter.

   ---

2. claims: 4-9, 21

   A method for immunotherapy of a subject with cancer comprising the testing of a tissue sample, assessing the portion of cells in the sample that express PD-L1 and administering a composition comprising a therapeutically effective amount of an anti-PD-1 antibody to a selected subject and related subject-matter.

   ---

3. claim: 10

   A method for immunotherapy of a subject with cancer comprising selecting a subject who is not suitable of anti-PD-1 antibody immunotherapy and administering to the subject a standard-of-care therapeutic other than an anti-PD-1 antibody.

   ---

4. claims: 11-13

   A monoclonal antibody, or an antigen-binding portion thereof, that binds specifically to a cell surface-expressed PD-L1 in a sample and related subject-matter.

   ---

5. claims: 14-20

   A method of treating a subject with cancer comprising administering to the subject an antibody, or an antigen-binding portion thereof, that specifically binds and inhibits PD-1 and an antibody, or an antigen-binding portion thereof, that specifically binds and inhibits CTLA-4, each antibody be administered at a specific dosage range in a concurrent regimen and related subject-matter.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2013/040764

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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(74) Agents: **KOLOM, Melissa E.** et al.; Leydig, Voit & Mayer, Ltd., Two Prudential Plaza, Suite 4900, 180 North Stetson Avenue, Chicago, Illinois 60601-6731 (US).

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(54) Title: ANTIBODIES DIRECTED AGAINST PROGRAMMED DEATH-1 (PD-1)

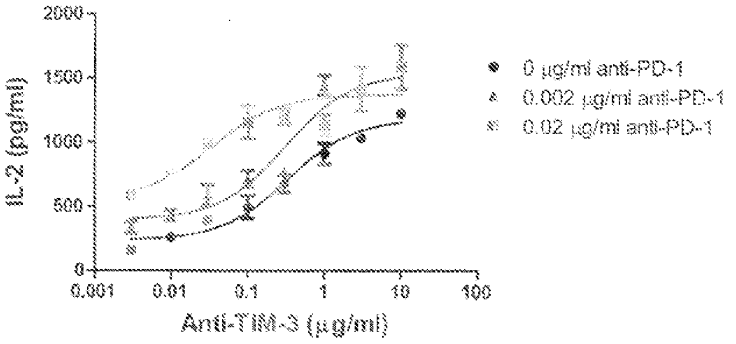


FIG. 2

(57) Abstract: The invention relates to an isolated immunoglobulin heavy chain polypeptide and an isolated immunoglobulin light chain polypeptide that bind to a programmed death-1 (PD-1) protein. The invention provides a PD-1-binding agent that comprises the aforementioned immunoglobulin heavy chain polypeptide and immunoglobulin light chain polypeptide. The invention also provides related vectors, compositions, and methods of using the PD-1-binding agent to treat a cancer or an infectious disease.

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ANTIBODIES DIRECTED AGAINST PROGRAMMED DEATH-1 (PD-1)

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0001] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 45,084 Byte ASCII (Text) file named "716746_ST25.TXT," created on May 1, 2014.

BACKGROUND OF THE INVENTION

[0002] Programmed Death 1 (PD-1) (also known as Programmed Cell Death 1) is a type I transmembrane protein of 268 amino acids originally identified by subtractive hybridization of a mouse T cell line undergoing apoptosis (Ishida et al., *Embo J.*, *11*: 3887-95 (1992)). PD-1 is a member of the CD28/CTLA-4 family of T-cell regulators, and is expressed on activated T-cells, B-cells, and myeloid lineage cells (Greenwald et al., *Annu. Rev. Immunol.*, *23*: 515-548 (2005); and Sharpe et al., *Nat. Immunol.*, *8*: 239-245 (2007)).

[0003] Two ligands for PD-1 have been identified, PD ligand 1 (PD-L1) and PD ligand 2 (PD-L2), both of which belong to the B7 protein superfamily (Greenwald et al., *supra*). PD-L1 is expressed in a variety of cell types, including cells of the lung, heart, thymus, spleen, and kidney (see, e.g., Freeman et al., *J. Exp. Med.*, *192*(7): 1027-1034 (2000); and Yamazaki et al., *J. Immunol.*, *169*(10): 5538-5545 (2002)). PD-L1 expression is upregulated on macrophages and dendritic cells (DCs) in response to lipopolysaccharide (LPS) and GM-CSF treatment, and on T-cells and B-cells upon signaling via T-cell and B-cell receptors. PD-L1 also is expressed in a variety of murine tumor cell lines (see, e.g., Iwai et al., *Proc. Natl. Acad. Sci. USA*, *99*(19): 12293-12297 (2002); and Blank et al., *Cancer Res.*, *64*(3): 1140-1145 (2004)). In contrast, PD-L2 exhibits a more restricted expression pattern and is expressed primarily by antigen presenting cells (e.g., dendritic cells and macrophages), and some tumor cell lines (see, e.g., Latchman et al., *Nat. Immunol.*, *2*(3): 261-238 (2001)). High PD-L1 expression in tumors, whether on the tumor cell, stroma, or other cells within the tumor microenvironment, correlates with poor clinical prognosis, presumably by inhibiting effector T cells and upregulating regulatory T cells (Treg) in the tumor.

[0004] PD-1 negatively regulates T-cell activation, and this inhibitory function is linked to an immunoreceptor tyrosine-based switch motif (ITSM) in the cytoplasmic domain (see,

e.g., Greenwald et al., *supra*; and Parry et al., *Mol. Cell. Biol.*, *25*: 9543-9553 (2005)). PD-1 deficiency can lead to autoimmunity. For example, C57BL/6 PD-1 knockout mice have been shown to develop a lupus-like syndrome (see, e.g., Nishimura et al., *Immunity*, *11*: 141-1151 (1999)). In humans, a single nucleotide polymorphism in the PD-1 gene is associated with higher incidences of systemic lupus erythematosus, type 1 diabetes, rheumatoid arthritis, and progression of multiple sclerosis (see, e.g., Nielsen et al., *Tissue Antigens*, *62*(6): 492-497 (2003); Bertsias et al., *Arthritis Rheum.*, *60*(1): 207-218 (2009); Ni et al., *Hum. Genet.*, *121*(2): 223-232 (2007); Tahoori et al., *Clin. Exp. Rheumatol.*, *29*(5): 763-767 (2011); and Kroner et al., *Ann. Neurol.*, *58*(1): 50-57 (2005)). Abnormal PD-1 expression also has been implicated in T-cell dysfunctions in several pathologies, such as tumor immune evasion and chronic viral infections (see, e.g., Barber et al., *Nature*, *439*: 682-687 (2006); and Sharpe et al., *supra*).

[0005] Recent studies demonstrate that T-cell suppression induced by PD-1 also plays a role in the suppression of anti-tumor immunity. For example, PD-L1 is expressed on a variety of human and mouse tumors, and binding of PD-1 to PD-L1 on tumors results in T-cell suppression and tumor immune evasion and protection (Dong et al., *Nat. Med.*, *8*: 793-800 (2002)). Expression of PD-L1 by tumor cells has been directly associated with their resistance to lysis by anti-tumor T-cells *in vitro* (Dong et al., *supra*; and Blank et al., *Cancer Res.*, *64*: 1140-1145 (2004)). PD-1 knockout mice are resistant to tumor challenge (Iwai et al., *Int. Immunol.*, *17*: 133-144 (2005)), and T-cells from PD-1 knockout mice are highly effective in tumor rejection when adoptively transferred to tumor-bearing mice (Blank et al., *supra*). Blocking PD-1 inhibitory signals using a monoclonal antibody can potentiate host anti-tumor immunity in mice (Iwai et al., *supra*; and Hirano et al., *Cancer Res.*, *65*: 1089-1096 (2005)), and high levels of PD-L1 expression in tumors are associated with poor prognosis for many human cancer types (Hamanishi et al., *Proc. Natl. Acad. Sci. USA*, *104*: 3360-335 (2007), Brown et al., *J. Immunol.*, *170*: 1257-1266 (2003); and Flies et al., *Yale Journal of Biology and Medicine*, *84*(4): 409-421 (2011)).

[0006] In view of the foregoing, strategies for inhibiting PD-1 activity to treat various types of cancer and for immunopotentiation (e.g., to treat infectious diseases) have been developed (see, e.g., Ascierto et al., *Clin. Cancer. Res.*, *19*(5): 1009-1020 (2013)). In this respect, monoclonal antibodies targeting PD-1 have been developed for the treatment of cancer (see, e.g., Weber, *Semin. Oncol.*, *37*(5): 430-4309 (2010); and Tang et al., *Current Oncology Reports*, *15*(2): 98-104 (2013)). For example, nivolumab (also known as BMS-

936558) produced complete or partial responses in non-small-cell lung cancer, melanoma, and renal-cell cancer in a Phase I clinical trial (see, e.g., Topalian, *New England J. Med.*, *366*: 2443-2454 (2012)), and is currently in Phase III clinical trials. MK-3575 is a humanized monoclonal antibody directed against PD-1 that has shown evidence of antitumor activity in Phase I clinical trials (see, e.g., Patnaik et al., *2012 American Society of Clinical Oncology (ASCO) Annual Meeting*, Abstract # 2512). In addition, recent evidence suggests that therapies which target PD-1 may enhance immune responses against pathogens, such as HIV (see, e.g., Porichis et al., *Curr. HIV/AIDS Rep.*, *9*(1): 81-90 (2012)). Despite these advances, however, the efficacy of these potential therapies in humans may be limited.

[0007] Therefore, there is a need for a PD-1-binding agent (e.g., an antibody) that binds PD-1 with high affinity and effectively neutralizes PD-1 activity. The invention provides such PD-1-binding agents.

BRIEF SUMMARY OF THE INVENTION

[0008] The invention provides an isolated immunoglobulin heavy chain polypeptide which comprises a complementarity determining region 1 (CDR) amino acid sequence of SEQ ID NO: 1, a CDR2 amino acid sequence of SEQ ID NO: 2, and a CDR3 amino acid sequence of SEQ ID NO: 3, wherein optionally (a) residue 9 of SEQ ID NO: 1 is replaced with a different amino acid residue, (b) one or more of residues 7, 8, and 9 of SEQ ID NO: 2 is replaced with a different amino acid residue, (c) one or more of residues 1, 2, and 5 of SEQ ID NO: 3 is replaced with a different amino acid residue, or (d) any combination of (a)-(c).

[0009] The invention provides an isolated immunoglobulin heavy chain polypeptide which comprises a complementarity determining region 1 (CDR) amino acid sequence of SEQ ID NO: 12, a CDR2 amino acid sequence of SEQ ID NO: 13, and a CDR3 amino acid sequence of SEQ ID NO: 14, wherein optionally (a) residue 9 of SEQ ID NO: 12 is replaced with a different amino acid residue, (b) residue 8 and/or residue 9 of SEQ ID NO: 13 is replaced with a different amino acid residue, (c) residue 5 of SEQ ID NO: 14 is replaced with a different amino acid residue, or (d) any combination of (a)-(c).

[0010] The invention provides an isolated immunoglobulin heavy chain polypeptide which comprises a complementarity determining region 1 (CDR) amino acid sequence of SEQ ID NO: 19, a CDR2 amino acid sequence of SEQ ID NO: 20, and a CDR3 amino acid sequence of SEQ ID NO: 21.

[0011] The invention also provides an isolated immunoglobulin heavy chain polypeptide which comprises an amino acid sequence that is at least 90% identical to any one of SEQ ID NOs: 4-11, SEQ ID NOs: 15-18, and SEQ ID NOs: 22-25.

[0012] The invention provides an isolated immunoglobulin light chain polypeptide which comprises a complementarity determining region 1 (CDR) amino acid sequence of SEQ ID NO: 26 and a CDR2 amino acid sequence of SEQ ID NO: 27.

[0013] The invention provides an isolated immunoglobulin light chain polypeptide which comprises a complementarity determining region 1 (CDR) amino acid sequence of SEQ ID NO: 30 and a CDR2 amino acid sequence of SEQ ID NO: 31, wherein optionally residue 12 of SEQ ID NO: 30 is replaced with a different amino acid residue.

[0014] The invention provides an isolated immunoglobulin light chain polypeptide which comprises a complementarity determining region 1 (CDR) amino acid sequence of SEQ ID NO: 35, a CDR2 amino acid sequence of SEQ ID NO: 36, and a CDR3 amino acid sequence of SEQ ID NO: 37, wherein optionally (a) residue 5 of SEQ ID NO: 36 is replaced with a different amino acid residue, and/or (b) residue 4 of SEQ ID NO: 37 is replaced with a different amino acid residue.

[0015] The invention provides an isolated immunoglobulin light chain polypeptide which comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, or SEQ ID NO: 41.

[0016] In addition, the invention provides isolated or purified nucleic acid sequences encoding the foregoing immunoglobulin polypeptides, vectors comprising such nucleic acid sequences, isolated PD-1-binding agents comprising the foregoing immunoglobulin polypeptides, nucleic acid sequences encoding such PD-1-binding agents, vectors comprising such nucleic acid sequences, isolated cells comprising such vectors, compositions comprising such PD-1-binding agents or such vectors with a pharmaceutically acceptable carrier, and methods of treating cancer or infectious diseases in mammals by administering effective amounts of such compositions to mammals.

BRIEF DESCRIPTION THE DRAWINGS

[0017] Figure 1 is a diagram which schematically depicts different PD-1 antigen constructs utilized to generate anti-PD-1 monoclonal antibodies as described in Example 1.

[0018] Figure 2 is a graph which illustrates experimental results demonstrating increased activity of an anti-TIM-3 antagonist antibody in a human CD4+ T-cell MLR assay in the presence of low levels of anti-PD-1 antibody APE2058.

[0019] Figure 3 is a graph which illustrates experimental results demonstrating increased activity of an anti-LAG-3 antagonist antibody in a human CD4+ T-cell MLR assay in the presence of low levels of anti-PD-1 APE2058.

DETAILED DESCRIPTION OF THE INVENTION

[0020] The invention provides an isolated immunoglobulin heavy chain polypeptide and/or an isolated immunoglobulin light chain polypeptide, or a fragment (e.g., antigen-binding fragment) thereof. The term "immunoglobulin" or "antibody," as used herein, refers to a protein that is found in blood or other bodily fluids of vertebrates, which is used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses. The polypeptide is "isolated" in that it is removed from its natural environment. In a preferred embodiment, an immunoglobulin or antibody is a protein that comprises at least one complementarity determining region (CDR). The CDRs form the "hypervariable region" of an antibody, which is responsible for antigen binding (discussed further below). A whole immunoglobulin typically consists of four polypeptides: two identical copies of a heavy (H) chain polypeptide and two identical copies of a light (L) chain polypeptide. Each of the heavy chains contains one N-terminal variable (V_H) region and three C-terminal constant (C_H1, C_H2, and C_H3) regions, and each light chain contains one N-terminal variable (V_L) region and one C-terminal constant (C_L) region. The light chains of antibodies can be assigned to one of two distinct types, either kappa (κ) or lambda (λ), based upon the amino acid sequences of their constant domains. In a typical immunoglobulin, each light chain is linked to a heavy chain by disulphide bonds, and the two heavy chains are linked to each other by disulphide bonds. The light chain variable region is aligned with the variable region of the heavy chain, and the light chain constant region is aligned with the first constant region of the heavy chain. The remaining constant regions of the heavy chains are aligned with each other.

[0021] The variable regions of each pair of light and heavy chains form the antigen binding site of an antibody. The V_H and V_L regions have the same general structure, with each region comprising four framework (FW or FR) regions. The term "framework region," as used herein, refers to the relatively conserved amino acid sequences within the variable

region which are located between the hypervariable or complementary determining regions (CDRs). There are four framework regions in each variable domain, which are designated FR1, FR2, FR3, and FR4. The framework regions form the β sheets that provide the structural framework of the variable region (see, e.g., C.A. Janeway et al. (eds.), *Immunobiology, 5th Ed.*, Garland Publishing, New York, NY (2001)).

[0022] The framework regions are connected by three complementarity determining regions (CDRs). As discussed above, the three CDRs, known as CDR1, CDR2, and CDR3, form the "hypervariable region" of an antibody, which is responsible for antigen binding. The CDRs form loops connecting, and in some cases comprising part of, the beta-sheet structure formed by the framework regions. While the constant regions of the light and heavy chains are not directly involved in binding of the antibody to an antigen, the constant regions can influence the orientation of the variable regions. The constant regions also exhibit various effector functions, such as participation in antibody-dependent complement-mediated lysis or antibody-dependent cellular toxicity via interactions with effector molecules and cells.

[0023] The isolated immunoglobulin heavy chain polypeptide and the isolated immunoglobulin light chain polypeptide of the invention desirably bind to PD-1. As discussed above, programmed death 1 (PD-1) (also known as programmed cell death 1) is a 268 amino acid type I transmembrane protein (Ishida et al., *supra*). PD-1 is a member of the CD28/CTLA-4 family of T-cell regulators and is expressed on activated T-cells, B-cells, and myeloid lineage cells (Greenwald et al., *supra*; and Sharpe et al., *supra*). PD-1 includes an extracellular IgV domain followed by short extracellular stalk, a transmembrane region and an intracellular tail. The intracellular tail contains two phosphorylation sites located in an immunoreceptor tyrosine-based inhibitory motif and an immunoreceptor tyrosine-based switch motif, which play a role in the ability of PD-1 to negatively regulate T-cell receptor signaling (see, e.g., Ishida et al., *supra*; and Blank et al., *supra*). The inventive isolated immunoglobulin heavy chain polypeptide and the inventive isolated immunoglobulin light chain polypeptide can form an agent that binds to PD-1 and another antigen, resulting in a "dual reactive" binding agent (e.g., a dual reactive antibody). For example, the agent can bind to PD-1 and to another negative regulator of the immune system such as, for example, lymphocyte-activation gene 3 (LAG-3) and/or T-cell immunoglobulin domain and mucin domain 3 protein (TIM-3).

[0024] Antibodies which bind to PD-1, and components thereof, are known in the art (see, e.g., U.S. Patent 8,168,757; Topalian et al., *supra*; and Patnaik et al., *supra*). Anti-PD-1 antibodies also are commercially available from sources such as, for example, Abcam (Cambridge, MA).

[0025] An amino acid "replacement" or "substitution" refers to the replacement of one amino acid at a given position or residue by another amino acid at the same position or residue within a polypeptide sequence.

[0026] Amino acids are broadly grouped as "aromatic" or "aliphatic." An aromatic amino acid includes an aromatic ring. Examples of "aromatic" amino acids include histidine (H or His), phenylalanine (F or Phe), tyrosine (Y or Tyr), and tryptophan (W or Trp). Non-aromatic amino acids are broadly grouped as "aliphatic." Examples of "aliphatic" amino acids include glycine (G or Gly), alanine (A or Ala), valine (V or Val), leucine (L or Leu), isoleucine (I or Ile), methionine (M or Met), serine (S or Ser), threonine (T or Thr), cysteine (C or Cys), proline (P or Pro), glutamic acid (E or Glu), aspartic acid (A or Asp), asparagine (N or Asn), glutamine (Q or Gln), lysine (K or Lys), and arginine (R or Arg).

[0027] Aliphatic amino acids may be sub-divided into four sub-groups. The "large aliphatic non-polar sub-group" consists of valine, leucine, and isoleucine. The "aliphatic slightly-polar sub-group" consists of methionine, serine, threonine, and cysteine. The "aliphatic polar/charged sub-group" consists of glutamic acid, aspartic acid, asparagine, glutamine, lysine, and arginine. The "small-residue sub-group" consists of glycine and alanine. The group of charged/polar amino acids may be sub-divided into three sub-groups: the "positively-charged sub-group" consisting of lysine and arginine, the "negatively-charged sub-group" consisting of glutamic acid and aspartic acid, and the "polar sub-group" consisting of asparagine and glutamine.

[0028] Aromatic amino acids may be sub-divided into two sub-groups: the "nitrogen ring sub-group" consisting of histidine and tryptophan and the "phenyl sub-group" consisting of phenylalanine and tyrosine.

[0029] The amino acid replacement or substitution can be conservative, semi-conservative, or non-conservative. The phrase "conservative amino acid substitution" or "conservative mutation" refers to the replacement of one amino acid by another amino acid with a common property. A functional way to define common properties between individual amino acids is to analyze the normalized frequencies of amino acid changes between corresponding proteins of homologous organisms (Schulz and Schirmer, *Principles of*

Protein Structure, Springer-Verlag, New York (1979)). According to such analyses, groups of amino acids may be defined where amino acids within a group exchange preferentially with each other, and therefore resemble each other most in their impact on the overall protein structure (Schulz and Schirmer, *supra*).

[0030] Examples of conservative amino acid substitutions include substitutions of amino acids within the sub-groups described above, for example, lysine for arginine and vice versa such that a positive charge may be maintained, glutamic acid for aspartic acid and vice versa such that a negative charge may be maintained, serine for threonine such that a free -OH can be maintained, and glutamine for asparagine such that a free -NH_2 can be maintained.

[0031] "Semi-conservative mutations" include amino acid substitutions of amino acids within the same groups listed above, but not within the same sub-group. For example, the substitution of aspartic acid for asparagine, or asparagine for lysine, involves amino acids within the same group, but different sub-groups. "Non-conservative mutations" involve amino acid substitutions between different groups, for example, lysine for tryptophan, or phenylalanine for serine, etc.

[0032] The invention provides an immunoglobulin heavy chain polypeptide that comprises a complementarity determining region 1 (CDR) amino acid sequence of SEQ ID NO: 1, a CDR2 amino acid sequence of SEQ ID NO: 2, and a CDR3 amino acid sequence of SEQ ID NO: 3. In one embodiment of the invention, the isolated immunoglobulin heavy chain polypeptide comprises, consists of, or consists essentially of a complementarity determining region 1 (CDR) amino acid sequence of SEQ ID NO: 1, a CDR2 amino acid sequence of SEQ ID NO: 2, and a CDR3 amino acid sequence of SEQ ID NO: 3, wherein optionally (a) residue 9 of SEQ ID NO: 1 is replaced with a different amino acid residue, (b) one or more of residues 7, 8, and 9 of SEQ ID NO: 2 is replaced with a different amino acid residue, (c) one or more of residues 1, 2, and 5 of SEQ ID NO: 3 is replaced with a different amino acid residue, or (d) any combination of (a)-(c). When the inventive immunoglobulin heavy chain polypeptide consists essentially of a CDR1 amino acid sequence of SEQ ID NO: 1, a CDR2 amino acid sequence of SEQ ID NO: 2, and a CDR3 amino acid sequence of SEQ ID NO: 3 and optional amino acid replacements, additional components can be included in the polypeptide that do not materially affect the polypeptide (e.g., protein moieties such as biotin that facilitate purification or isolation). When the inventive immunoglobulin heavy chain polypeptide consists of a CDR1 amino acid sequence of SEQ ID NO: 1, a CDR2 amino acid sequence of SEQ ID NO: 2, and a CDR3 amino acid sequence of SEQ ID NO: 3 and

optional amino acid replacements, the polypeptide does not comprise any additional components (i.e., components that are not endogenous to the inventive immunoglobulin heavy chain polypeptide).

[0033] In one embodiment of the invention, the isolated immunoglobulin polypeptide comprises a CDR1 amino acid sequence of SEQ ID NO: 1, a CDR2 amino acid sequence of SEQ ID NO: 2, and a CDR3 amino acid sequence of SEQ ID NO: 3, except that (a) residue 9 of SEQ ID NO: 1 is replaced with a different amino acid residue, (b) one or more of residues 7, 8, and 9 of SEQ ID NO: 2 is replaced with a different amino acid residue, (c) one or more of residues 1, 2, and 5 of SEQ ID NO: 3 is replaced with a different amino acid residue, or (d) any combination of (a)-(c). For example, the isolated immunoglobulin heavy chain polypeptide can comprise a CDR1 amino acid sequence of SEQ ID NO: 1, a CDR2 amino acid sequence of SEQ ID NO: 2, and a CDR3 amino acid sequence of SEQ ID NO: 3, except that residue 9 of SEQ ID NO: 1 is replaced with a different amino acid residue and one or more of residues 7, 8, and 9 of SEQ ID NO: 2 is replaced with a different amino acid residue. Alternatively, the isolated immunoglobulin heavy chain polypeptide can comprise a CDR1 amino acid sequence of SEQ ID NO: 1, a CDR2 amino acid sequence of SEQ ID NO: 2, and a CDR3 amino acid sequence of SEQ ID NO: 3, except that residue 9 of SEQ ID NO: 1 is replaced with a different amino acid residue, one or more of residues 7, 8, and 9 of SEQ ID NO: 2 is replaced with a different amino acid residue, and one or more of residues 1, 2, and 5 of SEQ ID NO: 3 is replaced with a different amino acid residue. In another embodiment, the isolated immunoglobulin heavy chain polypeptide can comprise a CDR1 amino acid sequence of SEQ ID NO: 1, a CDR2 amino acid sequence of SEQ ID NO: 2, and a CDR3 amino acid sequence of SEQ ID NO: 3, except that one or more of residues 1, 2, and 5 of SEQ ID NO: 3 is replaced with a different amino acid residue. Each of residue 9 of SEQ ID NO: 1, residues 7, 8, and 9 of SEQ ID NO: 2, and residues 1, 2, and 5 of SEQ ID NO: 3 can be replaced with any suitable amino acid residue that can be the same or different in each position. For example, the amino acid residue of a first position can be replaced with a first different amino acid residue, and the amino acid residue of a second position can be replaced with a second different amino acid residue, wherein the first and second different amino acid residues are the same or different.

[0034] In one embodiment, the isolated immunoglobulin heavy chain polypeptide comprises a CDR1 amino acid sequence of SEQ ID NO: 1, a CDR2 amino acid sequence of SEQ ID NO: 2, and a CDR3 amino acid sequence of SEQ ID NO: 3, except that residue 9 of

SEQ ID NO: 1 is replaced with a methionine (M) residue. In another embodiment, the isolated immunoglobulin heavy chain polypeptide comprises a CDR1 amino acid sequence of SEQ ID NO: 1, a CDR2 amino acid sequence of SEQ ID NO: 2, and a CDR3 amino acid sequence of SEQ ID NO: 3, except that (a) residue 7 of SEQ ID NO: 2 is replaced with an asparagine (N) residue, (b) residue 8 of SEQ ID NO: 2 is replaced with a serine (S) residue, (c)residue 9 of SEQ ID NO: 2 is replaced with a threonine (T) residue, or (d) any combination of (a)-(c). In another embodiment, the isolated immunoglobulin heavy chain polypeptide comprises a CDR1 amino acid sequence of SEQ ID NO: 1, a CDR2 amino acid sequence of SEQ ID NO: 2, and a CDR3 amino acid sequence of SEQ ID NO: 3, except that (a) residue 1 of SEQ ID NO: 3 is replaced with a glutamic acid (E) residue, (b) residue 2 of SEQ ID NO: 3 is replaced with a tyrosine (Y) residue, (c) residue 5 of SEQ ID NO: 3 is replaced with a serine (S) residue, or (d) any combination of (a)-(c).

[0035] Exemplary immunoglobulin heavy chain polypeptides as described above can comprise any one of the following amino acid sequences: SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, or SEQ ID NO: 11.

[0036] The invention provides an isolated immunoglobulin heavy chain polypeptide comprises, consists essentially of, or consists of a complementarity determining region 1 (CDR) amino acid sequence of SEQ ID NO: 12, a CDR2 amino acid sequence of SEQ ID NO: 13, and a CDR3 amino acid sequence of SEQ ID NO: 14, wherein optionally (a) residue 9 of SEQ ID NO: 12 is replaced with a different amino acid residue, (b) residue 8 and/or residue 9 of SEQ ID NO: 13 is replaced with a different amino acid residue, (c) residue 5 of SEQ ID NO: 14 is replaced with a different amino acid residue, or (d) any combination of (a)-(c). When the inventive immunoglobulin heavy chain polypeptide consists essentially of a CDR1 amino acid sequence of SEQ ID NO: 12, a CDR2 amino acid sequence of SEQ ID NO: 13, and a CDR3 amino acid sequence of SEQ ID NO: 14 and optional amino acid replacements, additional components can be included in the polypeptide that do not materially affect the polypeptide (e.g., protein moieties such as biotin that facilitate purification or isolation). When the inventive immunoglobulin heavy chain polypeptide consists of a CDR1 amino acid sequence of SEQ ID NO: 12, a CDR2 amino acid sequence of SEQ ID NO: 13, and a CDR3 amino acid sequence of SEQ ID NO: 14 and optional amino acid replacements, the polypeptide does not comprise any additional components (i.e.,

components that are not endogenous to the inventive immunoglobulin heavy chain polypeptide).

[0037] In one embodiment, the isolated immunoglobulin heavy chain polypeptide can comprise a CDR1 amino acid sequence of SEQ ID NO: 12, a CDR2 amino acid sequence of SEQ ID NO: 13, and a CDR3 amino acid sequence of SEQ ID NO: 14, except that (a) residue 9 of SEQ ID NO: 12 is replaced with a different amino acid residue, (b) residue 8 and/or residue 9 of SEQ ID NO: 13 is replaced with a different amino acid residue, (c) residue 5 of SEQ ID NO: 14 is replaced with a different amino acid residue, or (d) any combination of (a)-(c). For example, the isolated immunoglobulin heavy chain polypeptide can comprise a CDR1 amino acid sequence of SEQ ID NO: 12, a CDR2 amino acid sequence of SEQ ID NO: 13, and a CDR3 amino acid sequence of SEQ ID NO: 14, except that residue 9 of SEQ ID NO: 12 is replaced with a different amino acid residue, residue 8 of SEQ ID NO: 13, and residue 9 of SEQ ID NO: 13 is replaced with a different amino acid residue. Alternatively, the isolated immunoglobulin heavy chain polypeptide can comprise a CDR1 amino acid sequence of SEQ ID NO: 12, a CDR2 amino acid sequence of SEQ ID NO: 13, and a CDR3 amino acid sequence of SEQ ID NO: 14, except that residue 9 of SEQ ID NO: 12 is replaced with a different amino acid residue and residue 5 of SEQ ID NO: 14 is replaced with a different amino acid residue. In another embodiment, the isolated immunoglobulin heavy chain polypeptide can comprise a CDR1 amino acid sequence of SEQ ID NO: 12, a CDR2 amino acid sequence of SEQ ID NO: 13, and a CDR3 amino acid sequence of SEQ ID NO: 14, except that residue 9 of SEQ ID NO: 12 is replaced with a different amino acid residue, residue 8 of SEQ ID NO: 13 is replaced with a different amino acid residue, residue 9 of SEQ ID NO: 13 is replaced with a different amino acid residue, and residue 5 of SEQ ID NO: 14 is replaced with a different amino acid residue. Each of residue 9 of SEQ ID NO: 12, residues 8 and 9 of SEQ ID NO: 13, and residue 5 of SEQ ID NO: 14 can be replaced with any suitable amino acid residue that can be the same or different in each position. For example, the amino acid residue of a first position can be replaced with a first different amino acid residue, and the amino acid residue of a second position can be replaced with a second different amino acid residue, wherein the first and second different amino acid residues are the same or different. In one embodiment, the isolated immunoglobulin heavy chain polypeptide comprises a CDR1 amino acid sequence of SEQ ID NO: 12, a CDR2 amino acid sequence of SEQ ID NO: 13, and a CDR3 amino acid sequence of SEQ ID NO: 14, except that residue 9 of SEQ ID NO: 12 is replaced with a leucine (L) residue. In another embodiment, the

isolated immunoglobulin heavy chain polypeptide comprises a CDR1 amino acid sequence of SEQ ID NO: 12, a CDR2 amino acid sequence of SEQ ID NO: 13, and a CDR3 amino acid sequence of SEQ ID NO: 14, except that (a) residue 8 of SEQ ID NO: 13 is replaced with a tyrosine (Y) residue, and/or (b) residue 9 of SEQ ID NO: 13 is replaced with an alanine (A) residue. In another embodiment, the isolated immunoglobulin heavy chain polypeptide comprises a CDR1 amino acid sequence of SEQ ID NO: 12, a CDR2 amino acid sequence of SEQ ID NO: 13, and a CDR3 amino acid sequence of SEQ ID NO: 14, except that residue 5 of SEQ ID NO: 14 is replaced with a threonine (T) residue.

[0038] Exemplary immunoglobulin heavy chain polypeptides as described above can comprise any one of the following amino acid sequences: SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, or SEQ ID NO: 18.

[0039] The invention provides an isolated immunoglobulin heavy chain polypeptide comprises, consists essentially of, or consists of a complementarity determining region 1 (CDR) amino acid sequence of SEQ ID NO: 19, a CDR2 amino acid sequence of SEQ ID NO: 20, and a CDR3 amino acid sequence of SEQ ID NO: 21. When the inventive immunoglobulin heavy chain polypeptide consists essentially of a CDR1 amino acid sequence of SEQ ID NO: 19, a CDR2 amino acid sequence of SEQ ID NO: 20, and a CDR3 amino acid sequence of SEQ ID NO: 21, additional components can be included in the polypeptide that do not materially affect the polypeptide (e.g., protein moieties such as biotin that facilitate purification or isolation). When the inventive immunoglobulin heavy chain polypeptide consists of a CDR1 amino acid sequence of SEQ ID NO: 19, a CDR2 amino acid sequence of SEQ ID NO: 20, and a CDR3 amino acid sequence of SEQ ID NO: 21, the polypeptide does not comprise any additional components (i.e., components that are not endogenous to the inventive immunoglobulin heavy chain polypeptide). Exemplary immunoglobulin heavy chain polypeptides as described above can comprise any one of the following amino acid sequences: SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, or SEQ ID NO: 25.

[0040] In addition, one or more amino acids can be inserted into the aforementioned immunoglobulin heavy chain polypeptides. Any number of any suitable amino acids can be inserted into the amino acid sequence of the immunoglobulin heavy chain polypeptide. In this respect, at least one amino acid (e.g., 2 or more, 5 or more, or 10 or more amino acids), but not more than 20 amino acids (e.g., 18 or less, 15 or less, or 12 or less amino acids), can be inserted into the amino acid sequence of the immunoglobulin heavy chain polypeptide.

Preferably, 1-10 amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids) are inserted into the amino acid sequence of the immunoglobulin heavy chain polypeptide. In this respect, the amino acid(s) can be inserted into any one of the aforementioned immunoglobulin heavy chain polypeptides in any suitable location. Preferably, the amino acid(s) are inserted into a CDR (e.g., CDR1, CDR2, or CDR3) of the immunoglobulin heavy chain polypeptide.

[0041] The invention provides an isolated immunoglobulin heavy chain polypeptide which comprises an amino acid sequence that is at least 90% identical (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to any one of SEQ ID NOs: 4-11, SEQ ID NOs: 15-18, and SEQ ID NOs: 22-25. Nucleic acid or amino acid sequence "identity," as described herein, can be determined by comparing a nucleic acid or amino acid sequence of interest to a reference nucleic acid or amino acid sequence. The percent identity is the number of nucleotides or amino acid residues that are the same (i.e., that are identical) as between the sequence of interest and the reference sequence divided by the length of the longest sequence (i.e., the length of either the sequence of interest or the reference sequence, whichever is longer). A number of mathematical algorithms for obtaining the optimal alignment and calculating identity between two or more sequences are known and incorporated into a number of available software programs. Examples of such programs include CLUSTAL-W, T-Coffee, and ALIGN (for alignment of nucleic acid and amino acid sequences), BLAST programs (e.g., BLAST 2.1, BL2SEQ, and later versions thereof) and FASTA programs (e.g., FASTA3x, FASTM, and SSEARCH) (for sequence alignment and sequence similarity searches). Sequence alignment algorithms also are disclosed in, for example, Altschul et al., *J. Molecular Biol.*, *215*(3): 403-410 (1990), Beigert et al., *Proc. Natl. Acad. Sci. USA*, *106*(10): 3770-3775 (2009), Durbin et al., eds., *Biological Sequence Analysis: Probalistic Models of Proteins and Nucleic Acids*, Cambridge University Press, Cambridge, UK (2009), Soding, *Bioinformatics*, *21*(7): 951-960 (2005), Altschul et al., *Nucleic Acids Res.*, *25*(17): 3389-3402 (1997), and Gusfield, *Algorithms on Strings, Trees and Sequences*, Cambridge University Press, Cambridge UK (1997)).

[0042] The invention provides an immunoglobulin light chain polypeptide that comprises a complementarity determining region 1 (CDR) amino acid sequence of SEQ ID NO: 26 and a CDR2 amino acid sequence of SEQ ID NO: 27. In one embodiment of the invention, the isolated immunoglobulin light chain polypeptide comprises, consists essentially of, or consists of a CDR1 amino acid sequence of SEQ ID NO: 26 and a CDR2 amino acid

sequence of SEQ ID NO: 27. When the inventive immunoglobulin light chain polypeptide consists essentially of a CDR1 amino acid sequence of SEQ ID NO: 26 and a CDR2 amino acid sequence of SEQ ID NO: 27, additional components can be included in the polypeptide that do not materially affect the polypeptide (e.g., protein moieties such as biotin that facilitate purification or isolation). When the inventive immunoglobulin light chain polypeptide consists of a CDR1 amino acid sequence of SEQ ID NO: 26 and a CDR2 amino acid sequence of SEQ ID NO: 27, the polypeptide does not comprise any additional components (i.e., components that are not endogenous to the inventive immunoglobulin light chain polypeptide). Exemplary immunoglobulin light chain polypeptides as described above can comprise SEQ ID NO: 28 or SEQ ID NO: 29.

[0043] The invention provides an isolated immunoglobulin light chain polypeptide comprises a complementarity determining region 1 (CDR) amino acid sequence of SEQ ID NO: 30 and a CDR2 amino acid sequence of SEQ ID NO: 31. In one embodiment of the invention, the isolated immunoglobulin light chain polypeptide comprises, consists of, or consists essentially of a CDR1 amino acid sequence of SEQ ID NO: 30 and a CDR2 amino acid sequence of SEQ ID NO: 31, wherein optionally residue 12 of SEQ ID NO: 30 is replaced with a different amino acid residue. When the inventive immunoglobulin light chain polypeptide consists essentially of a CDR1 amino acid sequence of SEQ ID NO: 30 and a CDR2 amino acid sequence of SEQ ID NO: 31and optional amino acid replacements, additional components can be included in the polypeptide that do not materially affect the polypeptide (e.g., protein moieties such as biotin that facilitate purification or isolation). When the inventive immunoglobulin light chain polypeptide consists of a CDR1 amino acid sequence of SEQ ID NO: 30 and a CDR2 amino acid sequence of SEQ ID NO: 31 and optional amino acid replacements, the polypeptide does not comprise any additional components (i.e., components that are not endogenous to the inventive immunoglobulin light chain polypeptide).

[0044] In this respect, for example, the isolated immunoglobulin light chain polypeptide can comprise a CDR1 amino acid sequence of SEQ ID NO: 30 and a CDR2 amino acid sequence of SEQ ID NO: 31, except that residue 12 of SEQ ID NO: 30 is replaced with a different amino acid residue. Residue 12 of SEQ ID NO: 30 can be replaced with any suitable amino acid residue. In one embodiment, the isolated immunoglobulin light chain polypeptide can comprise a CDR1 amino acid sequence of SEQ ID NO: 30 and a CDR2 amino acid sequence of SEQ ID NO: 31, except that residue 12 of SEQ ID NO: 30 is

replaced with a threonine (T) residue. Exemplary immunoglobulin light chain polypeptides as described above can comprise any one of the following amino acid sequences: SEQ ID NO: 32, SEQ ID NO: 33, or SEQ ID NO: 34.

[0045] The invention provides an isolated immunoglobulin light chain polypeptide comprises a complementarity determining region 1 (CDR) amino acid sequence of SEQ ID NO: 35, a CDR2 amino acid sequence of SEQ ID NO: 36, and a CDR3 amino acid sequence of SEQ ID NO: 37. In one embodiment, the immunoglobulin light chain polypeptide comprises, consists essentially of, or consists of a CDR1 amino acid sequence of SEQ ID NO: 35, a CDR2 amino acid sequence of SEQ ID NO: 36, and a CDR3 amino acid sequence of SEQ ID NO: 37, wherein optionally (a) residue 5 of SEQ ID NO: 36 is replaced with a different amino acid residue, and/or (b) residue 4 of SEQ ID NO: 37 is replaced with a different amino acid residue. When the inventive immunoglobulin light chain polypeptide consists essentially of a CDR1 amino acid sequence of SEQ ID NO: 35, a CDR2 amino acid sequence of SEQ ID NO: 36, and a CDR3 amino acid sequence of SEQ ID NO: 37 and optional amino acid replacements, additional components can be included in the polypeptide that do not materially affect the polypeptide (e.g., protein moieties such as biotin that facilitate purification or isolation). When the inventive immunoglobulin light chain polypeptide consists of a CDR1 amino acid sequence of SEQ ID NO: 35, a CDR2 amino acid sequence of SEQ ID NO: 36, and a CDR3 amino acid sequence of SEQ ID NO: 37 and optional amino acid replacements, the polypeptide does not comprise any additional components (i.e., components that are not endogenous to the inventive immunoglobulin light chain polypeptide). In this respect, for example, the isolated immunoglobulin light chain polypeptide can comprise a CDR1 amino acid sequence of SEQ ID NO: 35, a CDR2 amino acid sequence of SEQ ID NO: 36, and a CDR3 amino acid sequence of SEQ ID NO: 37. Alternatively, the isolated immunoglobulin light chain polypeptide can comprise a CDR1 amino acid sequence of SEQ ID NO: 35, a CDR2 amino acid sequence of SEQ ID NO: 36, and a CDR3 amino acid sequence of SEQ ID NO: 37, except that (a) residue 5 of SEQ ID NO: 36 is replaced with a different amino acid residue, and/or (b) residue 4 of SEQ ID NO: 37 is replaced with a different amino acid residue. Each of residue 5 of SEQ ID NO: 36 and residue 4 of SEQ ID NO: 37 can be replaced with any suitable amino acid residue that can be the same or different in each position. For example, the amino acid residue of a first position can be replaced with a first different amino acid residue, and the amino acid residue of a

second position can be replaced with a second different amino acid residue, wherein the first and second different amino acid residues are the same or different.

[0046] In one embodiment, the isolated immunoglobulin light chain polypeptide comprises a CDR1 amino acid sequence of SEQ ID NO: 35, a CDR2 amino acid sequence of SEQ ID NO: 36, and a CDR3 amino acid sequence of SEQ ID NO: 37, except that (a) residue 5 of SEQ ID NO: 36 is replaced with a leucine (L) residue, and/or (b) residue 4 of SEQ ID NO: 37 is replaced with an asparagine (N) residue. Exemplary immunoglobulin light chain polypeptides as described above can comprise any one of the following amino acid sequences: SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, or SEQ ID NO: 41.

[0047] In addition, one or more amino acids can be inserted into the aforementioned immunoglobulin light chain polypeptides. Any number of any suitable amino acids can be inserted into the amino acid sequence of the immunoglobulin light chain polypeptide. In this respect, at least one amino acid (e.g., 2 or more, 5 or more, or 10 or more amino acids), but not more than 20 amino acids (e.g., 18 or less, 15 or less, or 12 or less amino acids), can be inserted into the amino acid sequence of the immunoglobulin light chain polypeptide. Preferably, 1-10 amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids) are inserted in to the amino acid sequence of the immunoglobulin light chain polypeptide. In this respect, the amino acid(s) can be inserted into any one of the aforementioned immunoglobulin light chain polypeptides in any suitable location. Preferably, the amino acid(s) are inserted into a CDR (e.g., CDR1, CDR2, or CDR3) of the immunoglobulin light chain polypeptide.

[0048] The invention provides an isolated immunoglobulin light chain polypeptide which comprises an amino acid sequence that is at least 90% identical (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to any one of SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, and SEQ ID NO: 41. Nucleic acid or amino acid sequence "identity," as described herein, can be determined using the methods described herein.

[0049] The invention provides an isolated programmed death 1 (PD-1)-binding agent comprising, consisting essentially of, or consisting of the inventive isolated amino acid sequences described herein. By "programmed death 1 (PD-1)-binding agent" is meant a molecule, preferably a proteinaceous molecule, that binds specifically to the programmed death 1 protein (PD-1). Preferably, the PD-1-binding agent is an antibody or a fragment (e.g., immunogenic fragment) thereof. The isolated PD-1-binding agent of the invention

comprises, consists essentially of, or consists of the inventive isolated immunoglobulin heavy chain polypeptide and/or the inventive isolated immunoglobulin light chain polypeptide. In one embodiment, the isolated PD-1-binding agent comprises, consists essentially of, or consists of the inventive immunoglobulin heavy chain polypeptide or the inventive immunoglobulin light chain polypeptide. In another embodiment, the isolated PD-1-binding agent comprises, consists essentially of, or consists of the inventive immunoglobulin heavy chain polypeptide and the inventive immunoglobulin light chain polypeptide.

[0050] The invention is not limited to an isolated PD-1-binding agent that comprises, consists essentially of, or consists of an immunoglobulin heavy chain polypeptide and/or light chain polypeptide having replacements, insertions, and/or deletions of the specific amino acid residues disclosed herein. Indeed, any amino acid residue of the inventive immunoglobulin heavy chain polypeptide and/or the inventive immunoglobulin light chain polypeptide can be replaced, in any combination, with a different amino acid residue, or can be deleted or inserted, so long as the biological activity of the PD-1-binding agent is enhanced or improved as a result of the amino acid replacements, insertions, and/or deletions. The "biological activity" of an PD-1-binding agent refers to, for example, binding affinity for PD-1 or a particular PD-1 epitope, neutralization or inhibition of PD-1 protein binding to its ligands PD-L1 and PD-L1, neutralization or inhibition of PD-1 protein activity *in vivo* (e.g., IC_{50}), pharmacokinetics, and cross-reactivity (e.g., with non-human homologs or orthologs of the PD-1 protein, or with other proteins or tissues). Other biological properties or characteristics of an antigen-binding agent recognized in the art include, for example, avidity, selectivity, solubility, folding, immunotoxicity, expression, and formulation. The aforementioned properties or characteristics can be observed, measured, and/or assessed using standard techniques including, but not limited to, ELISA, competitive ELISA, surface plasmon resonance analysis (BIACORE™), or KINEXA™, *in vitro* or *in vivo* neutralization assays, receptor-ligand binding assays, cytokine or growth factor production and/or secretion assays, and signal transduction and immunohistochemistry assays.

[0051] The terms "inhibit" or "neutralize," as used herein with respect to the activity of a PD-1-binding agent, refer to the ability to substantially antagonize, prohibit, prevent, restrain, slow, disrupt, alter, eliminate, stop, or reverse the progression or severity of, for example, the biological activity of a PD-1 protein, or a disease or condition associated with an PD-1 protein. The isolated PD-1-binding agent of the invention preferably inhibits or neutralizes the activity of a PD-1 protein by at least about 20%, about 30%, about 40%, about 50%,

about 60%, about 70%, about 80%, about 90%, about 95%, about 100%, or a range defined by any two of the foregoing values.

[0052] The isolated PD-1-binding agent of the invention can be a whole antibody, as described herein, or an antibody fragment. The terms "fragment of an antibody," "antibody fragment," and "functional fragment of an antibody" are used interchangeably herein to mean one or more fragments of an antibody that retain the ability to specifically bind to an antigen (see, generally, Holliger et al., *Nat. Biotech.*, *23*(9): 1126-1129 (2005)). The isolated PD-1 binding agent can contain any PD-1-binding antibody fragment. The antibody fragment desirably comprises, for example, one or more CDRs, the variable region (or portions thereof), the constant region (or portions thereof), or combinations thereof. Examples of antibody fragments include, but are not limited to, (i) a Fab fragment, which is a monovalent fragment consisting of the V_L, V_H, C_L, and CH_1 domains, (ii) a $F(ab')_2$ fragment, which is a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region, (iii) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (iv) a Fab' fragment, which results from breaking the disulfide bridge of an $F(ab')_2$ fragment using mild reducing conditions, (v) a disulfide-stabilized Fv fragment (dsFv), and (vi) a domain antibody (dAb), which is an antibody single variable region domain (VH or VL) polypeptide that specifically binds antigen.

[0053] In embodiments where the isolated PD-1-binding agent comprises a fragment of the immunoglobulin heavy chain or light chain polypeptide, the fragment can be of any size so long as the fragment binds to, and preferably inhibits the activity of, a PD-1 protein. In this respect, a fragment of the immunoglobulin heavy chain polypeptide desirably comprises between about 5 and 18 (e.g., about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or a range defined by any two of the foregoing values) amino acids. Similarly, a fragment of the immunoglobulin light chain polypeptide desirably comprises between about 5 and 18 (e.g., about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or a range defined by any two of the foregoing values) amino acids.

[0054] When the PD-1-binding agent is an antibody or antibody fragment, the antibody or antibody fragment desirably comprises a heavy chain constant region (F_c) of any suitable class. Preferably, the antibody or antibody fragment comprises a heavy chain constant region that is based upon wild-type IgG1, IgG2, or IgG4 antibodies, or variants thereof.

[0055] The PD-1-binding agent also can be a single chain antibody fragment. Examples of single chain antibody fragments include, but are not limited to, (i) a single chain Fv (scFv),

which is a monovalent molecule consisting of the two domains of the Fv fragment (i.e., V_L and V_H) joined by a synthetic linker which enables the two domains to be synthesized as a single polypeptide chain (see, e.g., Bird et al., *Science*, *242*: 423-426 (1988); Huston et al., *Proc. Natl. Acad. Sci. USA*, *85*: 5879-5883 (1988); and Osbourn et al., *Nat. Biotechnol.*, *16*: 778 (1998)) and (ii) a diabody, which is a dimer of polypeptide chains, wherein each polypeptide chain comprises a V_H connected to a V_L by a peptide linker that is too short to allow pairing between the V_H and V_L on the same polypeptide chain, thereby driving the pairing between the complementary domains on different V_H -V_L polypeptide chains to generate a dimeric molecule having two functional antigen binding sites. Antibody fragments are known in the art and are described in more detail in, e.g., U.S. Patent Application Publication 2009/0093024 A1.

[0056] The isolated PD-1-binding agent also can be an intrabody or fragment thereof. An intrabody is an antibody which is expressed and which functions intracellularly. Intrabodies typically lack disulfide bonds and are capable of modulating the expression or activity of target genes through their specific binding activity. Intrabodies include single domain fragments such as isolated V_H and V_L domains and scFvs. An intrabody can include sub-cellular trafficking signals attached to the N or C terminus of the intrabody to allow expression at high concentrations in the sub-cellular compartments where a target protein is located. Upon interaction with a target gene, an intrabody modulates target protein function and/or achieves phenotypic/functional knockout by mechanisms such as accelerating target protein degradation and sequestering the target protein in a non-physiological sub-cellular compartment. Other mechanisms of intrabody-mediated gene inactivation can depend on the epitope to which the intrabody is directed, such as binding to the catalytic site on a target protein or to epitopes that are involved in protein-protein, protein-DNA, or protein-RNA interactions.

[0057] The isolated PD-1-binding agent also can be an antibody conjugate. In this respect, the isolated PD-1-binding agent can be a conjugate of (1) an antibody, an alternative scaffold, or fragments thereof, and (2) a protein or non-protein moiety comprising the PD-1-binding agent. For example, the PD-1-binding agent can be all or part of an antibody conjugated to a peptide, a fluorescent molecule, or a chemotherapeutic agent.

[0058] The isolated PD-1-binding agent can be, or can be obtained from, a human antibody, a non-human antibody, or a chimeric antibody. By "chimeric" is meant an antibody or fragment thereof comprising both human and non-human regions. Preferably, the

isolated PD-1-binding agent is a humanized antibody. A "humanized" antibody is a monoclonal antibody comprising a human antibody scaffold and at least one CDR obtained or derived from a non-human antibody. Non-human antibodies include antibodies isolated from any non-human animal, such as, for example, a rodent (e.g., a mouse or rat). A humanized antibody can comprise, one, two, or three CDRs obtained or derived from a non-human antibody. In a preferred embodiment of the invention, CDRH3 of the inventive PD-1-binding agent is obtained or derived from a mouse monoclonal antibody, while the remaining variable regions and constant region of the inventive PD-1-binding agent are obtained or derived from a human monoclonal antibody.

[0059] A human antibody, a non-human antibody, a chimeric antibody, or a humanized antibody can be obtained by any means, including via *in vitro* sources (e.g., a hybridoma or a cell line producing an antibody recombinantly) and *in vivo* sources (e.g., rodents). Methods for generating antibodies are known in the art and are described in, for example, Köhler and Milstein, *Eur. J. Immunol.*, *5*: 511-519 (1976); Harlow and Lane (eds.), *Antibodies: A Laboratory Manual*, CSH Press (1988); and Janeway et al. (eds.), *Immunobiology, 5th Ed.*, Garland Publishing, New York, NY (2001)). In certain embodiments, a human antibody or a chimeric antibody can be generated using a transgenic animal (e.g., a mouse) wherein one or more endogenous immunoglobulin genes are replaced with one or more human immunoglobulin genes. Examples of transgenic mice wherein endogenous antibody genes are effectively replaced with human antibody genes include, but are not limited to, the Medarex HUMAB-MOUSE™, the Kirin TC MOUSE™, and the Kyowa Kirin KM-MOUSE™ (see, e.g., Lonberg, *Nat. Biotechnol.*, *23(9)*: 1117-25 (2005), and Lonberg, *Handb. Exp. Pharmacol.*, *181*: 69-97 (2008)). A humanized antibody can be generated using any suitable method known in the art (see, e.g., An, Z. (ed.), *Therapeutic Monoclonal Antibodies: From Bench to Clinic*, John Wiley & Sons, Inc., Hoboken, New Jersey (2009)), including, e.g., grafting of non-human CDRs onto a human antibody scaffold (see, e.g., Kashmiri et al., *Methods*, *36*(1): 25-34 (2005); and Hou et al., *J. Biochem.*, *144*(1): 115-120 (2008)). In one embodiment, a humanized antibody can be produced using the methods described in, e.g., U.S. Patent Application Publication 2011/0287485 A1.

[0060] In a preferred embodiment, the PD-1-binding agent binds an epitope of a PD-1 protein which blocks the binding of PD-1 to PD-L1. The invention also provides an isolated or purified epitope of a PD-1 protein which blocks the binding of PD-1 to PD-L1 in an indirect or allosteric manner.

[0061] The invention also provides one or more isolated or purified nucleic acid sequences that encode the inventive immunoglobulin heavy chain polypeptide, the inventive immunoglobulin light chain polypeptide, and the inventive PD-1-binding agent.

[0062] The term "nucleic acid sequence" is intended to encompass a polymer of DNA or RNA, i.e., a polynucleotide, which can be single-stranded or double-stranded and which can contain non-natural or altered nucleotides. The terms "nucleic acid" and "polynucleotide" as used herein refer to a polymeric form of nucleotides of any length, either ribonucleotides (RNA) or deoxyribonucleotides (DNA). These terms refer to the primary structure of the molecule, and thus include double- and single-stranded DNA, and double- and single-stranded RNA. The terms include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs and modified polynucleotides such as, though not limited to, methylated and/or capped polynucleotides. Nucleic acids are typically linked via phosphate bonds to form nucleic acid sequences or polynucleotides, though many other linkages are known in the art (e.g., phosphorothioates, boranophosphates, and the like). Nucleic acid sequences encoding the inventive immunoglobulin heavy chain polypeptides include, for example, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, and SEQ ID NO: 55. Nucleic acid sequences encoding the inventive immunoglobulin light chain polypeptides include, for example, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, and SEQ ID NO: 64.

[0063] The invention further provides a vector comprising one or more nucleic acid sequences encoding the inventive immunoglobulin heavy chain polypeptide, the inventive immunoglobulin light chain polypeptide, and/or the inventive PD-1-binding agent. The vector can be, for example, a plasmid, episome, cosmid, viral vector (e.g., retroviral or adenoviral), or phage. Suitable vectors and methods of vector preparation are well known in the art (see, e.g., Sambrook et al., *Molecular Cloning, a Laboratory Manual, 3rd edition*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (2001), and Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons, New York, N.Y. (1994)).

[0064] In addition to the nucleic acid sequence encoding the inventive immunoglobulin heavy polypeptide, the inventive immunoglobulin light chain polypeptide, and/or the inventive PD-1-binding agent, the vector preferably comprises expression control sequences,

such as promoters, enhancers, polyadenylation signals, transcription terminators, internal ribosome entry sites (IRES), and the like, that provide for the expression of the coding sequence in a host cell. Exemplary expression control sequences are known in the art and described in, for example, Goeddel, *Gene Expression Technology: Methods in Enzymology*, Vol. 185, Academic Press, San Diego, Calif. (1990).

[0065] A large number of promoters, including constitutive, inducible, and repressible promoters, from a variety of different sources are well known in the art. Representative sources of promoters include for example, virus, mammal, insect, plant, yeast, and bacteria, and suitable promoters from these sources are readily available, or can be made synthetically, based on sequences publicly available, for example, from depositories such as the ATCC as well as other commercial or individual sources. Promoters can be unidirectional (i.e., initiate transcription in one direction) or bi-directional (i.e., initiate transcription in either a 3' or 5' direction). Non-limiting examples of promoters include, for example, the T7 bacterial expression system, pBAD (araA) bacterial expression system, the cytomegalovirus (CMV) promoter, the SV40 promoter, the RSV promoter. Inducible promoters include, for example, the Tet system (U.S. Patents 5,464,758 and 5,814,618), the Ecdysone inducible system (No et al., *Proc. Natl. Acad. Sci., 93*: 3346-3351 (1996)), the T-REX™ system (Invitrogen, Carlsbad, CA), LACSWITCH™ system (Stratagene, San Diego, CA), and the Cre-ERT tamoxifen inducible recombinase system (Indra et al., *Nuc. Acid. Res., 27*: 4324-4327 (1999); *Nuc. Acid. Res., 28*: e99 (2000); U.S. Patent 7,112,715; and Kramer & Fussenegger, *Methods Mol. Biol., 308*: 123-144 (2005)).

[0066] The term "enhancer" as used herein, refers to a DNA sequence that increases transcription of, for example, a nucleic acid sequence to which it is operably linked. Enhancers can be located many kilobases away from the coding region of the nucleic acid sequence and can mediate the binding of regulatory factors, patterns of DNA methylation, or changes in DNA structure. A large number of enhancers from a variety of different sources are well known in the art and are available as or within cloned polynucleotides (from, e.g., depositories such as the ATCC as well as other commercial or individual sources). A number of polynucleotides comprising promoters (such as the commonly-used CMV promoter) also comprise enhancer sequences. Enhancers can be located upstream, within, or downstream of coding sequences.

[0067] The vector also can comprise a "selectable marker gene." The term "selectable marker gene," as used herein, refers to a nucleic acid sequence that allow cells expressing the

nucleic acid sequence to be specifically selected for or against, in the presence of a corresponding selective agent. Suitable selectable marker genes are known in the art and described in, e.g., International Patent Application Publications WO 1992/008796 and WO 1994/028143; Wigler et al., *Proc. Natl. Acad. Sci. USA*, *77*: 3567-3570 (1980); O'Hare et al., *Proc. Natl. Acad. Sci. USA*, *78*: 1527-1531 (1981); Mulligan & Berg, *Proc. Natl. Acad. Sci. USA*, *78*: 2072-2076 (1981); Colberre-Garapin et al., *J. Mol. Biol.*, *150*: 1-14 (1981); Santerre et al., *Gene*, *30*: 147-156 (1984); Kent et al., *Science*, *237*: 901-903 (1987); Wigler et al., *Cell*, *11*: 223-232 (1977); Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA*, *48*: 2026-2034 (1962); Lowy et al., *Cell*, *22*: 817-823 (1980); and U.S. Patents 5,122,464 and 5,770,359.

[0068] In some embodiments, the vector is an "episomal expression vector" or "episome," which is able to replicate in a host cell, and persists as an extrachromosomal segment of DNA within the host cell in the presence of appropriate selective pressure (see, e.g., Conese et al., *Gene Therapy*, *11*: 1735-1742 (2004)). Representative commercially available episomal expression vectors include, but are not limited to, episomal plasmids that utilize Epstein Barr Nuclear Antigen 1 (EBNA1) and the Epstein Barr Virus (EBV) origin of replication (oriP). The vectors pREP4, pCEP4, pREP7, and pcDNA3.1 from Invitrogen (Carlsbad, CA) and pBK-CMV from Stratagene (La Jolla, CA) represent non-limiting examples of an episomal vector that uses T-antigen and the SV40 origin of replication in lieu of EBNA1 and oriP.

[0069] Other suitable vectors include integrating expression vectors, which may randomly integrate into the host cell's DNA, or may include a recombination site to enable the specific recombination between the expression vector and the host cell's chromosome. Such integrating expression vectors may utilize the endogenous expression control sequences of the host cell's chromosomes to effect expression of the desired protein. Examples of vectors that integrate in a site specific manner include, for example, components of the flp-in system from Invitrogen (Carlsbad, CA) (e.g., pcDNA™5/FRT), or the cre-lox system, such as can be found in the pExchange-6 Core Vectors from Stratagene (La Jolla, CA). Examples of vectors that randomly integrate into host cell chromosomes include, for example, pcDNA3.1 (when introduced in the absence of T-antigen) from Invitrogen (Carlsbad, CA), UCOE from Millipore (Billerica, MA), and pCI or pFN10A (ACT) FLEXI™ from Promega (Madison, WI).

[0070] Viral vectors also can be used. Representative commercially available viral expression vectors include, but are not limited to, the adenovirus-based Per.C6 system

available from Crucell, Inc. (Leiden, The Netherlands), the lentiviral-based pLP1 from Invitrogen (Carlsbad, CA), and the retroviral vectors pFB-ERV plus pCFB-EGSH from Stratagene (La Jolla, CA).

[0071] Nucleic acid sequences encoding the inventive amino acid sequences can be provided to a cell on the same vector (i.e., in *cis*). A unidirectional promoter can be used to control expression of each nucleic acid sequence. In another embodiment, a combination of bidirectional and unidirectional promoters can be used to control expression of multiple nucleic acid sequences. Nucleic acid sequences encoding the inventive amino acid sequences alternatively can be provided to the population of cells on separate vectors (i.e., in *trans*). Each of the nucleic acid sequences in each of the separate vectors can comprise the same or different expression control sequences. The separate vectors can be provided to cells simultaneously or sequentially.

[0072] The vector(s) comprising the nucleic acid(s) encoding the inventive amino acid sequences can be introduced into a host cell that is capable of expressing the polypeptides encoded thereby, including any suitable prokaryotic or eukaryotic cell. As such, the invention provides an isolated cell comprising the inventive vector. Preferred host cells are those that can be easily and reliably grown, have reasonably fast growth rates, have well characterized expression systems, and can be transformed or transfected easily and efficiently.

[0073] Examples of suitable prokaryotic cells include, but are not limited to, cells from the genera *Bacillus* (such as *Bacillus subtilis* and *Bacillus brevis*), *Escherichia* (such as *E. coli*), *Pseudomonas*, *Streptomyces*, *Salmonella*, and *Erwinia*. Particularly useful prokaryotic cells include the various strains of *Escherichia coli* (e.g., K12, HB101 (ATCC No. 33694), DH5α, DH10, MC1061 (ATCC No. 53338), and CC102).

[0074] Preferably, the vector is introduced into a eukaryotic cell. Suitable eukaryotic cells are known in the art and include, for example, yeast cells, insect cells, and mammalian cells. Examples of suitable yeast cells include those from the genera *Kluyveromyces*, *Pichia*, *Rhino-sporidium*, *Saccharomyces*, and *Schizosaccharomyces*. Preferred yeast cells include, for example, *Saccharomyces cerivisae* and *Pichia pastoris*.

[0075] Suitable insect cells are described in, for example, Kitts et al., *Biotechniques*, *14*: 810-817 (1993); Lucklow, *Curr. Opin. Biotechnol.*, *4*: 564-572 (1993); and Lucklow et al., *J. Virol.*, *67*: 4566-4579 (1993). Preferred insect cells include Sf-9 and HI5 (Invitrogen, Carlsbad, CA).

[0076] Preferably, mammalian cells are utilized in the invention. A number of suitable mammalian host cells are known in the art, and many are available from the American Type Culture Collection (ATCC, Manassas, VA). Examples of suitable mammalian cells include, but are not limited to, Chinese hamster ovary cells (CHO) (ATCC No. CCL61), CHO DHFR- cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA*, *97*: 4216-4220 (1980)), human embryonic kidney (HEK) 293 or 293T cells (ATCC No. CRL1573), and 3T3 cells (ATCC No. CCL92). Other suitable mammalian cell lines are the monkey COS-1 (ATCC No. CRL1650) and COS-7 cell lines (ATCC No. CRL1651), as well as the CV-1 cell line (ATCC No. CCL70). Further exemplary mammalian host cells include primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from in vitro culture of primary tissue, as well as primary explants, are also suitable. Other suitable mammalian cell lines include, but are not limited to, mouse neuroblastoma N2A cells, HeLa, mouse L-929 cells, and BHK or HaK hamster cell lines, all of which are available from the ATCC. Methods for selecting suitable mammalian host cells and methods for transformation, culture, amplification, screening, and purification of cells are known in the art.

[0077] Most preferably, the mammalian cell is a human cell. For example, the mammalian cell can be a human lymphoid or lymphoid derived cell line, such as a cell line of pre-B lymphocyte origin. Examples of human lymphoid cells lines include, without limitation, RAMOS (CRL-1596), Daudi (CCL-213), EB-3 (CCL-85), DT40 (CRL-2111), 18-81 (Jack et al., *Proc. Natl. Acad. Sci. USA*, *85*: 1581-1585 (1988)), Raji cells (CCL-86), and derivatives thereof.

[0078] A nucleic acid sequence encoding the inventive amino acid sequence may be introduced into a cell by "transfection," "transformation," or "transduction." "Transfection," "transformation," or "transduction," as used herein, refer to the introduction of one or more exogenous polynucleotides into a host cell by using physical or chemical methods. Many transfection techniques are known in the art and include, for example, calcium phosphate DNA co-precipitation (see, e.g., Murray E.J. (ed.), *Methods in Molecular Biology, Vol. 7, Gene Transfer and Expression Protocols*, Humana Press (1991)); DEAE-dextran; electroporation; cationic liposome-mediated transfection; tungsten particle-facilitated microparticle bombardment (Johnston, *Nature*, *346*: 776-777 (1990)); and strontium phosphate DNA co-precipitation (Brash et al., *Mol. Cell Biol.*, *7*: 2031-2034 (1987)). Phage or viral vectors can be introduced into host cells, after growth of infectious particles in suitable packaging cells, many of which are commercially available.

[0079] The invention provides a composition comprising an effective amount of the inventive immunoglobulin heavy chain polypeptide, the inventive immunoglobulin light chain polypeptide, the inventive PD-1-binding agent, the inventive nucleic acid sequence encoding any of the foregoing, or the inventive vector comprising the inventive nucleic acid sequence. Preferably, the composition is a pharmaceutically acceptable (e.g., physiologically acceptable) composition, which comprises a carrier, preferably a pharmaceutically acceptable (e.g., physiologically acceptable) carrier, and the inventive amino acid sequences, antigen-binding agent, or vector. Any suitable carrier can be used within the context of the invention, and such carriers are well known in the art. The choice of carrier will be determined, in part, by the particular site to which the composition may be administered and the particular method used to administer the composition. The composition optionally can be sterile. The composition can be frozen or lyophilized for storage and reconstituted in a suitable sterile carrier prior to use. The compositions can be generated in accordance with conventional techniques described in, e.g., Remington: *The Science and Practice of Pharmacy, 21st Edition*, Lippincott Williams & Wilkins, Philadelphia, PA (2001).

[0080] The invention further provides a method of treating any disease or disorder in which the improper expression (e.g., overexpression) or increased activity of a PD-1 protein causes or contributes to the pathological effects of the disease, or a decrease in PD-1 protein levels or activity has a therapeutic benefit in mammals, preferably humans. The invention also provides a method of treating a cancer or an infectious disease in a mammal. The method comprises administering the aforementioned composition to a mammal having a cancer or an infectious disease, whereupon the cancer or infectious disease is treated in the mammal. As discussed herein, PD-1 is abnormally expressed in a variety of cancers (see, e.g., Brown et al., *J. Immunol.*, *170*: 1257-1266 (2003); and Flies et. al., *Yale Journal of Biology and Medicine*, *84*: 409-421 (2011)), and PD-L1 expression in some renal cell carcinoma patients correlates with tumor aggressiveness. The inventive method can be used to treat any type of cancer known in the art, such as, for example, melanoma, renal cell carcinoma, lung cancer, bladder cancer, breast cancer, cervical cancer, colon cancer, gall bladder cancer, laryngeal cancer, liver cancer, thyroid cancer, stomach cancer, salivary gland cancer, prostate cancer, pancreatic cancer, or Merkel cell carcinoma (see, e.g., Bhatia et al., *Curr. Oncol. Rep.*, *13*(6): 488-497 (2011)). The inventive method can be used to treat any type of infectious disease (i.e., a disease or disorder caused by a bacterium, a virus, a fungus, or a parasite). Examples of infectious diseases that can be treated by the inventive method

include, but are not limited to, diseases caused by a human immunodeficiency virus (HIV), a respiratory syncytial virus (RSV), an influenza virus, a dengue virus, a hepatitis B virus (HBV, or a hepatitis C virus (HCV)). Administration of a composition comprising the inventive immunoglobulin heavy chain polypeptide, the inventive immunoglobulin light chain polypeptide, the inventive PD-1-binding agent, the inventive nucleic acid sequence encoding any of the foregoing, or the inventive vector comprising the inventive nucleic acid sequence induces an immune response against a cancer or infectious disease in a mammal. An "immune response" can entail, for example, antibody production and/or the activation of immune effector cells (e.g., T-cells).

[0081] As used herein, the terms "treatment," "treating," and the like refer to obtaining a desired pharmacologic and/or physiologic effect. Preferably, the effect is therapeutic, i.e., the effect partially or completely cures a disease and/or adverse symptom attributable to the disease. To this end, the inventive method comprises administering a "therapeutically effective amount" of the PD-1-binding agent. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. The therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the PD-1-binding agent to elicit a desired response in the individual. For example, a therapeutically effective amount of a PD-1-binding agent of the invention is an amount which decreases PD-1 protein bioactivity in a human and/or enhances the immune response against a cancer or infectious disease.

[0082] Alternatively, the pharmacologic and/or physiologic effect may be prophylactic, i.e., the effect completely or partially prevents a disease or symptom thereof. In this respect, the inventive method comprises administering a "prophylactically effective amount" of the PD-1-binding agent. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired prophylactic result (e.g., prevention of disease onset).

[0083] A typical dose can be, for example, in the range of 1 pg/kg to 20 mg/kg of animal or human body weight; however, doses below or above this exemplary range are within the scope of the invention. The daily parenteral dose can be about 0.00001 μg/kg to about 20 mg/kg of total body weight (e.g., about 0.001 μg /kg, about 0.1 μg /kg , about 1 μg /kg, about 5 μg /kg, about 10 μg/kg, about 100 μg /kg, about 500 μg/kg, about 1 mg/kg, about 5 mg/kg, about 10 mg/kg, or a range defined by any two of the foregoing values), preferably from

about 0.1 μg/kg to about 10 mg/kg of total body weight (e.g., about 0.5 μg/kg, about 1 μg/kg, about 50 μg/kg, about 150 μg/kg, about 300 μg/kg, about 750 μg/kg, about 1.5 mg/kg, about 5 mg/kg, or a range defined by any two of the foregoing values), more preferably from about 1 μg/kg to 5 mg/kg of total body weight (e.g., about 3 μg/kg, about 15 μg/kg, about 75 μg/kg, about 300 μg/kg, about 900 μg/kg, about 2 mg/kg, about 4 mg/kg, or a range defined by any two of the foregoing values), and even more preferably from about 0.5 to 15 mg/kg body weight per day (e.g., about 1 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 6 mg/kg, about 9 mg/kg, about 11 mg/kg, about 13 mg/kg, or a range defined by any two of the foregoing values). Therapeutic or prophylactic efficacy can be monitored by periodic assessment of treated patients. For repeated administrations over several days or longer, depending on the condition, the treatment can be repeated until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful and are within the scope of the invention. The desired dosage can be delivered by a single bolus administration of the composition, by multiple bolus administrations of the composition, or by continuous infusion administration of the composition.

[0084] The composition comprising an effective amount of the inventive immunoglobulin heavy chain polypeptide, the inventive immunoglobulin light chain polypeptide, the inventive PD-1-binding agent, the inventive nucleic acid sequence encoding any of the foregoing, or the inventive vector comprising the inventive nucleic acid sequence can be administered to a mammal using standard administration techniques, including oral, intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository administration. The composition preferably is suitable for parenteral administration. The term "parenteral," as used herein, includes intravenous, intramuscular, subcutaneous, rectal, vaginal, and intraperitoneal administration. More preferably, the composition is administered to a mammal using peripheral systemic delivery by intravenous, intraperitoneal, or subcutaneous injection.

[0085] Once administered to a mammal (e.g., a human), the biological activity of the inventive PD-1-binding agent can be measured by any suitable method known in the art. For example, the biological activity can be assessed by determining the stability of a particular PD-1-binding agent. In one embodiment of the invention, the PD-1-binding agent (e.g., an antibody) has an *in vivo* half life between about 30 minutes and 45 days (e.g., about 30 minutes, about 45 minutes, about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 10 hours, about 12 hours, about 1 day, about 5 days, about 10 days, about 15 days, about 25

days, about 35 days, about 40 days, about 45 days, or a range defined by any two of the foregoing values). In another embodiment, the PD-1-binding agent has an *in vivo* half life between about 2 hours and 20 days (e.g., about 5 hours, about 10 hours, about 15 hours, about 20 hours, about 2 days, about 3 days, about 7 days, about 12 days, about 14 days, about 17 days, about 19 days, or a range defined by any two of the foregoing values). In another embodiment, the PD-1-binding agent has an *in vivo* half life between about 10 days and about 40 days (e.g., about 10 days, about 13 days, about 16 days, about 18 days, about 20 days, about 23 days, about 26 days, about 29 days, about 30 days, about 33 days, about 37 days, about 38 days, about 39 days, about 40 days, or a range defined by any two of the foregoing values).

[0086] The biological activity of a particular PD-1-binding agent also can be assessed by determining its binding affinity to a PD-1 protein or an epitope thereof. The term "affinity" refers to the equilibrium constant for the reversible binding of two agents and is expressed as the dissociation constant (K_D). Affinity of a binding agent to a ligand, such as affinity of an antibody for an epitope, can be, for example, from about 1 picomolar (pM) to about 100 micromolar (μM) (e.g., from about 1 picomolar (pM) to about 1 nanomolar (nM), from about 1 nM to about 1 micromolar (μM), or from about 1 μM to about 100 μM). In one embodiment, the PD-1-binding agent can bind to an PD-1 protein with a K_D less than or equal to 1 nanomolar (e.g., 0.9 nM, 0.8 nM, 0.7 nM, 0.6 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, 0.1 nM, 0.05 nM, 0.025 nM, 0.01 nM, 0.001 nM, or a range defined by any two of the foregoing values). In another embodiment, the PD-1-binding agent can bind to PD-1 with a K_D less than or equal to 200 pM (e.g., 190 pM, 175 pM, 150 pM, 125 pM, 110 pM, 100 pM, 90 pM, 80 pM, 75 pM, 60 pM, 50 pM, 40 pM, 30 pM, 25 pM, 20 pM, 15 pM, 10 pM, 5 pM, 1 pM, or a range defined by any two of the foregoing values). Immunoglobulin affinity for an antigen or epitope of interest can be measured using any art-recognized assay. Such methods include, for example, fluorescence activated cell sorting (FACS), separable beads (e.g., magnetic beads), surface plasmon resonance (SPR), solution phase competition (KINEXA™), antigen panning, and/or ELISA (see, e.g., Janeway et al. (eds.), *Immunobiology*, 5th ed., Garland Publishing, New York, NY, 2001).

[0087] The PD-1-binding agent of the invention may be administered alone or in combination with other drugs (e.g., as an adjuvant). For example, the PD-1-binding agent can be administered in combination with other agents for the treatment or prevention of the diseases disclosed herein. In this respect, the PD-1-binding agent can be used in combination

with at least one other anticancer agent including, for example, any chemotherapeutic agent known in the art, ionization radiation, small molecule anticancer agents, cancer vaccines, biological therapies (e.g., other monoclonal antibodies, cancer-killing viruses, gene therapy, and adoptive T-cell transfer), and/or surgery. When the inventive method treats an infectious disease, the PD-1-binding agent can be administered in combination with at least one anti-bacterial agent or at least one anti-viral agent. In this respect, the anti-bacterial agent can be any suitable antibiotic known in the art. The anti-viral agent can be any vaccine of any suitable type that specifically targets a particular virus (e.g., live-attenuated vaccines, subunit vaccines, recombinant vector vaccines, and small molecule anti-viral therapies (e.g., viral replication inhibitors and nucleoside analogs).

[0088] In another embodiment, the inventive PD-1 binding agent can be administered in combination with other agents that inhibit immune checkpoint pathways. For example, the inventive PD-1 binding agent can be administered in combination with agents that inhibit or antagonize the CTLA-4, TIM-3 or LAG-3 pathways. Combination treatments that simultaneously target two or more of these immune checkpoint pathways have demonstrated improved and potentially synergistic antitumor activity (see, e.g., Sakuishi et al., *J. Exp. Med.*, *207*: 2187-2194 (2010); Ngiow et al., *Cancer Res.*, *71*: 3540-3551 (2011); and Woo et al., *Cancer Res.*, *72*: 917-927 (2012)). In one embodiment, the inventive PD-1 binding agent is administered in combination with an antibody that binds to TIM-3 and/or an antibody that binds to LAG-3. In this respect, the inventive method of treating a cancer or an infectious disease in a mammal can further comprise administering to the mammal a composition comprising (i) an antibody that binds to a TIM-3 protein and (ii) a pharmaceutically acceptable carrier or a composition comprising (i) an antibody that binds to a LAG-3 protein and (ii) a pharmaceutically acceptable carrier.

[0089] In addition to therapeutic uses, the PD-1-binding agent described herein can be used in diagnostic or research applications. In this respect, the PD-1-binding agent can be used in a method to diagnose a cancer or infectious disease. In a similar manner, the PD-1-binding agent can be used in an assay to monitor PD-1 protein levels in a subject being tested for a disease or disorder that is associated with abnormal PD-1 expression. Research applications include, for example, methods that utilize the PD-1-binding agent and a label to detect a PD-1 protein in a sample, e.g., in a human body fluid or in a cell or tissue extract. The PD-1-binding agent can be used with or without modification, such as covalent or non-covalent labeling with a detectable moiety. For example, the detectable moiety can be a

radioisotope (e.g., ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I), a fluorescent or chemiluminescent compound (e.g., fluorescein isothiocyanate, rhodamine, or luciferin), an enzyme (e.g., alkaline phosphatase, beta-galactosidase, or horseradish peroxidase), or prosthetic groups. Any method known in the art for separately conjugating an antigen-binding agent (e.g., an antibody) to a detectable moiety may be employed in the context of the invention (see, e.g., Hunter et al., *Nature*, *194*: 495-496 (1962); David et al., *Biochemistry*, *13*: 1014-1021 (1974); Pain et al., *J. Immunol. Meth.*, *40*: 219-230 (1981); and Nygren, *J. Histochem. and Cytochem.*, *30*: 407-412 (1982)).

[0090] PD-1 protein levels can be measured using the inventive PD-1-binding agent by any suitable method known in the art. Such methods include, for example, radioimmunoassay (RIA), and FACS. Normal or standard expression values of PD-1 protein can be established using any suitable technique, e.g., by combining a sample comprising, or suspected of comprising, a PD-1 polypeptide with a PD-1-specific antibody under conditions suitable to form an antigen-antibody complex. The antibody is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, and radioactive materials (see, e.g., Zola, *Monoclonal Antibodies: A Manual of Techniques*, CRC Press, Inc. (1987)). The amount of PD-1 polypeptide expressed in a sample is then compared with a standard value.

[0091] The PD-1-binding agent can be provided in a kit, i.e., a packaged combination of reagents in predetermined amounts with instructions for performing a diagnostic assay. If the PD-1-binding agent is labeled with an enzyme, the kit desirably includes substrates and cofactors required by the enzyme (e.g., a substrate precursor which provides a detectable chromophore or fluorophore). In addition, other additives may be included in the kit, such as stabilizers, buffers (e.g., a blocking buffer or lysis buffer), and the like. The relative amounts of the various reagents can be varied to provide for concentrations in solution of the reagents which substantially optimize the sensitivity of the assay. The reagents may be provided as dry powders (typically lyophilized), including excipients which on dissolution will provide a reagent solution having the appropriate concentration.

[0092] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

[0093] This example demonstrates a method of generating monoclonal antibodies directed against human PD-1.

[0094] Several forms of genes encoding human PD-1 and its ligands PD-L1 and PD-L2 were generated as antigens for use in mouse immunization, hybridoma screening, and affinity maturation of CDR-grafted antibodies, and are schematically depicted in Figure 1. Full-length human and cynomolgus monkey PD-1 genes were expressed with their native leader sequence and no added tags using a ubiquitous chromatin opening element (UCOE) single expression vector with hygromycin selection (Millipore, Billerica, MA). CHO-K1 cells were stably transfected with Lipofectamine LTX (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. Following selection with hygromycin, cells expressing PD-1 on the cell surface were identified by flow cytometry using a PE-conjugated mouse antibody to human PD-1 (BD Bioscience, Franklin Lakes, NJ) and subcloned. Subclones were then selected for high-level and uniform PD-1 expression.

[0095] Nucleic acid sequences encoding soluble monomeric forms of the extracellular domain (ECD) of human and cynomolgus monkey PD-1 were constructed with His tags appended to the C-terminus of the ECD or as soluble dimeric fusion proteins with mouse IgG2a Fc as indicated in Figure 1. Nucleic acid sequences encoding soluble dimeric forms of the ECDs of human PD-L1 and PD-L2 were constructed as fusion proteins with mouse IgG1 Fc as indicated in Figure 1. Soluble proteins were expressed transiently in HEK 293 cells or in stable CHO cell lines using standard techniques. His-tagged proteins were purified from cell culture supernatant via Ni-affinity column chromatography followed by size exclusion chromatography. IgG-Fc fusion proteins were purified using protein A/G affinity chromatography. Purified proteins were analyzed by SDS-PAGE and size-exclusion chromatography to ensure homogeneity. Additionally, identity and size were confirmed by mass spectrometry.

[0096] For FACS sorting experiments, purified proteins were labeled with biotin using an NHS ester crosslinker (Thermo-Fisher Scientific, Inc., Waltham, MA) or the fluorescent dye DyLight 650 (Thermo-Fisher Scientific, Inc., Waltham, MA) using standard techniques.

[0097] Mice were immunized with either CHO cells expressing full-length PD-1 on the cell surface or the PD-1 ECD His protein. Specifically, female BALB/c mice (7 weeks old) were purchased from Harlan Laboratories, Inc. (Indianapolis, IN) and divided into two groups. After six days of acclimatization, one group of animals was immunized with four

weekly doses of purified human PD-1 ECD-His at 50 μg/mouse, as a 1:1 emulsion with TITERMAX GOLD™ (Sigma Aldrich, St. Louis, MO). Immunization was carried out subcutaneously around the armpits and inguinal regions. The second group of animals was injected with four weekly doses of CHO-K1 cells stably expressing full length human PD-1 (5×10^6 cells/mouse) subcutaneously around the inguinal regions. After ten days, animals were bled for measurement of the serum titer to PD-1, and one animal from each group was boosted with soluble human PD-1 after a 3-week rest. After three days, spleens, axillary/brachial lymph nodes, and inguinal lymph nodes were collected from each animal. Single cell suspensions of cells from all tissues collected from both animals were pooled and used for generation of hybridomas by cell fusion using standard techniques. Two different myeloma cell lines were used for fusion, F0 (as described in de St. Groth and Scheidegger, *J. Immunol. Methods*, *35*: 1-21 (1980)) and P3X63Ag8.653 (as described in Kearney et al., *J. Immunol.*, *123*: 1548-1550 (1979)).

[0098] Hybridoma supernatants from ten 96-well plates were screened for binding to a CHO-K1 cell clone stably transfected with a nucleic acid sequence encoding full length human PD-1 and compared to binding to untransfected CHO-K1 cells. Specifically, hybridoma supernatants were diluted 1:1 with PBS/2% FBS and incubated with an equal volume of PD-1 CHO-K1 cells (2.5×10^5 cells in PBS, 2% FBS) for 30 minutes at 4 °C. Cells were centrifuged, washed once with PBS/1% FBS, and incubated with APC-conjugated goat anti-mouse IgG (H+L) (Southern Biotechnology, Birmingham, AL) for 30 minutes at 4 °C. Cells were washed twice in PBS/2% FBS, resuspended in PBS, 2% FBS, 1% paraformaldehyde, and fluorescence analyzed on a BD FACSARRAY™ Bioanalyzer (BD Biosciences, Franklin Lakes, NJ). Mouse IgG levels were quantified by ELISA.

[0099] Based on strong binding to PD-1 CHO cells, 46 parental wells were expanded, and the supernatants were tested for the ability to block binding of DyL650-labeled PD-L1-mIgG1 Fc fusion protein to PD-1 CHO cells. Specifically, purified mouse monoclonal antibodies were incubated in a dose response with the E_{C30} concentration of PD-L1-DyL650 (10 nM), and inhibition was quantified by flow cytometry. Cells from wells showing the best PD-L1 blocking activity and highest levels of mouse IgG were subcloned for further analysis, including purification and heavy and light chain (V_H and V_L) sequencing. Eleven of the strongest blockers of PD-1/PD-L1 interaction were selected for subcloning. Following re-confirmation of PD-1 binding and PD-L1 blocking, selected subclones were scaled up, and supernatant was submitted for antibody purification. Purified antibodies were verified for

binding to both human and cynomolgus monkey PD-1 and for PD-L1 blocking activity. K_D values were determined by surface plasmon resonance on a BIACORE™ T200 instrument (GE Healthcare, Waukesha, WI), and kinetic constants were determined using the BIACORE ™ T200 evaluation software (GE Healthcare, Waukesha, WI). In this respect, antibodies were captured on a BIACORE ™ CM5 chip to which GE anti-mouse IgG was coupled. PD-1-His monomer was flowed over the captured antibody using two- or three-fold serial dilutions beginning with 500 nM at the highest concentration. The resulting sensorgrams were fit globally using a 1:1 binding model to calculate on- and off-rates and the subsequent affinities (K_D).

[0100] The results of this example demonstrate a method of producing monoclonal antibodies that bind to human and cynomolgus monkey PD-1 and block PD-1 ligand binding.

EXAMPLE 2

[0101] This example describes the design and generation of CDR-grafted and chimeric anti-PD-1 monoclonal antibodies.

[0102] Subclones of the hybridomas which produced PD-1-binding antibodies with PD-L1 blocking activity as described in Example 1 were isotyped, subjected to RT-PCR for cloning the antibody heavy chain variable region (V_H) and light chain variable region (V_L), and sequenced. Specifically, RNA was isolated from cell pellets of hybridoma clones (5 x 10^5 cells/pellet) using the RNEASY™ kit (Qiagen, Venlo, Netherlands), and cDNA was prepared using oligo-dT-primed SUPERSCRIPT™ III First-Strand Synthesis System (Life Technologies, Carlsbad, CA). PCR amplification of the V_L utilized a pool of 9 or 11 degenerate mouse V_L forward primers (see Kontermann and Dubel, eds., *Antibody Engineering*, Springer-Verlag, Berlin (2001)) and a mouse κ constant region reverse primer. PCR amplification of the V_H utilized a pool of 12 degenerate mouse V_H forward primers (Kontermann and Dubel, *supra*) and a mouse γ1 or γ2a constant region reverse primer (based on isotyping of purified antibody from each clone) with the protocol recommended in the SUPERSCRIPT™ III First-Stand Synthesis System (Life Technologies, Carlsbad, CA). PCR products were purified and cloned into pcDNA3.3-TOPO (Life Technologies, Carlsbad, CA). Individual colonies from each cell pellet (24 heavy chains and 48 light chains) were selected and sequenced using standard Sanger sequencing methodology (Genewiz, Inc., South Plainfield, NJ). Variable region sequences were examined and aligned with the closest human heavy chain or light chain V-region germline sequence. Three antibodies were

selected for CDR-grafting: (1) 9A2, comprising a V_H of SEQ ID NO: 4 and a V_L of SEQ ID NO: 28, (2) 10B11, comprising a V_H of SEQ ID NO: 15 and a V_L of SEQ ID NO: 32, and (3) 6E9, comprising a V_H of SEQ ID NO: 22 and a V_L of SEQ ID NO: 38.

[0103] CDR-grafted antibody sequences were designed by grafting CDR residues from each of the above-described mouse antibodies into the closest human germline homologue. CDR-grafted antibody variable regions were synthesized and expressed with human IgG1/κ constant regions for analysis. In addition, mouse:human chimeric antibodies were constructed using the variable regions of the above-described mouse antibodies linked to human IgG1/κ constant regions. Chimeric and CDR-grafted antibodies were characterized for binding to human and cynomolgus monkey PD-1 antigens and for activity in the PD-1/PD-L1 blocking assay as described above.

[0104] The functional antagonist activity of chimeric and CDR-grafted antibodies also was tested in a human $CD4^+$ T-cell mixed lymphocyte reaction (MLR) assay in which activation of CD4+ T-cells in the presence of anti-PD-1 antibodies is assessed by measuring IL-2 secretion. Because PD-1 is a negative regulator of T-cell function, antagonism of PD-1 was expected to result in increased T-cell activation as measured by increased IL-2 production. The 9A2, 10B11, and 6E9 CDR-grafted antibodies demonstrated antagonistic activity and were selected for affinity maturation.

[0105] The results of this example demonstrate a method of generating chimeric and CDR-grafted monoclonal antibodies that specifically bind to and inhibit PD-1.

EXAMPLE 3

[0106] This example demonstrates affinity maturation of monoclonal antibodies directed against PD-1.

[0107] CDR-grafted antibodies derived from the original murine monoclonal antibodies, (9A2, 10B11, and 6E9) were subjected to affinity maturation via *in vitro* somatic hypermutation. Each antibody was displayed on the surface of HEK 293c18 cells using the SHM-XEL deciduous system (see Bowers et al., *Proc. Natl. Acad. Sci. USA, 108*: 20455-20460 (2011); and U.S. Patent Application Publication No. 2013/0035472). After establishment of stable episomal lines, a vector for expression of activation-induced cytosine deaminase (AID) was transfected into the cells to initiate somatic hypermutation as described in Bowers et al., *supra*. After multiple rounds of FACS sorting under conditions of increasing antigen binding stringency, a number of mutations in the variable region of each

antibody were identified and recombined to produce mature humanized antibodies with improved properties.

[0108] A panel of six affinity-matured humanized heavy and light chain variable region sequences were paired (denoted APE1922, APE1923, APE1924, APE1950, APE1963 and APE2058) and selected for characterization, and are set forth in Table 1. The PD-1 binding properties of each of these antibody sequences were assayed using surface plasmon resonance (SPR) and solution-based affinity analysis. Antibodies were expressed from HEK 293 cells as human IgG1 antibodies and compared to the reference antibody, a human IgG1 version of BMS-936558, designated BMS.

[0109] SPR analyses were carried out using a BIACORE™ T200 instrument, and kinetic constants were determined using the BIACORE™ T200 evaluation software. Experimental parameters were chosen to ensure that saturation would be reached at the highest antigen concentrations and that R_{max} values would be kept under 30 RU. GE anti-Human IgG (Fc-specific, approximately 7,000 RU) was immobilized on a BIACORE™ CM5 chip using EDC-activated amine coupling chemistry. Antibodies (0.5 µg/mL, 60 second capture time) were then captured using this surface. Next, monomeric soluble human PD1-Avi-His was flowed over captured antibody (300 second association, 300 second dissociation) using a three-fold serial dilution series from 500 nM to 2 nM. Captured antibody and antigen were removed between each cycle using 3 M $MgCl_2$ (60 second contact time) in order to ensure a fresh binding surface for each concentration of antigen. The resulting sensorgrams were fit globally using a 1:1 binding model in order to calculate on- and off-rates (k_a and k_d, respectively), as well as affinities (K_D).

[0110] Solution-based affinity analyses were carried out using a KINEXA™ 3000 assay (Sapidyne Instruments, Boise, Idaho), and results were analyzed using KINEXA™ Pro Software 3.2.6. Experimental parameters were selected to reach a maximum signal with antibody alone between 0.8 and 1.2 V, while limiting nonspecific binding signal with buffer alone to less than 10% of the maximum signal. Azlactone beads (50 mg) were coated with antigen by diluting in a solution of PD-1-Avi-His (50 µg/mL in 1 mL) in 50 mM Na_2CO_3. The solution was rotated at room temperature for 2 hours, and beads were pelleted in a picofuge and washed twice with blocking solution (10 mg/mL BSA, 1 M Tris-HCl, pH 8.0). Beads were resuspended in blocking solution (1 mL), rotated at room temperature for 1 hour, and diluted in 25 volumes PBS/0.02% NaN_3. For affinity measurement, the secondary antibody was ALEXFLUOR™ 647 dye-anti-human IgG (500 ng/mL). Sample antibody

concentrations were held constant (50 pM or 75 pM), while antigen PD1-Avi-His was titrated using a three-fold dilutions series from 1 μM to 17 pM. All samples were diluted in PBS, 0.2% NaN_3, 1 mg/mL BSA and allowed to equilibrate at room temperature for 30 hours. Additionally, samples containing only antibody and only buffer were tested in order to determine maximum signal and nonspecific binding signal, respectively. The results of the affinity analyses are set forth in Table 1. All of the selected antibodies exhibited higher affinities for PD-1 than the BMS reference antibody, with the highest affinity antibody being APE2058.

Table 1

Antibody	V_H SEQ ID NO:	V_L SEQ ID NO:	BIACORE™ k_a $(Ms)^{-1}$	BIACORE™ k_d (s^{-1})	BIACORE™ K_D (nM)	KINEXA™ K_D (nM)
BMS	n/a	n/a	8.8×10^{4}	2.1×10^{-3}	23	2
APE1922	6	29	1.3×10^{5}	1.8×10^{-3}	15	-
APE1923	7	29	1.9×10^{5}	1.7×10^{-3}	9	1
APE1924	8	29	1.8×10^{5}	1.8×10^{-3}	10	-1
APE1950	9	29	1.5×10^{5}	2.5×10^{-3}	17	-
APE1963	10	29	5.8×10^{4}	1.0×10^{-3}	17	-
APE2058	23	40	3.0×10^{5}	6.4×10^{-4}	2	0.2

[0111] To assess binding of the antibodies to cell surface PD-1, binding to CHO cells expressing either human or cynomolgus monkey PD-1 was determined by flow cytometry analysis as described above. In addition, blocking of the PD-1/PD-L1 interaction was assessed using DyL650 labeled PD-L1 (mouse IgG1 Fc fusion protein) and PD-1-expressing CHO cells as described above. High binding affinities for cell-surface PD-1 were observed for all tested affinity-matured antibody sequences, with reactivity to cynomolgus monkey PD-1 within a factor of 3-4 fold of human. Blocking of the PD-1/PD-L1 interaction was also efficient with all of the tested affinity-matured antibody sequences, with IC_{50} values in the low nM range. These results were consistent with binding affinities assayed both by the BIACORE™ and KINEXA™ systems as well as cell surface EC_{50} values.

[0112] Thermal stability of the selected antibodies was assessed using a Thermofluor assay as described in McConnell et al., *Protein Eng. Des. Sel.*, *26*: 151 (2013). This assay assesses stability through the ability of a hydrophobic fluorescent dye to bind to hydrophobic patches on the protein surface which are exposed as the protein unfolds. The temperature at which 50% of the protein unfolds is determined (Tm) to measure thermal stability. This assay demonstrated that all of the tested affinity-matured antibody sequences had high

thermal stability, and all were more stable than the reference antibody. APE2058 was the most stable antibody, exhibiting a Tm more than 10 °C greater than the Tm of the IgG1 version of BMS-936558.

[0113] De-risking of potential issues related to *in vivo* pharmokinetics of the tested antibodies was undertaken through (a) assessment of non-specific binding to target negative cells (see, e.g., Hotzel et al., *mAbs*, *4*: 753-760 (2012)) and (b) measurement of differential neonatal Fc receptor (FcRn) dissociation properties (see, e.g., Wang et al., *Drug Metab. Disp.*, *39*: 1469-1477 (2011)). To assess non-specific binding, antibodies were tested for binding to HEK 293f cells using a flow cytometry-based assay. The tested antibodies were compared to two FDA-approved antibodies, infliximab and denosumab. The results indicated that non-specific binding was low for all of the antibodies. To assess FcRn binding and dissociation, both human FcRn and cynomolgus FcRn were tested in a BIACORE™-based assay. Antibodies were bound to FcRn at pH 6.0, and after pH adjustment to 7.4, residual bound antibody was determined. The results of this assay are shown in Table 2.

Table 2

Antibody	**% Residual Binding at pH 7.4**	
	Human FcRn	**Cyno FcRn**
BMS	2.0	1.7
APE1922	2.7	2.9
APE1923	4.0	5.0
APE1924	3.6	4.0
APE1950	34.0	36.5
APE1963	9.0	11.9
APE2058	2.1	2.0

[0114] The results of this example demonstrate a method of generating the inventive immunoglobulin heavy and light chain polypeptides, which exhibit thermostability and high affinity for PD-1.

EXAMPLE 4

[0115] This example demonstrates the activity of the inventive immunoglobulin heavy and light chain polypeptides *in vitro*.

[0116] Functional antagonist activity of the V_H and V_L sequences described in Example 3 was tested in a human $CD4^+$ T-cell MLR assay as described above. For determination of

functional potency, the EC_{50} for each antibody was determined in five separate experiments using different human donors. The results are shown in Table 3 and demonstrate potent activity for each of the selected antibodies, which was indistinguishable from the activity of the reference antibody.

Table 3

EC_{50} Values (μg/mL)						
BMS Reference	**APE2058**	**APE1922**	**APE1923**	**APE1924**	**APE1950**	**APE1963**
0.01	0.01	0.01	0.01	0.01	0.01	0.01
0.03	0.10	0.10	0.2	0.03	0.20	0.10
0.02	0.04	0.03	0.01	0.04	0.02	0.02
0.01	0.01	0.01	0.02	0.01	0.01	0.07
0.01	0.01	0.01	0.01	0.01	0.01	0.01
Each line represents an independent experiment using different human donors for the responder $CD4^+$ T cells. Shaded line with one responder produced higher IL-2 levels in the presence of the affinity-matured mAbs than in the other experiments, artificially raising the EC_{50} values.						

[0117] The results of this example demonstrate that the inventive immunoglobulin heavy and light chain polypeptides can antagonize PD-1 signaling, resulting in increased T-cell activation.

EXAMPLE 5

[0118] This example demonstrates that a combination of the inventive PD-1 binding agent and either an anti-LAG-3 antibody or an anti-TIM-3 antibody enhances T-cell activation *in vitro*.

[0119] To establish parameters for combination studies, the anti-PD-1 antibody APE2058 was titrated in a dose response in the human CD4+ T-cell MLR assay described above. Antagonism of PD-1 signaling resulted in increased T-cell activation and a corresponding 4- to 5-fold increase in the production of IL-2.

[0120] Based on the results from titrating the APE2058 antibody in multiple MLR assays, an EC_{50} value of 20 ng/mL and a concentration 10-fold lower that represents an approximate EC_{10} value (2 ng/mL) were selected for combination studies with antagonist antibodies to the TIM-3 or LAG-3 checkpoint molecules.

[0121] A fully human anti-TIM-3 antibody was characterized in a CD4+ T cell *in vitro* assay as having antagonist activity as measured by increased IL-2 production in the presence of low levels of anti-CD3 and anti-CD28 antibodies. The anti-TIM-3 antibody demonstrated activity in the MLR assay with an EC_{50} value of approximately 0.3 μg/mL, as shown in Figure 2 and Table 4, which is approximately 15-fold less activity than the anti-PD-1 APE2058 antibody alone (EC_{50} approximately 0.02 μg/mL). In combination with 0.02 μg/mL of APE2058, the anti-TIM-3 antagonist antibody stimulated increased amounts of IL-2 production as compared to APE2058 or anti-TIM-3 alone, resulting in a 10-fold decrease in the EC_{50} values, as shown in Figure 2 and Table 4. These results demonstrate that enhanced T-cell activation occurs with combination inhibition of the PD-1 and TIM-3 checkpoint pathways.

[0122] A fully human antagonist anti-LAG-3 antibody (described in U.S. Patent Application Publication 2011/0150892) has demonstrated potent activity in blocking binding of recombinant soluble LAG-3 to MHC Class II positive cells. This antibody, designated herein as APE03109, was evaluated for functional activity in the human $CD4^+$ T-cell MLR assay. APE03109 demonstrated activity in the MLR with an EC_{50} value of approximately 0.05 μg/mL, as shown in Figure 3 and Table 4, which was similar to the activity of the anti-PD-1 antibody alone. In combination with 0.02 μg/mL of the anti-PD-1 APE2058 antibody, the APE03109 antibody stimulated increased amounts of IL-2 production over APE2058 or APE03109 alone, resulting in a 5-fold decrease in the EC_{50} values.

[0123] A time course of IL-2 production with the anti-LAG-3 APE03109 antibody alone and the combination of APE2058 with APE03109 also was characterized in a human $CD4^+$ MLR assay. A similar decrease in EC_{50} value for the combination of 0.02 μg/mL APE2058 and APE03109 was observed after 72 hours of culture, as shown in Figure 3. After 96 hours of culture the differences in EC_{50} values were not as pronounced; however, the levels of IL-2 produced in the cultures treated with 0.02 μg/mL of the anti-PD-1 APE2058 antibody and the anti-LAG-3 APE03109 antibody almost doubled as compared to cultures treated with APE03109 alone (2,200 pg/mL versus 1,200 pg/mL). Consistent with the time course of LAG-3 expression, no increased IL-2 production from adding APE03109 to APE2058 was observed after 24 hours, although APE2058 alone produced a dose responsive increase in IL-2 production at this time. In separate MLR experiments it was also demonstrated that the combination of APE2058 and APE03109 enhanced the levels of production of the T-cell cytokine IFN-γ by over 50% after 48 hours.

[0124] To demonstrate that the combined effects of the anti-TIM-3 antibody or the anti-LAG-3 antibody in the $CD4^+$ T-cell MLR were due to target specificity, an irrelevant human IgG1 antibody, APE0422, was tested in combination with 0.02 μg/mL anti-PD-1 antibody APE2058. At the highest concentration tested (30 μg/mL), the APE0422 antibody exhibited no effect on IL-2 production over anti-PD-1 alone.

Table 4

Antibody	MLR Assay EC_{50} Single agent	MLR Assay EC_{50} with 2 ng/mL anti-PD-1	MLR Assay EC_{50} with 20 ng/mL anti-PD-1	Fold Improvement
Anti-TIM-3	330 ng/mL	310 ng/mL	33 ng/mL	10
Anti-LAG-3	53 ng/mL	44 ng/mL	11 ng/mL	4.8

[0125] The results of this example demonstrate that the inventive PD-1-binding agent combined with antagonistic antibodies directed against TIM-3 or LAG-3 enhances $CD4^+$ T-cell activation *in vitro*.

[0126] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0127] The use of the terms "a" and "an" and "the" and "at least one" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The use of the term "at least one" followed by a list of one or more items (for example, "at least one of A and B") is to be construed to mean one item selected from the listed items (A or B) or any combination of two or more of the listed items (A and B), unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly

contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0128] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIMS:

1. An isolated immunoglobulin heavy chain polypeptide which comprises a complementarity determining region 1 (CDR) amino acid sequence of SEQ ID NO: 1, a CDR2 amino acid sequence of SEQ ID NO: 2, and a CDR3 amino acid sequence of SEQ ID NO: 3, wherein optionally

(a) residue 9 of SEQ ID NO: 1 is replaced with a different amino acid residue,

(b) one or more of residues 7, 8, and 9 of SEQ ID NO: 2 is replaced with a different amino acid residue,

(c) one or more of residues 1, 2, and 5 of SEQ ID NO: 3 is replaced with a different amino acid residue, or

(d) any combination of (a)-(c).

2. The isolated immunoglobulin heavy chain polypeptide of claim 1, which comprises a CDR1 amino acid sequence of SEQ ID NO: 1, a CDR2 amino acid sequence of SEQ ID NO: 2, and a CDR3 amino acid sequence of SEQ ID NO: 3, except that residue 9 of SEQ ID NO: 1 is replaced with a different amino acid residue.

3. The isolated immunoglobulin heavy chain polypeptide of claim 2, wherein residue 9 of SEQ ID NO: 1 is replaced with a methionine (M) residue.

4. The isolated immunoglobulin heavy chain polypeptide of any one of claims 1-3, which comprises a CDR1 amino acid sequence of SEQ ID NO: 1, a CDR2 amino acid sequence of SEQ ID NO: 2, and a CDR3 amino acid sequence of SEQ ID NO: 3, except that one or more of resides 7, 8, and 9 of SEQ ID NO: 2 is replaced with a different amino acid residue.

5. The isolated immunoglobulin heavy chain polypeptide of claim 4, wherein

(a) residue 7 of SEQ ID NO: 2 is replaced with an asparagine (N) residue,

(b) residue 8 of SEQ ID NO: 2 is replaced with a serine (S) residue,

(c) residue 9 of SEQ ID NO: 2 is replaced with a threonine (T) residue, or

(d) any combination of (a)-(c).

6. The isolated immunoglobulin heavy chain polypeptide of any one of claims 1-5, which comprises a CDR1 amino acid sequence of SEQ ID NO: 1, a CDR2 amino acid sequence of SEQ ID NO: 2, and a CDR3 amino acid sequence of SEQ ID NO: 3, except that one or more of residues 1, 2, and 5 of SEQ ID NO: 3 is replaced with a different amino acid residue.

7. The isolated immunoglobulin heavy chain polypeptide of claim 6, wherein

(a) residue 1 of SEQ ID NO: 3 is replaced with a glutamic acid (E) residue,

(b) residue 2 of SEQ ID NO: 3 is replaced with a tyrosine (Y) residue,

(c) residue 5 of SEQ ID NO: 3 is replaced with a serine (S) residue, or

(d) any combination of (a)-(c).

8. The isolated immunoglobulin heavy chain polypeptide of any one of claims 1-7, which comprises an amino acid sequence of any one of SEQ ID NOs: 4-11.

9. An isolated immunoglobulin heavy chain polypeptide which comprises a complementarity determining region 1 (CDR) amino acid sequence of SEQ ID NO: 12, a CDR2 amino acid sequence of SEQ ID NO: 13, and a CDR3 amino acid sequence of SEQ ID NO: 14, wherein optionally

(a) residue 9 of SEQ ID NO: 12 is replaced with a different amino acid residue,

(b) residue 8 and/or residue 9 of SEQ ID NO: 13 is replaced with a different amino acid residue,

(c) residue 5 of SEQ ID NO: 14 is replaced with a different amino acid residue, or

(d) any combination of (a)-(c).

10. The isolated immunoglobulin heavy chain polypeptide of claim 9, which comprises a CDR1 amino acid sequence of SEQ ID NO: 12, a CDR2 amino acid sequence of SEQ ID NO: 13, and a CDR3 amino acid sequence of SEQ ID NO:14, except that residue 9 of SEQ ID NO: 12 is replaced with a different amino acid residue.

11. The isolated immunoglobulin heavy chain polypeptide of claim 10, wherein residue 9 of SEQ ID NO: 12 is replaced with a leucine (L) residue.

12. The isolated immunoglobulin heavy chain polypeptide of any one of claims 9-11, which comprises a CDR1 amino acid sequence of SEQ ID NO: 12, a CDR2 amino acid sequence of SEQ ID NO: 13, and a CDR3 amino acid sequence of SEQ ID NO: 14, except that residue 8 and/or residue 9 of SEQ ID NO: 13 is replaced with a different amino residue.

13. The isolated immunoglobulin heavy chain polypeptide of claim 12, wherein

(a) residue 8 of SEQ ID NO: 13 is replaced with a tyrosine (Y) residue, and/or

(b) residue 9 of SEQ ID NO: 13 is replaced with an alanine (A) residue.

14. The isolated immunoglobulin heavy chain polypeptide of any one of claims 9-13, which comprises a CDR1 amino acid sequence of SEQ ID NO: 12, a CDR2 amino acid sequence of SEQ ID NO: 13, and a CDR3 amino acid sequence of SEQ ID NO: 14, except that residue 5 of SEQ ID NO: 3 is replaced with a different residue.

15. The isolated immunoglobulin heavy chain polypeptide of claim 15, wherein residue 5 of SEQ ID NO: 14 is replaced with a threonine (T) residue.

16. The isolated immunoglobulin heavy chain polypeptide of any one of claims 1-7, which comprises an amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, or SEQ ID NO: 18.

17. An isolated immunoglobulin heavy chain polypeptide which comprises a complementarity determining region 1 (CDR) amino acid sequence of SEQ ID NO: 19, a CDR2 amino acid sequence of SEQ ID NO: 20, and a CDR3 amino acid sequence of SEQ ID NO: 21.

18. The isolated immunoglobulin heavy chain polypeptide of claim 17, which comprises an amino acid sequence of SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, or SEQ ID NO: 25.

19. An isolated immunoglobulin heavy chain polypeptide which comprises an amino acid sequence that is at least 90% identical to any one of SEQ ID NOs: 4-11, SEQ ID NOs: 15-18, and SEQ ID NOs: 22-25.

20. An isolated immunoglobulin light chain polypeptide which comprises a complementarity determining region 1 (CDR) amino acid sequence of SEQ ID NO: 26 and a CDR2 amino acid sequence of SEQ ID NO: 27.

21. The isolated immunoglobulin light chain polypeptide of claim 20, which comprises an amino acid sequence of SEQ ID NO: 28 or SEQ ID NO: 29.

22. An isolated immunoglobulin light chain polypeptide which comprises a complementarity determining region 1 (CDR) amino acid sequence of SEQ ID NO: 30 and a CDR2 amino acid sequence of SEQ ID NO: 31, wherein optionally residue 12 of SEQ ID NO: 30 is replaced with a different amino acid residue.

23. The isolated immunoglobulin light chain polypeptide of claim 22, wherein residue 12 of SEQ ID NO: 30 is replaced with a different amino acid residue.

24. The isolated immunoglobulin light chain polypeptide of claim 23, wherein residue 12 of SEQ ID NO: 30 is replaced with a threonine (T) residue.

25. The isolated immunoglobulin light chain polypeptide of any one of claims 22-24, which comprises the amino acid sequence of SEQ ID NO: 32, SEQ ID NO: 33, or SEQ ID NO: 34.

26. An isolated immunoglobulin light chain polypeptide which comprises a complementarity determining region 1 (CDR) amino acid sequence of SEQ ID NO: 35, a CDR2 amino acid sequence of SEQ ID NO: 36, and a CDR3 amino acid sequence of SEQ ID NO: 37, wherein optionally

(a) residue 5 of SEQ ID NO: 36 is replaced with a different amino acid residue, and/or

(b) residue 4 of SEQ ID NO: 37 is replaced with a different amino acid residue.

27. The isolated immunoglobulin light chain polypeptide of claim 26, which comprises a CDR1 amino acid sequence of SEQ ID NO: 35, a CDR2 amino acid sequence of SEQ ID NO: 36, and a CDR3 amino acid sequence of SEQ ID NO: 37, except that residue 5 of SEQ ID NO: 36 is replaced with a different amino acid residue.

28. The isolated immunoglobulin light chain polypeptide of claim 27, wherein residue 5 of SEQ ID NO: 36 is replaced with a leucine (L) residue.

29. The isolated immunoglobulin light chain polypeptide of any one of claims 26-28, which comprises a CDR1 amino acid sequence of SEQ ID NO: 35, a CDR2 amino acid sequence of SEQ ID NO: 36, and a CDR3 amino acid sequence of SEQ ID NO: 37, except that residue 4 of SEQ ID NO: 37 is replaced with a different amino acid residue.

30. The isolated immunoglobulin light chain polypeptide of claim 29, wherein residue 4 of SEQ ID NO: 37 is replaced with an asparagine (N) residue.

31. The isolated immunoglobulin light chain polypeptide of any one of claims 26-30, which comprises an amino acid sequence of SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, or SEQ ID NO: 41.

32. An isolated immunoglobulin light chain polypeptide which comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, or SEQ ID NO: 41.

33. An isolated or purified nucleic acid sequence encoding the isolated immunoglobulin heavy chain polypeptide of any one of claims 1-19.

34. An isolated or purified nucleic acid sequence encoding the isolated immunoglobulin light chain polypeptide of any one of claims 20-32.

35. A vector comprising the isolated or purified nucleic acid molecule of claim 33 or claim 34.

36. An isolated programmed death-1 protein (PD-1)-binding agent comprising (a) the immunoglobulin heavy chain polypeptide of any one of claims 1-19 and (b) the immunoglobulin light chain polypeptide of any one of claims 20-32.

37. The isolated PD-1-binding agent of claim 36, which is an antibody, an antibody conjugate, or an antigen-binding fragment thereof.

38. The isolated PD-1-binding agent of claim 36 or claim 37, which is an antibody fragment selected from $F(ab')_2$, Fab', Fab, Fv, scFv, dsFv, dAb, and a single chain binding polypeptide.

39. An isolated or purified nucleic acid sequence encoding the PD-1-binding agent of any one of claims 36-38.

40. A vector comprising the isolated or purified nucleic acid sequence of claim 39.

41. An isolated cell comprising the vector of claim 40.

42. A composition comprising the isolated PD-1-binding agent of any one of claims 36-38 or the vector of claim 40 and a pharmaceutically acceptable carrier.

43. A method of treating a cancer or an infectious disease in a mammal, which method comprises administering an effective amount of the composition of claim 42 to a mammal having a cancer or an infectious disease, whereupon the cancer or infectious disease is treated in the mammal.

44. The method of claim 43, wherein the composition is administered to a mammal having a cancer.

45. The method of claim 44, wherein the cancer is melanoma, renal cell carcinoma, lung cancer, bladder cancer, breast cancer, cervical cancer, colon cancer, gall bladder cancer, laryngeal cancer, liver cancer, thyroid cancer, stomach cancer, salivary gland cancer, prostate cancer, pancreatic cancer, or Merkel cell carcinoma.

46. The method of claim 43, wherein the composition is administered to a mammal having an infectious disease.

47. The method of claim 46, wherein the infectious disease is caused by a virus or a bacterium.

48. The method of claim 47, wherein the virus is human immunodeficiency virus (HIV), respiratory syncytial virus (RSV), influenza virus, dengue virus, or hepatitis B virus (HBV).

49. The method of any one of claims 43-48, wherein the half-life of the PD-1-binding agent in the mammal is between 30 minutes and 45 days.

50. The method of any one of claims 43-49, wherein the PD-1-binding agent binds to PD-1 with a K_D between about 1 picomolar (pM) and about 100 micromolar (μM).

51. The method of any one of claims 43-50, further comprising administering to the mammal a composition comprising (i) an antibody that binds to a TIM-3 protein and (ii) a pharmaceutically acceptable carrier.

52. The method of any one of claims 43-50, further comprising administering to the mammal a composition comprising (i) an antibody that binds to a LAG-3 protein and (ii) a pharmaceutically acceptable carrier.

1. Full-length human PD-1, expressed on the surface on CHO cells
2. Human PD-1 extracellular domain with C-terminal tags G/S-avi-$(His)_6$
3. Human PD-1 extracellular domain - Mouse IgG2a Fc
4. Human PD-L1 extracellular domain - mouse IgG1 Fc
5. Human PD-L2 extracellular domain - mouse IgG1 Fc
6. Full-length cynomolgus monkey PD-1 expressed on the surface of CHO cells

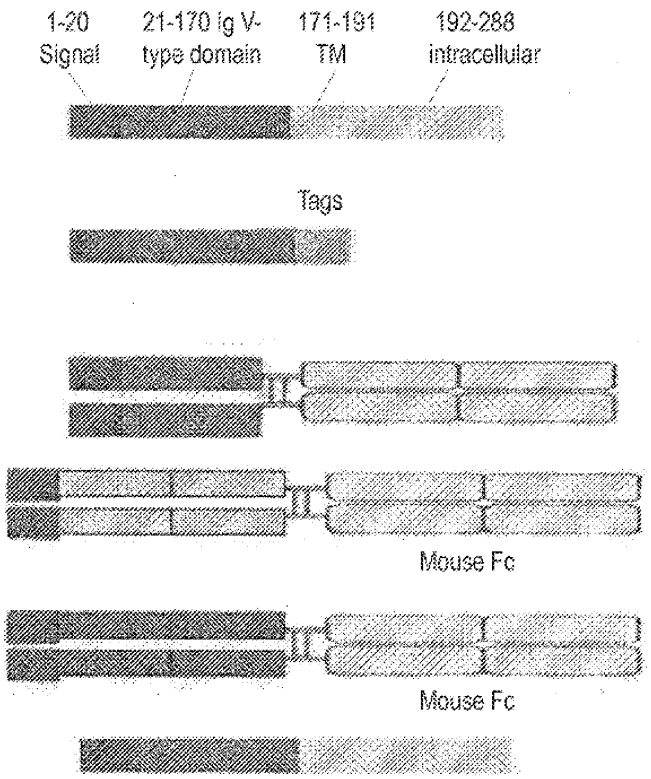


FIG. 1

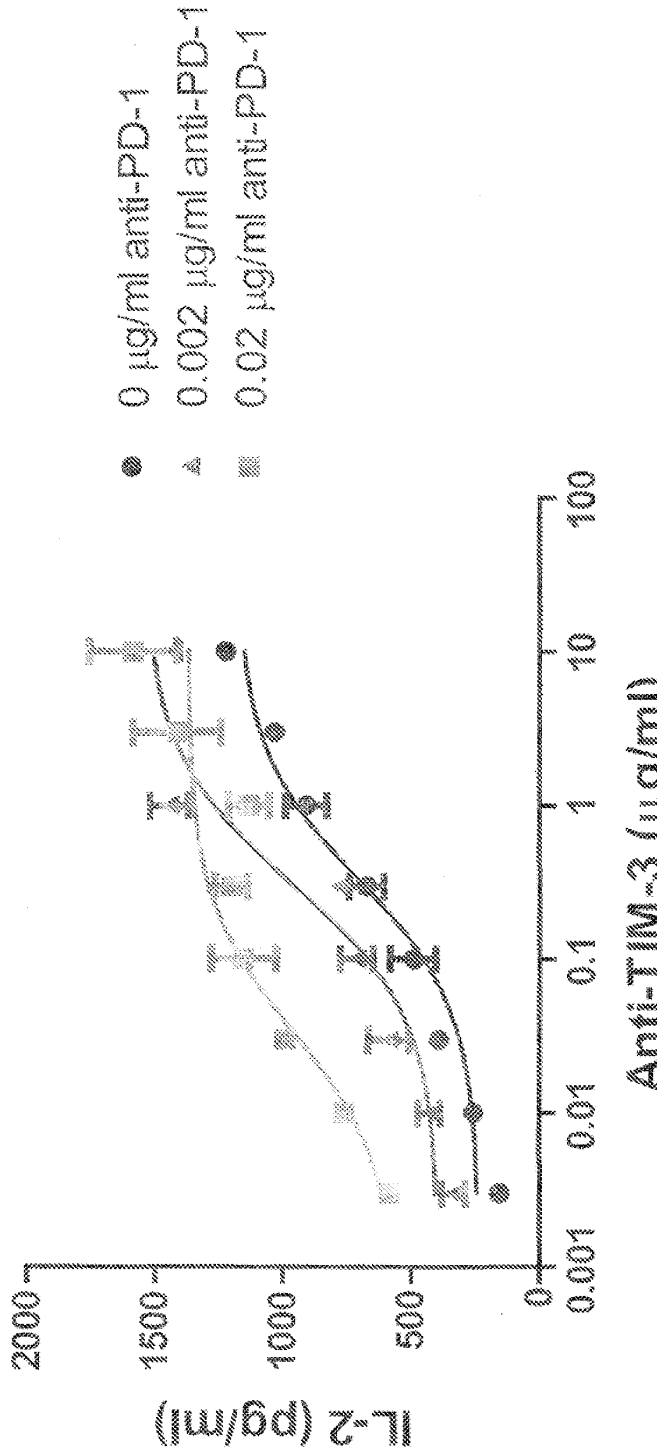

0 μg/ml anti-PD-1
0.002 μg/ml anti-PD-1
0.02 μg/ml anti-PD-1
IL-2 (pg/ml)
2000
1500
1000
500
0
0.001
0.01
0.1
1
10
100
Anti-TIM-3 (μg/ml)

FIG. 2

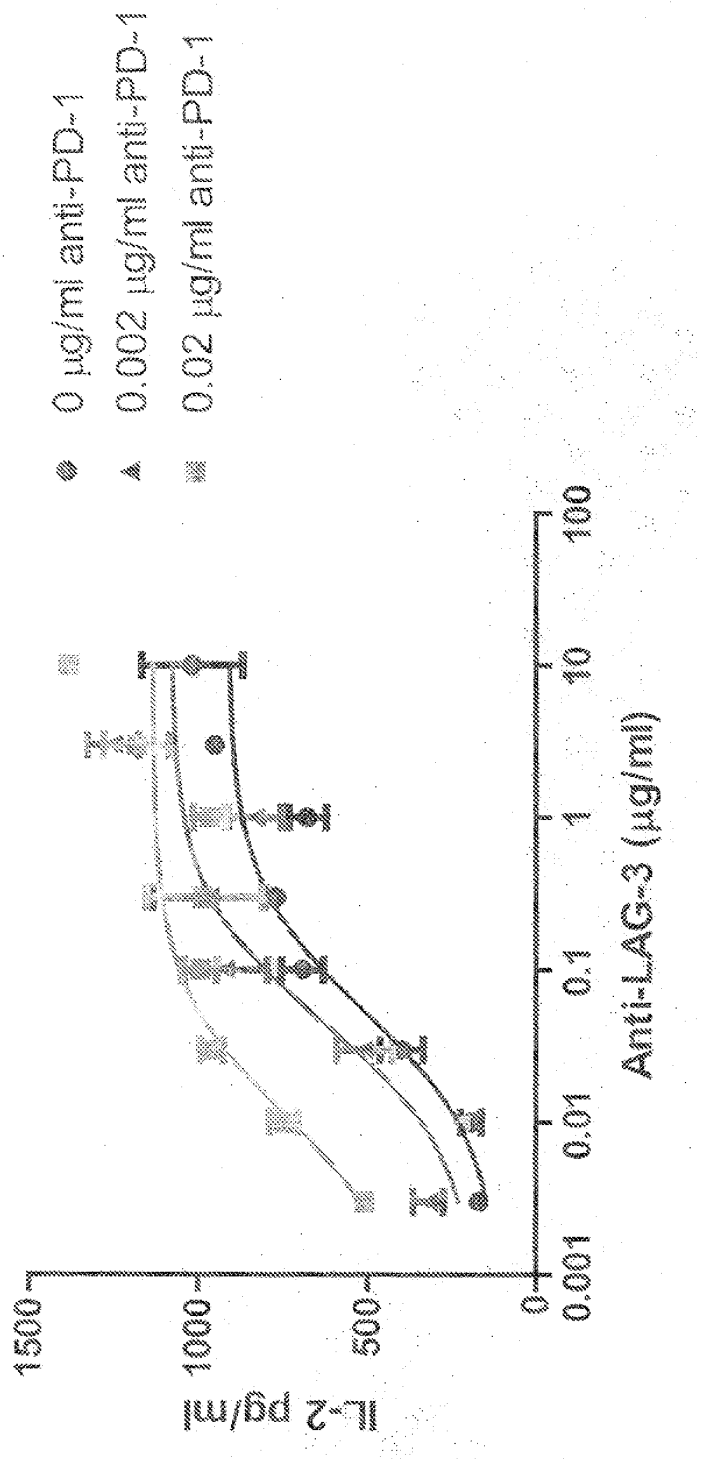

0 µg/ml anti-PD-1
0.002 µg/ml anti-PD-1
0.02 µg/ml anti-PD-1
IL-2 pg/ml
1500
1000
500
0
0.001
0.01
0.1
1
10
100
Anti-LAG-3 (µg/ml)

FIG. 3

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(54) Title: ANTIGEN BINDING PROTEINS THAT BIND PD-1

(57) Abstract: There is disclosed compositions and methods relating to or derived from anti-PD-1 antibodies. More specifically, there is disclosed fully human antibodies that bind PD-1, PD-1 -binding fragments and derivatives of such antibodies, and PD-1 -binding polypeptides comprising such fragments. Further still, there is disclosed nucleic acids encoding such antibodies, antibody fragments and derivatives and polypeptides, cells comprising such polynucleotides, methods of making such antibodies, antibody fragments and derivatives and polypeptides, and methods of using such antibodies, antibody fragments and derivatives and polypeptides, including methods of treating or diagnosing subjects having PD-1 related disorders or conditions, including various inflammatory disorders and various cancers.

Antigen Binding Proteins that Bind PD-1

Technical Field

The present disclosure provides compositions and methods relating to or derived from anti-PD-1 antibodies. More specifically, the present disclosure provides human antibodies that bind PD-1, PD-1-binding fragments and derivatives of such antibodies, and PD-1-binding polypeptides comprising such fragments. Further still, the present disclosure provides nucleic acids encoding such antibodies, antibody fragments and derivatives and polypeptides, cells comprising such polynucleotides, methods of making such antibodies, antibody fragments and derivatives and polypeptides, and methods of using such antibodies, antibody fragments and derivatives and polypeptides, including methods of treating or diagnosing subjects having PD-1 related disorders or conditions, including various inflammatory disorders and various cancers.

Background

Programmed cell death protein-1 (PD-1) is a type I membrane protein of 268 amino acids and is a member of the extended CD28/CTLA-4 family of T cell regulators PD-1 (*The EMBO Journal* (1992), vol. 11, issue 11, p. 3887-3895,). Human PD-1 cDNA is composed of the base sequence shown in EMBL/GenBank Acc. No. NM_005018 and mouse PD-1 cDNA is composed of the base sequence shown in Acc. No. NM_008798, and those expressions are observed when thymus cells differentiate from CD4-CD8- cell into CD4+CD8+ cell (*International Immunology* (1996), vol. 18, issue 5, p. 773-780., *J. Experimental Med.* (2000), vol. 191, issue 5, p. 891-898.). It is reported that PD-1 expression in periphery is observed in myeloid cells including T cells or B lymphocytes activated by stimulation from antigen receptors, or activated macrophages (*International Immunology* (1996), vol. 18, issue 5, p. 765-772.).

PD-1 is a member of the CD28 family of receptors, which includes CD28, CTLA-4, ICOS, PD-1, and BTLA. The initial member of the family, CD28, was discovered by functional effect on augmenting T cell proliferation following the addition of monoclonal antibodies (Hutloff et al. (1999) *Nature* 397:263-266; Hansen et al. (1980) *Immunogenics* 10:247-260). Two cell surface glycoprotein ligands for PD-1 have been identified, PD-1 and PDL-2, and have been shown to down-regulate T cell activation and cytokine secretion occur upon binding to PD-1 (Freeman et al. (2000) *J. Exp. Med.* 192:1027-34; Latchman et al. (2001) *Nat. Immunol.* 2:261-8; Carter et al. (2002) *Eur. J. Immunol.* 32:634-43; Ohigashi et al. (2005) *Clin. Cancer Res.* 11:2947-53). Both PD-1 (B7-H1) and PD-L2 (B7-DC) are B7 homologs that

bind to PD-1. Expression of PD-1 on the cell surface has also been shown to be upregulated through IFN-γ stimulation.

Summary

The present disclosure provides a fully human antibody of an IgG class that binds to a PD-1 epitope with a binding affinity of at least 10^{-6}M, which has a heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, and combinations thereof. Preferably, the fully human antibody has both a heavy chain and a light chain wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2 (called GA1 herein), SEQ ID NO. 3/SEQ ID NO. 4 (called GA2 herein), SEQ ID NO. 5/SEQ ID NO. 6 (called GB1 herein), SEQ ID NO. 7/SEQ ID NO. 8 (called GB6 herein), SEQ ID NO. 9/SEQ ID NO. 10 (called GH1 herein), SEQ ID NO. 11/SEQ ID NO. 12 (called A2 herein), SEQ ID NO. 13/SEQ ID NO. 14 (called C7 herein), SEQ ID NO. 15/SEQ ID NO. 16 (called H7 herein), SEQ ID NO. 17/SEQ ID NO. 18 (called SH-A4 herein), SEQ ID NO. 19/SEQ ID NO. 20 (called SH-A9 herein), SEQ ID NO. 21/SEQ ID NO. 22 (called RG1B3 herein), SEQ ID NO. 23/SEQ ID NO. 24 (called RG1H10 herein), SEQ ID NO. 25/SEQ ID NO. 26 (called RG1H11 herein), SEQ ID NO. 27/SEQ ID NO. 28 (called RG2H7 herein), SEQ ID NO. 29/SEQ ID NO. 30 (called RG2H10 herein), SEQ ID NO. 31/SEQ ID NO. 32 (called RG3E12 herein), SEQ ID NO. 33/SEQ ID NO. 34 (called RG4A6 herein), SEQ ID NO. 35/SEQ ID NO. 36 (called RG5D9 herein), SEQ ID NO. 37/SEQ ID NO. 24 (called RG1H10-H2A-22-15 herein), SEQ ID NO. 38/SEQ ID NO. 24 (called RG1H10-H2A-27-25 herein), SEQ ID NO. 39/SEQ ID NO. 24 (called RG1H10-3C herein), SEQ ID NO. 40/SEQ ID NO. 24 (called RG1H10-16C herein), SEQ ID NO. 41/SEQ ID NO. 24 (called RG1H10-17C herein), SEQ ID NO. 42/SEQ ID NO. 24 (called RG1H10-19C herein), SEQ ID NO. 43/SEQ ID NO. 24 (called RG1H10-21C

herein), SEQ ID NO. 44/SEQ ID NO. 24 (called RG1H10-23C2 herein), and combinations thereof.

The present disclosure provides a Fab fully human antibody fragment, having a variable domain region from a heavy chain and a variable domain region from a light chain, wherein the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, and combinations thereof. Preferably, the fully human antibody Fab fragment has both a heavy chain variable domain region and a light chain variable domain region wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 24, SEQ ID NO. 38/SEQ ID NO. 24, SEQ ID NO. 39/SEQ ID NO. 24, SEQ ID NO. 40/SEQ ID NO. 24, SEQ ID NO. 41/SEQ ID NO. 24, SEQ ID NO. 42/SEQ ID NO. 24, SEQ ID NO. 43/SEQ ID NO. 24, SEQ ID NO. 44/SEQ ID NO. 24, and combinations thereof.

The present disclosure provides a single chain human antibody, having a variable domain region from a heavy chain and a variable domain region from a light chain and a peptide linker connection the heavy chain and light chain variable domain regions, wherein the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO.

5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, and combinations thereof. Preferably, the fully human single chain antibody has both a heavy chain variable domain region and a light chain variable domain region, wherein the single chain fully human antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 24, SEQ ID NO. 38/SEQ ID NO. 24, SEQ ID NO. 39/SEQ ID NO. 24, SEQ ID NO. 40/SEQ ID NO. 24, SEQ ID NO. 41/SEQ ID NO. 24, SEQ ID NO. 42/SEQ ID NO. 24, SEQ ID NO. 43/SEQ ID NO. 24, SEQ ID NO. 44/SEQ ID NO. 24, and combinations thereof.

The present disclosure further provides a method for treating a broad spectrum of mammalian cancers or a broad-spectrum of inflammatory diseases and autoimmune diseases, comprising administering an effective amount of an anti-PD-1 polypeptide, wherein the anti-PD-1 polypeptide is selected from the group consisting of a fully human antibody of an IgG class that binds to a PD-1 epitope with a binding affinity of at least 10^{-6}M, a Fab fully human antibody fragment, having a variable domain region from a heavy chain and a variable domain region from a light chain, a single chain human antibody, having a variable domain region from a heavy chain and a variable domain region from a light chain and a peptide linker connection the heavy chain and light chain variable domain regions, and combinations thereof;

wherein the fully human antibody has a heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID

NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, and combinations thereof;

wherein the Fab fully human antibody fragment has the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, and combinations thereof, and that has the light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, and combinations thereof; and

wherein the single chain human antibody has the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, and combinations thereof, and that has the light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID

NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, and combinations thereof.

Preferably, the fully human antibody has both a heavy chain and a light chain wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2 (called GA1 herein), SEQ ID NO. 3/SEQ ID NO. 4 (called GA2 herein), SEQ ID NO. 5/SEQ ID NO. 6 (called GB1 herein), SEQ ID NO. 7/SEQ ID NO. 8 (called GB6 herein), SEQ ID NO. 9/SEQ ID NO. 10 (called GH1 herein), SEQ ID NO. 11/SEQ ID NO. 12 (called A2 herein), SEQ ID NO. 13/SEQ ID NO. 14 (called C7 herein), SEQ ID NO. 15/SEQ ID NO. 16 (called H7 herein), SEQ ID NO. 17/SEQ ID NO. 18 (called SH-A4 herein), SEQ ID NO. 19/SEQ ID NO. 20 (called SH-A9 herein), SEQ ID NO. 21/SEQ ID NO. 22 (called RG1B3 herein), SEQ ID NO. 23/SEQ ID NO. 24 (called RG1H10 herein), SEQ ID NO. 25/SEQ ID NO. 26 (called RG1H11 herein), SEQ ID NO. 27/SEQ ID NO. 28 (called RG2H7 herein), SEQ ID NO. 29/SEQ ID NO. 30 (called RG2H10 herein), SEQ ID NO. 31/SEQ ID NO. 32 (called RG3E12 herein), SEQ ID NO. 33/SEQ ID NO. 34 (called RG4A6 herein), SEQ ID NO. 35/SEQ ID NO. 36 (called RG5D9 herein), SEQ ID NO. 37/SEQ ID NO. 24 (called RG1H10-H2A-22-15 herein), SEQ ID NO. 38/SEQ ID NO. 24 (called RG1H10-H2A-27-25 herein), SEQ ID NO. 39/SEQ ID NO. 24 (called RG1H10-3C herein), SEQ ID NO. 40/SEQ ID NO. 24 (called RG1H10-16C herein), SEQ ID NO. 41/SEQ ID NO. 24 (called RG1H10-17C herein), SEQ ID NO. 42/SEQ ID NO. 24 (called RG1H10-19C herein), SEQ ID NO. 43/SEQ ID NO. 24 (called RG1H10-21C herein), SEQ ID NO. 44/SEQ ID NO. 24 (called RG1H10-23C2 herein), and combinations thereof. Preferably, the fully human antibody Fab fragment has both a heavy chain variable domain region and a light chain variable domain region wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2 (called GA1 herein), SEQ ID NO. 3/SEQ ID NO. 4 (called GA2 herein), SEQ ID NO. 5/SEQ ID NO. 6 (called GB1 herein), SEQ ID NO. 7/SEQ ID NO. 8 (called GB6 herein), SEQ ID NO. 9/SEQ ID NO. 10 (called GH1 herein), SEQ ID NO. 11/SEQ ID NO. 12 (called A2 herein), SEQ ID NO. 13/SEQ ID NO. 14 (called C7 herein), SEQ ID NO. 15/SEQ ID NO. 16 (called H7 herein), SEQ ID NO. 17/SEQ ID NO. 18 (called SH-A4 herein), SEQ ID NO. 19/SEQ ID NO. 20 (called SH-A9 herein), SEQ ID NO. 21/SEQ ID NO. 22 (called RG1B3 herein), SEQ ID NO. 23/SEQ ID NO. 24 (called RG1H10 herein), SEQ ID NO. 25/SEQ ID NO. 26 (called RG1H11 herein), SEQ ID NO. 27/SEQ ID NO. 28 (called RG2H7 herein), SEQ ID NO. 29/SEQ ID NO. 30 (called RG2H10 herein), SEQ ID NO. 31/SEQ ID NO. 32 (called RG3E12 herein), SEQ ID NO. 33/SEQ ID NO. 34 (called RG4A6 herein), SEQ ID NO. 35/SEQ ID NO.

36 (called RG5D9 herein), SEQ ID NO. 37/SEQ ID NO. 24 (called RG1H10-H2A-22-15 herein), SEQ ID NO. 38/SEQ ID NO. 24 (called RG1H10-H2A-27-25 herein), SEQ ID NO. 39/SEQ ID NO. 24 (called RG1H10-3C herein), SEQ ID NO. 40/SEQ ID NO. 24 (called RG1H10-16C herein), SEQ ID NO. 41/SEQ ID NO. 24 (called RG1H10-17C herein), SEQ ID NO. 42/SEQ ID NO. 24 (called RG1H10-19C herein), SEQ ID NO. 43/SEQ ID NO. 24 (called RG1H10-21C herein), SEQ ID NO. 44/SEQ ID NO. 24 (called RG1H10-23C2 herein), and combinations thereof. Preferably, the fully human single chain antibody has both a heavy chain variable domain region and a light chain variable domain region, wherein the single chain fully human antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 24, SEQ ID NO. 38/SEQ ID NO. 24, SEQ ID NO. 39/SEQ ID NO. 24, SEQ ID NO. 40/SEQ ID NO. 24, SEQ ID NO. 41/SEQ ID NO. 24, SEQ ID NO. 42/SEQ ID NO. 24, SEQ ID NO. 43/SEQ ID NO. 24, SEQ ID NO. 44/SEQ ID NO. 24, and combinations thereof.

Preferably, the broad spectrum of mammalian cancers to be treated is selected from the group consisting of ovarian, colon, breast, lung cancers, myelomas, neuroblastic-derived CNS tumors, monocytic leukemias, B-cell derived leukemias, T-cell derived leukemias, B-cell derived lymphomas, T-cell derived lymphomas, mast cell derived tumors, and combinations thereof. Preferably, the autoimmune disease or inflammatory disease is selected from the group consisting of intestinal mucosal inflammation, wasting disease associated with colitis, multiple sclerosis, systemic lupus erythematosus, viral infections, rheumatoid arthritis, osteoarthritis, psoriasis, Cohn's disease, and inflammatory bowel disease.

Brief Description of the Figures

Figure 1 shows a mixed lymphocyte reaction (MLR) was employed to evaluate the effect of antibody RG1H10 (SEQ ID NO.23/SEQ ID NO. 24) on lymphocyte activity by the anti-PD-1 on lymphocyte effector cells. T cell activation was measured in the presence or absence of the anti-PD-1 human monoclonal antibody.

Figure 2 shows a mixed lymphocyte reaction (MLR) to evaluate the effect of the RG1H10 antibody on lymphocyte activity in lymphocyte effector cells. IL-2 secretion was

measured in the presence or absence of the anti-PD-1 human monoclonal antibody. The ordinate is IFNγ in pg/ml.

Figure 3 shows a mixed lymphocyte reaction (MLR) to demonstrate the effect of blocking the PD-L1/PD-1 pathway by the listed anti-PD-1 RG1H10 antibody on lymphocyte effector cells. IFN-γ secretion was measured in the presence or absence of the anti-PD-1 human monoclonal antibody. The ordinant is IL-2 pg/ml.

Figure 4 shows a cell binding of anti-PD-1 antibody to CHO-PD-1 cells in a dose-dependent manner.

Figure 5 shows how inhibition of recombinant PD-1 binding to recombinant PD-L1 by anti-PD-1 antibodies.

Figure 6 shows how specific binding of the anti-PD-1 antibody RG1H10 to recombinant PD-1 proteins.

Figure 7 shows a mixed lymphocyte reaction (MLR) was employed to compare the effect of antibody RG1H10 (SEQ ID NO.23/SEQ ID NO. 24) as IgG1or IgG4 (S228P) on lymphocyte activity by the anti-PD-1 on lymphocyte effector cells. T cell activation was measured as increased expression of the interleukin-2 (IL-2) receptor (CD25) in the presence or absence of the anti-PD-1 human monoclonal antibody.

Figure 8 shows a mixed lymphocyte reaction (MLR) to evaluate the effect of the RG1H10 antibody as IgG1or IgG4 (S228P) on lymphocyte activity in lymphocyte effector cells. IL-2 secretion was measured in the presence or absence of the anti-PD-1 human monoclonal antibody.

Figure 9 shows a mixed lymphocyte reaction (MLR) to demonstrate the effect of blocking the PD-L1/PD-1 pathway by the listed anti-PD-1 RG1H10 antibody as IgG1or IgG4 (S228P) on lymphocyte effector cells. IFN-γ secretion was measured in the presence or absence of the anti-PD-1 human monoclonal antibody.

Figure 10 shows the cross reactivity, or lack of cross reactivity as between human and murine anti-PD-1 antibodies disclosed herein.

Detailed Description

The present disclosure provides a fully human antibody of an IgG class that binds to a PD-1 epitope with a binding affinity of 10^{-6}M or less, that has a heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31,

SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, and combinations thereof. Preferably, the fully human antibody has both a heavy chain and a light chain wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2 (called GA1 herein), SEQ ID NO. 3/SEQ ID NO. 4 (called GA2 herein), SEQ ID NO. 5/SEQ ID NO. 6 (called GB1 herein), SEQ ID NO. 7/SEQ ID NO. 8 (called GB6 herein), SEQ ID NO. 9/SEQ ID NO. 10 (called GI1 herein), SEQ ID NO. 11/SEQ ID NO. 12 (called A2 herein), SEQ ID NO. 13/SEQ ID NO. 14 (called C7 herein), SEQ ID NO. 15/SEQ ID NO. 16 (called H7 herein), SEQ ID NO. 17/SEQ ID NO. 18 (called SH-A4 herein), SEQ ID NO. 19/SEQ ID NO. 20 (called SH-A9 herein), SEQ ID NO. 21/SEQ ID NO. 22 (called RG1B3 herein), SEQ ID NO. 23/SEQ ID NO. 24 (called RG1H10 herein), SEQ ID NO. 25/SEQ ID NO. 26 (called RG1H11 herein), SEQ ID NO. 27/SEQ ID NO. 28 (called RG2H7 herein), SEQ ID NO. 29/SEQ ID NO. 30 (called RG2H10 herein), SEQ ID NO. 31/SEQ ID NO. 32 (called RG3E12 herein), SEQ ID NO. 33/SEQ ID NO. 34 (called RG4A6 herein), SEQ ID NO. 35/SEQ ID NO. 36 (called RG5D9 herein), SEQ ID NO. 37/SEQ ID NO. 24 (called RG1H10-H2A-22-15 herein), SEQ ID NO. 38/SEQ ID NO. 24 (called RG1H10-H2A-27-25 herein), SEQ ID NO. 39/SEQ ID NO. 24 (called RG1H10-3C herein), SEQ ID NO. 40/SEQ ID NO. 24 (called RG1H10-16C herein), SEQ ID NO. 41/SEQ ID NO. 24 (called RG1H10-17C herein), SEQ ID NO. 42/SEQ ID NO. 24 (called RG1H10-19C herein), SEQ ID NO. 43/SEQ ID NO. 24 (called RG1H10-21C herein), SEQ ID NO. 44/SEQ ID NO. 24 (called RG1H10-23C2 herein), and combinations thereof.

The present disclosure provides a Fab fully human antibody fragment, having a variable domain region from a heavy chain and a variable domain region from a light chain, wherein the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37,

SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, and combinations thereof. Preferably, the fully human antibody Fab fragment has both a heavy chain variable domain region and a light chain variable domain region wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 24, SEQ ID NO. 38/SEQ ID NO. 24, SEQ ID NO. 39/SEQ ID NO. 24, SEQ ID NO. 40/SEQ ID NO. 24, SEQ ID NO. 41/SEQ ID NO. 24, SEQ ID NO. 42/SEQ ID NO. 24, SEQ ID NO. 43/SEQ ID NO. 24, SEQ ID NO. 44/SEQ ID NO. 24, and combinations thereof.

The present disclosure provides a single chain human antibody, having a variable domain region from a heavy chain and a variable domain region from a light chain and a peptide linker connection the heavy chain and light chain variable domain regions, wherein the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO.

34, SEQ ID NO. 36, and combinations thereof. Preferably, the fully human single chain antibody has both a heavy chain variable domain region and a light chain variable domain region, wherein the single chain fully human antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 24, SEQ ID NO. 38/SEQ ID NO. 24, SEQ ID NO. 39/SEQ ID NO. 24, SEQ ID NO. 40/SEQ ID NO. 24, SEQ ID NO. 41/SEQ ID NO. 24, SEQ ID NO. 42/SEQ ID NO. 24, SEQ ID NO. 43/SEQ ID NO. 24, SEQ ID NO. 44/SEQ ID NO. 24, and combinations thereof.

The present disclosure further provides a method for treating a broad spectrum of mammalian cancers or inflammatory diseases or autoimmune diseases, comprising administering an effective amount of an anti-PD-1 polypeptide, wherein the anti-PD-1 polypeptide is selected from the group consisting of a fully human antibody of an IgG class that binds to a PD-1 epitope with a binding affinity of at least 10^{-6}M, a Fab fully human antibody fragment, having a variable domain region from a heavy chain and a variable domain region from a light chain, a single chain human antibody, having a variable domain region from a heavy chain and a variable domain region from a light chain and a peptide linker connection the heavy chain and light chain variable domain regions, and combinations thereof;

wherein the fully human antibody has a heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID

NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, and combinations thereof;

wherein the Fab fully human antibody fragment has the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, and combinations thereof, and that has the light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, and combinations thereof; and

wherein the single chain human antibody has the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, and combinations thereof, and that has the light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, and combinations thereof.

Preferably, the fully human antibody has both a heavy chain and a light chain wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO.

22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 24, SEQ ID NO. 38/SEQ ID NO. 24, SEQ ID NO. 39/SEQ ID NO. 24, SEQ ID NO. 40/SEQ ID NO. 24, SEQ ID NO. 41/SEQ ID NO. 24, SEQ ID NO. 42/SEQ ID NO. 24, SEQ ID NO. 43/SEQ ID NO. 24, SEQ ID NO. 44/SEQ ID NO. 24, and combinations thereof. Preferably, the fully human antibody Fab fragment has both a heavy chain variable domain region and a light chain variable domain region wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 24, SEQ ID NO. 38/SEQ ID NO. 24, SEQ ID NO. 39/SEQ ID NO. 24, SEQ ID NO. 40/SEQ ID NO. 24, SEQ ID NO. 41/SEQ ID NO. 24, SEQ ID NO. 42/SEQ ID NO. 24, SEQ ID NO. 43/SEQ ID NO. 24, SEQ ID NO. 44/SEQ ID NO. 24, and combinations thereof. Preferably, the fully human single chain antibody has both a heavy chain variable domain region and a light chain variable domain region, wherein the single chain fully human antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 24, SEQ ID NO. 38/SEQ ID NO. 24, SEQ ID NO. 39/SEQ ID NO. 24, SEQ ID NO. 40/SEQ ID NO. 24, SEQ ID NO. 41/SEQ ID NO. 24, SEQ ID NO. 42/SEQ ID NO. 24, SEQ ID NO. 43/SEQ ID NO. 24, SEQ ID NO. 44/SEQ ID NO. 24, and combinations thereof.

Preferably, the broad spectrum of mammalian cancers to be treated is selected from the group consisting of ovarian, colon, breast, lung cancers, myelomas, neuroblastic-derived CNS tumors, monocytic leukemias, B-cell derived leukemias, T-cell derived leukemias, B-cell

derived lymphomas, T-cell derived lymphomas, mast cell derived tumors, and combinations thereof. Preferably, the autoimmune disease or inflammatory disease is selected from the group consisting of intestinal mucosal inflammation, wasting disease associated with colitis, multiple sclerosis, systemic lupus erythematosus, viral infections, rheumatoid arthritis, osteoarthritis, psoriasis, Cohn's disease, and inflammatory bowel disease.

An "antigen binding protein" is a protein comprising a portion that binds to an antigen and, optionally, a scaffold or framework portion that allows the antigen binding portion to adopt a conformation that promotes binding of the antigen binding protein to the antigen. Examples of antigen binding proteins include antibodies, antibody fragments (*e.g.*, an antigen binding portion of an antibody), antibody derivatives, and antibody analogs. The antigen binding protein can comprise, for example, an alternative protein scaffold or artificial scaffold with grafted CDRs or CDR derivatives. Such scaffolds include, but are not limited to, antibody-derived scaffolds comprising mutations introduced to, for example, stabilize the three-dimensional structure of the antigen binding protein as well as wholly synthetic scaffolds comprising, for example, a biocompatible polymer. See, for example, Korndorfer et al., 2003, *Proteins: Structure, Function, and Bioinformatics*, Volume 53, Issue 1:121-129; Roque et al., 2004, *Biotechnol. Prog.* 20:639-654. In addition, peptide antibody mimetics ("PAMs") can be used, as well as scaffolds based on antibody mimetics utilizing fibronection components as a scaffold.

An antigen binding protein can have, for example, the structure of a naturally occurring immunoglobulin. An "immunoglobulin" is a tetrameric molecule. In a naturally occurring immunoglobulin, each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa or lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site such that an intact immunoglobulin has two binding sites.

The variable regions of naturally occurring immunoglobulin chains exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. From N-terminus to C-terminus, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat et al. in Sequences of Proteins of Immunological Interest, 5th Ed., US Dept. of Health and Human Services, PHS, NIH, NIH Publication no. 91-3242, 1991. Other numbering systems for the amino acids in immunoglobulin chains include IMGT.RTM. (international ImMunoGeneTics information system; Lefranc et al, *Dev. Comp. Immunol.* 29:185-203; 2005) and AHo (Honegger and Pluckthun, *J. Mol. Biol.* 309(3):657-670; 2001).

Antibodies can be obtained from sources such as serum or plasma that contain immunoglobulins having varied antigenic specificity. If such antibodies are subjected to affinity purification, they can be enriched for a particular antigenic specificity. Such enriched preparations of antibodies usually are made of less than about 10% antibody having specific binding activity for the particular antigen. Subjecting these preparations to several rounds of affinity purification can increase the proportion of antibody having specific binding activity for the antigen. Antibodies prepared in this manner are often referred to as "monospecific." Monospecfic antibody preparations can be made up of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 99.9% antibody having specific binding activity for the particular antigen.

An "antibody" refers to an intact immunoglobulin or to an antigen binding portion thereof that competes with the intact antibody for specific binding, unless otherwise specified. Antigen binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antigen binding portions include, *inter alia*, Fab, Fab', $F(ab')_2$, Fv, domain antibodies (dAbs), and complementarity determining region (CDR) fragments, single-chain antibodies (scFv), chimeric antibodies, diabodies, triabodies, tetrabodies, and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide.

A Fab fragment is a monovalent fragment having the V_L, V_H, C_L and C_{H1} domains; a $F(ab')_2$ fragment is a bivalent fragment having two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment has the V_H and C_{H1} domains; an Fv fragment has the V_L and V_H domains of a single arm of an antibody; and a dAb fragment has a V_H domain, a V_L domain, or an antigen-binding fragment of a V_H or VL domain (U.S. Patents 6,846,634;

6,696,245, US App. Pub.20/0202512; 2004/0202995; 2004/0038291; 2004/0009507;20 03/0039958, and Ward et al., *Nature* 341:544-546, 1989).

A single-chain antibody (scFv) is an antibody in which a V_L and a V_H region are joined via a linker (*e.g.*, a synthetic sequence of amino acid residues) to form a continuous protein chain wherein the linker is long enough to allow the protein chain to fold back on itself and form a monovalent antigen binding site (see, e.g., Bird et al., 1988, *Science* 242:423-26 and Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-83). Diabodies are bivalent antibodies comprising two polypeptide chains, wherein each polypeptide chain comprises V_H and V_L domains joined by a linker that is too short to allow for pairing between two domains on the same chain, thus allowing each domain to pair with a complementary domain on another polypeptide chain (see, e.g., Holliger et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:6444-48, and Poljak et al., 1994, *Structure* 2:1121-23). If the two polypeptide chains of a diabody are identical, then a diabody resulting from their pairing will have two identical antigen binding sites. Polypeptide chains having different sequences can be used to make a diabody with two different antigen binding sites. Similarly, tribodies and tetrabodies are antibodies comprising three and four polypeptide chains, respectively, and forming three and four antigen binding sites, respectively, which can be the same or different.

Complementarity determining regions (CDRs) and framework regions (FR) of a given antibody may be identified using the system described by Kabat et al. *supra*; Lefranc et al., *supra* and/or Honegger and Pluckthun, *supra*. One or more CDRs may be incorporated into a molecule either covalently or noncovalently to make it an antigen binding protein. An antigen binding protein may incorporate the CDR(s) as part of a larger polypeptide chain, may covalently link the CDR(s) to another polypeptide chain, or may incorporate the CDR(s) noncovalently. The CDRs permit the antigen binding protein to specifically bind to a particular antigen of interest.

An antigen binding protein may have one or more binding sites. If there is more than one binding site, the binding sites may be identical to one another or may be different. For example, a naturally occurring human immunoglobulin typically has two identical binding sites, while a "bispecific" or "bifunctional" antibody has two different binding sites.

The term "human antibody" includes all antibodies that have one or more variable and constant regions derived from human immunoglobulin sequences. In one embodiment, all of the variable and constant domains are derived from human immunoglobulin sequences (a fully human antibody). These antibodies may be prepared in a variety of ways, examples of which are described below, including through the immunization with an antigen of interest of a

mouse that is genetically modified to express antibodies derived from human heavy and/or light chain-encoding genes.

A humanized antibody has a sequence that differs from the sequence of an antibody derived from a non-human species by one or more amino acid substitutions, deletions, and/or additions, such that the humanized antibody is less likely to induce an immune response, and/or induces a less severe immune response, as compared to the non-human species antibody, when it is administered to a human subject. In one embodiment, certain amino acids in the framework and constant domains of the heavy and/or light chains of the non-human species antibody are mutated to produce the humanized antibody. In another embodiment, the constant domain(s) from a human antibody are fused to the variable domain(s) of a non-human species. In another embodiment, one or more amino acid residues in one or more CDR sequences of a non-human antibody are changed to reduce the likely immunogenicity of the non-human antibody when it is administered to a human subject, wherein the changed amino acid residues either are not critical for immunospecific binding of the antibody to its antigen, or the changes to the amino acid sequence that are made are conservative changes, such that the binding of the humanized antibody to the antigen is not significantly worse than the binding of the non-human antibody to the antigen. Examples of how to make humanized antibodies may be found in U.S. Patents 6,054,297, 5,886,152 and 5,877,293.

The term "chimeric antibody" refers to an antibody that contains one or more regions from one antibody and one or more regions from one or more other antibodies. In one embodiment, one or more of the CDRs are derived from a human anti-PD-1 antibody. In another embodiment, all of the CDRs are derived from a human anti-PD-1 antibody. In another embodiment, the CDRs from more than one human anti-PD-1 antibodies are mixed and matched in a chimeric antibody. For instance, a chimeric antibody may comprise a CDR1 from the light chain of a first human anti-PD-1 antibody, a CDR2 and a CDR3 from the light chain of a second human anti-PD-1 antibody, and the CDRs from the heavy chain from a third anti-PD-1 antibody. Other combinations are possible.

Further, the framework regions may be derived from one of the same anti-PD-1 antibodies, from one or more different antibodies, such as a human antibody, or from a humanized antibody. In one example of a chimeric antibody, a portion of the heavy and/or light chain is identical with, homologous to, or derived from an antibody from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is/are identical with, homologous to, or derived from an antibody (-ies) from another

species or belonging to another antibody class or subclass. Also included are fragments of such antibodies that exhibit the desired biological activity (*i.e.*, the ability to specifically bind PD-1).

A "neutralizing antibody" or an "inhibitory antibody" is an antibody that inhibits the proteolytic activation of PD-1 when an excess of the anti-PD-1 antibody reduces the amount of activation by at least about 20% using an assay such as those described herein in the Examples. In various embodiments, the antigen binding protein reduces the amount of amount of proteolytic activation of PD-1 by at least 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, and 99.9%.

Fragments or analogs of antibodies can be readily prepared by those of ordinary skill in the art following the teachings of this specification and using techniques known in the art. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Computerized comparison methods can be used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. See, Bowie et al., 1991, *Science* 253:164.

A "CDR grafted antibody" is an antibody comprising one or more CDRs derived from an antibody of a particular species or isotype and the framework of another antibody of the same or different species or isotype.

A "multi-specific antibody" is an antibody that recognizes more than one epitope on one or more antigens. A subclass of this type of antibody is a "bi-specific antibody" which recognizes two distinct epitopes on the same or different antigens.

An antigen binding protein "specifically binds" to an antigen (*e.g.*, human PD-1) if it binds to the antigen with a dissociation constant of 1 nanomolar or less.

An "antigen binding domain," "antigen binding region," or "antigen binding site" is a portion of an antigen binding protein that contains amino acid residues (or other moieties) that interact with an antigen and contribute to the antigen binding protein's specificity and affinity for the antigen. For an antibody that specifically binds to its antigen, this will include at least part of at least one of its CDR domains.

An "epitope" is the portion of a molecule that is bound by an antigen binding protein (*e.g.*, by an antibody). An epitope can comprise non-contiguous portions of the molecule (*e.g.*, in a polypeptide, amino acid residues that are not contiguous in the polypeptide's primary

sequence but that, in the context of the polypeptide's tertiary and quaternary structure, are near enough to each other to be bound by an antigen binding protein).

The "percent identity" of two polynucleotide or two polypeptide sequences is determined by comparing the sequences using the GAP computer program (a part of the GCG Wisconsin Package, version 10.3 (Accelrys, San Diego, Calif.)) using its default parameters.

The terms "polynucleotide," "oligonucleotide" and "nucleic acid" are used interchangeably throughout and include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs (*e.g.*, peptide nucleic acids and non-naturally occurring nucleotide analogs), and hybrids thereof. The nucleic acid molecule can be single-stranded or double-stranded. In one embodiment, the nucleic acid molecules of the invention comprise a contiguous open reading frame encoding an antibody, or a fragment, derivative, mutein, or variant thereof.

Two single-stranded polynucleotides are "the complement" of each other if their sequences can be aligned in an anti-parallel orientation such that every nucleotide in one polynucleotide is opposite its complementary nucleotide in the other polynucleotide, without the introduction of gaps, and without unpaired nucleotides at the 5' or the 3' end of either sequence. A polynucleotide is "complementary" to another polynucleotide if the two polynucleotides can hybridize to one another under moderately stringent conditions. Thus, a polynucleotide can be complementary to another polynucleotide without being its complement.

A "vector" is a nucleic acid that can be used to introduce another nucleic acid linked to it into a cell. One type of vector is a "plasmid," which refers to a linear or circular double stranded DNA molecule into which additional nucleic acid segments can be ligated. Another type of vector is a viral vector (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), wherein additional DNA segments can be introduced into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors comprising a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. An "expression vector" is a type of vector that can direct the expression of a chosen polynucleotide.

A nucleotide sequence is "operably linked" to a regulatory sequence if the regulatory sequence affects the expression (*e.g.*, the level, timing, or location of expression) of the nucleotide sequence. A "regulatory sequence" is a nucleic acid that affects the expression (*e.g.*, the level, timing, or location of expression) of a nucleic acid to which it is operably linked. The

regulatory sequence can, for example, exert its effects directly on the regulated nucleic acid, or through the action of one or more other molecules (*e.g.*, polypeptides that bind to the regulatory sequence and/or the nucleic acid). Examples of regulatory sequences include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Further examples of regulatory sequences are described in, for example, Goeddel, 1990, Gene Expression Technology: *Methods in Enzymology* 185, Academic Press, San Diego, Calif. and Baron et al., 1995, *Nucleic Acids Res.* 23:3605-06.

A "host cell" is a cell that can be used to express a nucleic acid, *e.g.*, a nucleic acid of the invention. A host cell can be a prokaryote, for example, *E. coli*, or it can be a eukaryote, for example, a single-celled eukaryote (*e.g.*, a yeast or other fungus), a plant cell (*e.g.*, a tobacco or tomato plant cell), an animal cell (*e.g.*, a human cell, a monkey cell, a hamster cell, a rat cell, a mouse cell, or an insect cell) or a hybridoma. Examples of host cells include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (see Gluzman et al., 1981, *Cell* 23:175), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells or their derivatives such as Veggie CHO and related cell lines which grow in serum-free media (see Rasmussen et al., 1998, *Cytotechnology* 28:31) or CHO strain DX-B11, which is deficient in DHFR (see Urlaub et al., 1980, *Proc. Natl. Acad. Sci. USA* 77:4216-20), HeLa cells, BHK (ATCC CRL 10) cell lines, the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) (see McMahan et al., 1991, *EMBO J.* 10:2821), human embryonic kidney cells such as 293,293 EBNA or MSR 293, human epidermal A431 cells, human Colo205 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HL-60, U937, HaK or Jurkat cells. Typically, a host cell is a cultured cell that can be transformed or transfected with a polypeptide-encoding nucleic acid, which can then be expressed in the host cell. The phrase "recombinant host cell" can be used to denote a host cell that has been transformed or transfected with a nucleic acid to be expressed. A host cell also can be a cell that comprises the nucleic acid but does not express it at a desired level unless a regulatory sequence is introduced into the host cell such that it becomes operably linked with the nucleic acid. It is understood that the term host cell refers not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to, *e.g.*, mutation or environmental influence, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

Preferably, the mammalian cancer to be treated is selected from the group consisting of ovarian, colon, breast or hepatic carcinoma cell lines, myelomas, neuroblastic-derived CNS

tumors, monocytic leukemias, B-cell derived leukemia's, T-cell derived leukemias, B-cell derived lymphomas, T-cell derived lymphomas, mast cell derived tumors, and combinations thereof.

Polypeptides of the present disclosure can be produced using any standard methods known in the art. In one example, the polypeptides are produced by recombinant DNA methods by inserting a nucleic acid sequence (*e.g.*, a cDNA) encoding the polypeptide into a recombinant expression vector and expressing the DNA sequence under conditions promoting expression.

Nucleic acids encoding any of the various polypeptides disclosed herein may be synthesized chemically. Codon usage may be selected so as to improve expression in a cell. Such codon usage will depend on the cell type selected. Specialized codon usage patterns have been developed for E. coli and other bacteria, as well as mammalian cells, plant cells, yeast cells and insect cells. See for example: Mayfield et al., *Proc. Natl. Acad. Sci. USA*. 2003 100(2):438-42; Sinclair et al. *Protein Expr. Purif.* 2002 (1):96-105; Connell N D. *Curr. Opin. Biotechnol.* 2001 12(5):446-9; Makrides et al. *Microbiol. Rev.* 1996 60(3):512-38; and Sharp et al. *Yeast.* 1991 7(7):657-78.

General techniques for nucleic acid manipulation are described for example in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Vols. 1-3, Cold Spring Harbor Laboratory Press, 2 ed., 1989, or F. Ausubel et al., *Current Protocols in Molecular Biology* (Green Publishing and Wiley-Interscience: New York, 1987) and periodic updates, herein incorporated by reference. The DNA encoding the polypeptide is operably linked to suitable transcriptional or translational regulatory elements derived from mammalian, viral, or insect genes. Such regulatory elements include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences that control the termination of transcription and translation. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants is additionally incorporated.

The recombinant DNA can also include any type of protein tag sequence that may be useful for purifying the protein. Examples of protein tags include but are not limited to a histidine tag, a FLAG tag, a myc tag, an HA tag, or a GST tag. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts can be found in *Cloning Vectors: A Laboratory Manual*, (Elsevier, N.Y., 1985).

The expression construct is introduced into the host cell using a method appropriate to the host cell. A variety of methods for introducing nucleic acids into host cells are known in

the art, including, but not limited to, electroporation; transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (where the vector is an infectious agent). Suitable host cells include prokaryotes, yeast, mammalian cells, or bacterial cells.

Suitable bacteria include gram negative or gram positive organisms, for example, *E. coli* or *Bacillus spp*. Yeast, preferably from the *Saccharomyces* species, such as *S. cerevisiae*, may also be used for production of polypeptides. Various mammalian or insect cell culture systems can also be employed to express recombinant proteins. *Baculovirus* systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, (*Bio/Technology*, 6:47, 1988). Examples of suitable mammalian host cell lines include endothelial cells, COS-7 monkey kidney cells, CV-1, L cells, C127, 3T3, Chinese hamster ovary (CHO), human embryonic kidney cells, HeLa, 293, 293T, and BHK cell lines. Purified polypeptides are prepared by culturing suitable host/vector systems to express the recombinant proteins. For many applications, the small size of many of the polypeptides disclosed herein would make expression in *E. coli* as the preferred method for expression. The protein is then purified from culture media or cell extracts.

Proteins disclosed herein can also be produced using cell-translation systems. For such purposes the nucleic acids encoding the polypeptide must be modified to allow in vitro transcription to produce mRNA and to allow cell-free translation of the mRNA in the particular cell-free system being utilized (eukaryotic such as a mammalian or yeast cell-free translation system or prokaryotic such as a bacterial cell-free translation system.

PD-1-binding polypeptides can also be produced by chemical synthesis (*e.g.*, by the methods described in Solid Phase Peptide Synthesis, 2nd ed., 1984, The Pierce Chemical Co., Rockford, Ill.). Modifications to the protein can also be produced by chemical synthesis.

The polypeptides of the present disclosure can be purified by isolation/purification methods for proteins generally known in the field of protein chemistry. Non-limiting examples include extraction, recrystallization, salting out (*e.g.*, with ammonium sulfate or sodium sulfate), centrifugation, dialysis, ultrafiltration, adsorption chromatography, ion exchange chromatography, hydrophobic chromatography, normal phase chromatography, reversed-phase chromatography, gel filtration, gel permeation chromatography, affinity chromatography, electrophoresis, countercurrent distribution or any combinations of these. After purification, polypeptides may be exchanged into different buffers and/or concentrated by any of a variety of methods known to the art, including, but not limited to, filtration and dialysis.

The purified polypeptide is preferably at least 85% pure, more preferably at least 95% pure, and most preferably at least 98% pure. Regardless of the exact numerical value of the purity, the polypeptide is sufficiently pure for use as a pharmaceutical product.

Post-Translational Modifications of Polypeptides

In certain embodiments, the binding polypeptides of the invention may further comprise post-translational modifications. Exemplary post-translational protein modifications include phosphorylation, acetylation, methylation, ADP-ribosylation, ubiquitination, glycosylation, carbonylation, sumoylation, biotinylation or addition of a polypeptide side chain or of a hydrophobic group. As a result, the modified soluble polypeptides may contain non-amino acid elements, such as lipids, poly- or mono-saccharide, and phosphates. A preferred form of glycosylation is sialylation, which conjugates one or more sialic acid moieties to the polypeptide. Sialic acid moieties improve solubility and serum half-life while also reducing the possible immunogeneticity of the protein. See Raju et al. *Biochemistry*. 2001 31; 40(30):8868-76. Effects of such non-amino acid elements on the functionality of a polypeptide may be tested for its antagonizing role in PD-L1 or PD-1 function, *e.g.*, its inhibitory effect on angiogenesis or on tumor growth.

In one specific embodiment, modified forms of the subject soluble polypeptides comprise linking the subject soluble polypeptides to nonproteinaceous polymers. In one specific embodiment, the polymer is polyethylene glycol ("PEG"), polypropylene glycol, or polyoxyalkylenes, in the manner as set forth in U.S. Patents 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337. Examples of the modified polypeptide include PEGylated VK-B8.

PEG is a water soluble polymer that is commercially available or can be prepared by ring-opening polymerization of ethylene glycol according to methods well known in the art (Sandler and Karo, Polymer Synthesis, Academic Press, New York, Vol. 3, pages 138-161). The term "PEG" is used broadly to encompass any polyethylene glycol molecule, without regard to size or to modification at an end of the PEG, and can be represented by the formula: X--O(CH_2CH_2O)$_n$-1CH_2CH_2OH (1), where n is 20 to 2300 and X is H or a terminal modification, *e.g.*, a C_{1-4} alkyl. In one embodiment, the PEG of the invention terminates on one end with hydroxy or methoxy, *i.e.*, X is H or CH_3 ("methoxy PEG"). A PEG can contain further chemical groups which are necessary for binding reactions; which results from the chemical synthesis of the molecule; or which is a spacer for optimal distance of parts of the molecule. In addition, such a PEG can consist of one or more PEG side-chains which are linked together. PEGs with more than one PEG chain are called multiarmed or branched PEGs.

Branched PEGs can be prepared, for example, by the addition of polyethylene oxide to various polyols, including glycerol, pentaerythriol, and sorbitol. For example, a four-armed branched PEG can be prepared from pentaerythriol and ethylene oxide. Branched PEG are described in, for example, EP-A 0 473 084 and U.S. Patent. 5,932,462. One form of PEGs includes two PEG side-chains (PEG2) linked via the primary amino groups of a lysine (Monfardini et al., *Bioconjugate Chem.* 6 (1995) 62-69).

Although PEG is well-known, this is, to our knowledge, the first demonstration that a pegylated ^{10F}n3 polypeptide can be pegylated and retain ligand binding activity. In a preferred embodiment, the pegylated ^{10F}n3 polypeptide is produced by site-directed pegylation, particularly by conjugation of PEG to a cysteine moiety at the N- or C-terminus. Accordingly, the present disclosure provides a target-binding ^{10F}n3 polypeptide with improved pharmacokinetic properties, the polypeptide comprising: a ^{10F}n3 domain having from about 80 to about 150 amino acids, wherein at least one of the loops of said ^{10F}n3 domain participate in target binding; and a covalently bound PEG moiety, wherein said ^{10F}n3 polypeptide binds to the target with a K_D of less than 100 nM and has a clearance rate of less than 30 mL/hr/kg in a mammal. The PEG moiety may be attached to the ^{10F}n3 polypeptide by site directed pegylation, such as by attachment to a Cys residue, where the Cys residue may be positioned at the N-terminus of the ^{0F}n3 polypeptide or between the N-terminus and the most N-terminal beta or beta-like strand or at the C-terminus of the ^{10F}n3 polypeptide or between the C-terminus and the most C-terminal beta or beta-like strand. A Cys residue may be situated at other positions as well, particularly any of the loops that do not participate in target binding. A PEG moiety may also be attached by other chemistry, including by conjugation to amines.

PEG conjugation to peptides or proteins generally involves the activation of PEG and coupling of the activated PEG-intermediates directly to target proteins/peptides or to a linker, which is subsequently activated and coupled to target proteins/peptides (see Abuchowski et al., *J. Biol. Chem.*, 252, 3571 (1977) and *J. Biol. Chem.*, 252, 3582 (1977), Zalipsky, et al., and Harris et. al., in: *Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications*; (J. M. Harris ed.) Plenum Press: New York, 1992; Chap.21 and 22). It is noted that a binding polypeptide containing a PEG molecule is also known as a conjugated protein, whereas the protein lacking an attached PEG molecule can be referred to as unconjugated.

A variety of molecular mass forms of PEG can be selected, *e.g.*, from about 1,000 Daltons (Da) to 100,000 Da (n is 20 to 2300), for conjugating to PD-1-binding polypeptides. The number of repeating units "n" in the PEG is approximated for the molecular mass described in Daltons. It is preferred that the combined molecular mass of PEG on an activated

linker is suitable for pharmaceutical use. Thus, in one embodiment, the molecular mass of the PEG molecules does not exceed 100,000 Da. For example, if three PEG molecules are attached to a linker, where each PEG molecule has the same molecular mass of 12,000 Da (each n is about 270), then the total molecular mass of PEG on the linker is about 36,000 Da (total n is about 820). The molecular masses of the PEG attached to the linker can also be different, *e.g.*, of three molecules on a linker two PEG molecules can be 5,000 Da each (each n is about 110) and one PEG molecule can be 12,000 Da (n is about 270).

In a specific embodiment of the disclosure an PD-1 binding polypeptide is covalently linked to one poly(ethylene glycol) group of the formula: --CO--$(CH_2)_x$--$(OCH_2CH_2)_m$--OR , with the --CO (*i.e.* carbonyl) of the poly(ethylene glycol) group forming an amide bond with one of the amino groups of the binding polypeptide; R being lower alkyl; x being 2 or 3; m being from about 450 to about 950; and n and m being chosen so that the molecular weight of the conjugate minus the binding polypeptide is from about 10 to 40 kDa. In one embodiment, a binding polypeptide's 6-amino group of a lysine is the available (free) amino group.

The above conjugates may be more specifically presented by formula (II): P--NHCO--$(CH_2)_x$--$(OCH_2CH_2)_m$--OR (II) , wherein P is the group of a binding polypeptide as described herein, (i.e. without the amino group or amino groups which form an amide linkage with the carbonyl shown in formula (II); and wherein R is lower alkyl; x is 2 or 3; m is from about 450 to about 950 and is chosen so that the molecular weight of the conjugate minus the binding polypeptide is from about 10 to about 40 kDa. As used herein, the given ranges of "m" have an orientational meaning. The ranges of "m" are determined in any case, and exactly, by the molecular weight of the PEG group.

One skilled in the art can select a suitable molecular mass for PEG, *e.g.*, based on how the pegylated binding polypeptide will be used therapeutically, the desired dosage, circulation time, resistance to proteolysis, immunogenicity, and other considerations. For a discussion of PEG and its use to enhance the properties of proteins, see Katre, *Advanced Drug Delivery Reviews* 10: 91-114 (1993).

In one embodiment, PEG molecules may be activated to react with amino groups on a binding polypeptide, such as with lysines (Bencham et al., *Anal. Biochem.*, 131, 25 (1983); Veronese et al., *Appl. Biochem.*, 11, 141 (1985).; Zalipsky et al., *Polymeric Drugs and Drug Delivery Systems*, adrs 9-110 ACS Symposium Series 469 (1999); Zalipsky et al., *Europ. Polym. J.*, 19, 1177-1183 (1983); Delgado et al., *Biotechnology and Applied Biochemistry*, 12, 119-128 (1990)).

In one specific embodiment, carbonate esters of PEG are used to form the PEG-binding polypeptide conjugates. N,N'-disuccinimidylcarbonate (DSC) may be used in the reaction with PEG to form active mixed PEG-succinimidyl carbonate that may be subsequently reacted with a nucleophilic group of a linker or an amino group of a binding polypeptide (see U.S. Patents 5,281,698 and 5,932,462). In a similar type of reaction, 1,1'-(dibenzotriazolyl)carbonate and di-(2-pyridyl)carbonate may be reacted with PEG to form PEG-benzotriazolyl and PEG-pyridyl mixed carbonate (U.S. Patent 5,382,657), respectively.

Pegylation of a ^{10F}n3 polypeptide can be performed according to the methods of the state of the art, for example by reaction of the binding polypeptide with electrophilically active PEGs (supplier: Shearwater Corp., USA, www.shearwatercorp.com). Preferred PEG reagents of the present invention are, e.g., N-hydroxysuccinimidyl propionates (PEG-SPA), butanoates (PEG-SBA), PEG-succinimidyl propionate or branched N-hydroxysuccinimides such as mPEG2-NHS (Monfardini et al., *Bioconjugate Chem.* 6 (1995) 62-69). Such methods may used to pegylated at an f-amino group of a binding polypeptide lysine or the N-terminal amino group of the binding polypeptide.

In another embodiment, PEG molecules may be coupled to sulfhydryl groups on a binding polypeptide (Sartore et al., *Appl. Biochem. Biotechnol.*, 27, 45 (1991); Morpurgo et al., *Biocon. Chem.*, 7, 363-368 (1996); Goodson et al., *Bio/Technology* (1990) 8, 343; U.S. Patent 5,766,897). U.S. Patents 6,610,281 and 5,766,897 describes exemplary reactive PEG species that may be coupled to sulfhydryl groups.

In some embodiments where PEG molecules are conjugated to cysteine residues on a binding polypeptide, the cysteine residues are native to the binding polypeptide, whereas in other embodiments, one or more cysteine residues are engineered into the binding polypeptide. Mutations may be introduced into a binding polypeptide coding sequence to generate cysteine residues. This might be achieved, for example, by mutating one or more amino acid residues to cysteine. Preferred amino acids for mutating to a cysteine residue include serine, threonine, alanine and other hydrophilic residues. Preferably, the residue to be mutated to cysteine is a surface-exposed residue. Algorithms are well-known in the art for predicting surface accessibility of residues based on primary sequence or a protein. Alternatively, surface residues may be predicted by comparing the amino acid sequences of binding polypeptides, given that the crystal structure of the framework based on which binding polypeptides are designed and evolved has been solved (see Himanen et al., *Nature*. (2001) 20-27; 414(6866):933-8) and thus the surface-exposed residues identified. In one embodiment, cysteine residues are introduced into binding polypeptides at or near the N- and/or C-terminus, or within loop regions.

In some embodiments, the pegylated binding polypeptide comprises a PEG molecule covalently attached to the alpha amino group of the N-terminal amino acid. Site specific N-terminal reductive amination is described in Pepinsky et al., (2001) *JPET*, 297, 1059, and U.S. Patent 5,824,784. The use of a PEG-aldehyde for the reductive amination of a protein utilizing other available nucleophilic amino groups is described in U.S. Patent 4,002,531, in Wieder et al., (1979) *J. Biol. Chem.* 254,12579, and in Chamow et al., (1994) *Bioconjugate Chem.* 5, 133.

In another embodiment, pegylated binding polypeptide comprises one or more PEG molecules covalently attached to a linker, which in turn is attached to the alpha amino group of the amino acid residue at the N-terminus of the binding polypeptide. Such an approach is disclosed in U.S. Patent Publication 2002/0044921 and in WO094/01451.

In one embodiment, a binding polypeptide is pegylated at the C-terminus. In a specific embodiment, a protein is pegylated at the C-terminus by the introduction of C-terminal azido-methionine and the subsequent conjugation of a methyl-PEG-triarylphosphine compound via the Staudinger reaction. This C-terminal conjugation method is described in Cazalis et al., *Bioconjug. Chem.* 2004; 15(5):1005-1009.

MonoPEGylation of a binding polypeptide can also be produced according to the general methods described in WO 94/01451. WO 94/01451 describes a method for preparing a recombinant polypeptide with a modified terminal amino acid alpha-carbon reactive group. The steps of the method involve forming the recombinant polypeptide and protecting it with one or more biologically added protecting groups at the N-terminal alpha-amine and C-terminal alpha-carboxyl. The polypeptide can then be reacted with chemical protecting agents to selectively protect reactive side chain groups and thereby prevent side chain groups from being modified. The polypeptide is then cleaved with a cleavage reagent specific for the biological protecting group to form an unprotected terminal amino acid alpha-carbon reactive group. The unprotected terminal amino acid alpha-carbon reactive group is modified with a chemical modifying agent. The side chain protected terminally modified single copy polypeptide is then deprotected at the side chain groups to form a terminally modified recombinant single copy polypeptide. The number and sequence of steps in the method can be varied to achieve selective modification at the N- and/or C-terminal amino acid of the polypeptide.

The ratio of a binding polypeptide to activated PEG in the conjugation reaction can be from about 1:0.5 to 1:50, between from about 1:1 to 1:30, or from about 1:5 to 1:15. Various aqueous buffers can be used in the present method to catalyze the covalent addition of PEG to the binding polypeptide. In one embodiment, the pH of a buffer used is from about 7.0 to 9.0.

In another embodiment, the pH is in a slightly basic range, *e.g.*, from about 7.5 to 8.5. Buffers having a pKa close to neutral pH range may be used, *e.g.*, phosphate buffer.

Conventional separation and purification techniques known in the art can be used to purify PEGylated binding polypeptide, such as size exclusion (*e.g.* gel filtration) and ion exchange chromatography. Products may also be separated using SDS-PAGE. Products that may be separated include mono-, di-, tri- poly- and un-PEGylated binding polypeptide, as well as free PEG. The percentage of mono-PEG conjugates can be controlled by pooling broader fractions around the elution peak to increase the percentage of mono-PEG in the composition. About ninety percent mono-PEG conjugates represents a good balance of yield and activity. Compositions in which, for example, at least ninety-two percent or at least ninety-six percent of the conjugates are mono-PEG species may be desired. In an embodiment of this invention the percentage of mono-PEG conjugates is from ninety percent to ninety-six percent.

In one embodiment, PEGylated binding polypeptide of the invention contain one, two or more PEG moieties. In one embodiment, the PEG moiety(ies) are bound to an amino acid residue which is on the surface of the protein and/or away from the surface that contacts the target ligand. In one embodiment, the combined or total molecular mass of PEG in PEG-binding polypeptide is from about 3,000 Da to 60,000 Da, optionally from about 10,000 Da to 36,000 Da. In a one embodiment, the PEG in PEGylated binding polypeptide is a substantially linear, straight-chain PEG.

In one embodiment of the invention, the PEG in PEGylated binding polypeptide is not hydrolyzed from the PEGylated amino acid residue using a hydroxylamine assay, *e.g.*, 450 mM hydroxylamine (pH 6.5) over 8 to 16 hours at room temperature, and is thus stable. In one embodiment, greater than 80% of the composition is stable mono-PEG-binding polypeptide, more preferably at least 90%, and most preferably at least 95%.

In another embodiment, the PEGylated binding polypeptides of the invention will preferably retain at least 25%, 50%, 60%, 70%, 80%, 85%, 90%, 95% or 100% of the biological activity associated with the unmodified protein. In one embodiment, biological activity refers to its ability to bind to PD-1, as assessed by KD, k_{on} or k_{off}. In one specific embodiment, the PEGylated binding polypeptide protein shows an increase in binding to PD-1 relative to unPEGylated binding polypeptide.

The serum clearance rate of PEG-modified polypeptide may be decreased by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or even 90%, relative to the clearance rate of the unmodified binding polypeptide. The PEG-modified polypeptide may have a half-life ($t_{1/2}$) which is enhanced relative to the half-life of the unmodified protein. The half-life of PEG-

binding polypeptide may be enhanced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 400% or 500%, or even by 1000% relative to the half-life of the unmodified binding polypeptide. In some embodiments, the protein half-life is determined *in vitro*, such as in a buffered saline solution or in serum. In other embodiments, the protein half-life is an *in vivo* half life, such as the half-life of the protein in the serum or other bodily fluid of an animal.

Therapeutic Formulations and Modes of Administration

The present disclosure features methods for treating conditions or preventing pre-conditions which respond to an inhibition of PD-1 biological activity. Preferred examples are conditions that are characterized by inflammation or cellular hyperproliferation. Techniques and dosages for administration vary depending on the type of specific polypeptide and the specific condition being treated but can be readily determined by the skilled artisan. In general, regulatory agencies require that a protein reagent to be used as a therapeutic is formulated so as to have acceptably low levels of pyrogens. Accordingly, therapeutic formulations will generally be distinguished from other formulations in that they are substantially pyrogen free, or at least contain no more than acceptable levels of pyrogen as determined by the appropriate regulatory agency (*e.g.*, FDA).

Therapeutic compositions of the present disclosure may be administered with a pharmaceutically acceptable diluent, carrier, or excipient, in unit dosage form. Administration may be parenteral (*e.g.*, intravenous, subcutaneous), oral, or topical, as non-limiting examples. In addition, any gene therapy technique, using nucleic acids encoding the polypeptides of the invention, may be employed, such as naked DNA delivery, recombinant genes and vectors, cell-based delivery, including ex vivo manipulation of patients' cells, and the like.

The composition can be in the form of a pill, tablet, capsule, liquid, or sustained release tablet for oral administration; or a liquid for intravenous, subcutaneous or parenteral administration; gel, lotion, ointment, cream, or a polymer or other sustained release vehicle for local administration.

Methods well known in the art for making formulations are found, for example, in "Remington: The Science and Practice of Pharmacy" (20th ed., ed. A. R. Gennaro A R., 2000, Lippincott Williams & Wilkins, Philadelphia, Pa.). Formulations for parenteral administration may, for example, contain excipients, sterile water, saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds.

Nanoparticulate formulations (*e.g.*, biodegradable nanoparticles, solid lipid nanoparticles, liposomes) may be used to control the biodistribution of the compounds. Other potentially useful parenteral delivery systems include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. The concentration of the compound in the formulation varies depending upon a number of factors, including the dosage of the drug to be administered, and the route of administration.

The polypeptide may be optionally administered as a pharmaceutically acceptable salt, such as non-toxic acid addition salts or metal complexes that are commonly used in the pharmaceutical industry. Examples of acid addition salts include organic acids such as acetic, lactic, pamoic, maleic, citric, malic, ascorbic, succinic, benzoic, palmitic, suberic, salicylic, tartaric, methanesulfonic, toluenesulfonic, or trifluoroacetic acids or the like; polymeric acids such as tannic acid, carboxymethyl cellulose, or the like; and inorganic acid such as hydrochloric acid, hydrobromic acid, sulfuric acid phosphoric acid, or the like. Metal complexes include zinc, iron, and the like. In one example, the polypeptide is formulated in the presence of sodium acetate to increase thermal stability.

Formulations for oral use include tablets containing the active ingredient(s) in a mixture with non-toxic pharmaceutically acceptable excipients. These excipients may be, for example, inert diluents or fillers (*e.g.*, sucrose and sorbitol), lubricating agents, glidants, and anti-adhesives (*e.g.*, magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc).

Formulations for oral use may also be provided as chewable tablets, or as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium.

A therapeutically effective dose refers to a dose that produces the therapeutic effects for which it is administered. The exact dose will depend on the disorder to be treated, and may be ascertained by one skilled in the art using known techniques. In general, the polypeptide is administered at about 0.01 μg/kg to about 50 mg/kg per day, preferably 0.01 mg/kg to about 30 mg/kg per day, most preferably 0.1 mg/kg to about 20 mg/kg per day. The polypeptide may be given daily (*e.g.*, once, twice, three times, or four times daily) or preferably less frequently (*e.g.*, weekly, every two weeks, every three weeks, monthly, or quarterly). In addition, as is known in the art, adjustments for age as well as the body weight, general health, sex, diet, time of administration, drug interaction, and the severity of the disease may be necessary, and will be ascertainable with routine experimentation by those skilled in the art.

Pharmaceutical Formulations of Disclosed Antibodies with Tumor Vaccines

A combined therapeutic product or formulation of a disclosed anti-PD-1 antibody with a therapeutic vaccine provides synergistic oncologic therapeutic benefit. For example, the present disclosure provides a combination of a disclosed anti-PD-1 antibody with "Neuvax" which is a E75-derived 9 mer synthetic peptide isolated from HER2/neu combined with GM-CSF as an adjuvant as described in U.S. Patent 8,222,214, the disclosure of which is incorporated by reference herein. In addition, the present disclosure provides a combination of a disclosed anti-PD-1 antibody with ALVAC-CEA vaccine, which is a canary pox virus combined with carcinoembryonic antigen.

Exemplary Uses

The PD-1 binding proteins described herein and their related variants are useful in a number of therapeutic and diagnostic applications. These include the inhibition of the biological activity of PD-1 by competing for or blocking the binding to a PD-1 as well as the delivery of cytotoxic or imaging moieties to cells, preferably cells expressing PD-1. The small size and stable structure of these molecules can be particularly valuable with respect to manufacturing of the drug, rapid clearance from the body for certain applications where rapid clearance is desired or formulation into novel delivery systems that are suitable or improved using a molecule with such characteristics.

On the basis of their efficacy as inhibitors of PD-1 biological activity, the polypeptides of this disclosure are effective against a number of cancer conditions as well as complications arising from cancer, such as pleural effusion and ascites. Preferably, the PD-1-binding polypeptides of the disclosure can be used for the treatment of prevention of hyperproliferative diseases or cancer and the metastatic spread of cancers. Preferred indications for the disclosed anti-PD-1 antibodies include colorectal cancers, head and neck cancers, small cell lung cancer, non-small cell lung cancer (NSCLC) and pancreatic cancer. Non-limiting examples of cancers include bladder, blood, bone, brain, breast, cartilage, colon kidney, liver, lung, lymph node, nervous tissue, ovary, pancreatic, prostate, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, uterus, or vaginal cancer.

In addition, various inflammatory disorders can be treated with the disclosed anti-PD-1 binding polypeptides disclosed herein. Such inflammatory disorders include, for example, intestinal mucosa inflammation wasting diseases associated with colitis, multiple sclerosis, systemic lupus erythematosus, viral infections, rheumatoid arthritis, osteoarthritis, psoriasis, and Crohn's disease.

A PD-1 binding polypeptide can be administered alone or in combination with one or more additional therapies such as chemotherapy radiotherapy, immunotherapy, surgical

intervention, or any combination of these. Long-term therapy is equally possible as is adjuvant therapy in the context of other treatment strategies, as described above.

The disclosed anti-PD-1 antibodies agents can be used alone or used in combination with other conventional anti-cancer therapeutic approaches directed to treatment or prevention of proliferative disorders (*e.g.*, tumor). For example, such methods can be used in prophylactic cancer prevention, prevention of cancer recurrence and metastases after surgery, and as an adjuvant of other conventional cancer therapy. The present disclosure recognizes that the effectiveness of conventional cancer therapies (*e.g.*, chemotherapy, radiation therapy, phototherapy, immunotherapy, and surgery) can be enhanced through the use of a subject polypeptide therapeutic agent.

A wide array of conventional compounds have been shown to have anti-neoplastic activities. These compounds have been used as pharmaceutical agents in chemotherapy to shrink solid tumors, prevent metastases and further growth, or decrease the number of malignant cells in leukemic or bone marrow malignancies. Although chemotherapy has been effective in treating various types of malignancies, many anti-neoplastic compounds induce undesirable side effects. It has been shown that when two or more different treatments are combined, the treatments may work synergistically and allow reduction of dosage of each of the treatments, thereby reducing the detrimental side effects exerted by each compound at higher dosages. In other instances, malignancies that are refractory to a treatment may respond to a combination therapy of two or more different treatments.

When a polypeptide therapeutic agent is administered in combination with another conventional anti-neoplastic agent, either concomitantly or sequentially, such therapeutic agent may be found to enhance the therapeutic effect of the anti-neoplastic agent or overcome cellular resistance to such anti-neoplastic agent. This allows decrease of dosage of an anti-neoplastic agent, thereby reducing the undesirable side effects, or restores the effectiveness of an anti-neoplastic agent in resistant cells.

Pharmaceutical compounds that may be used for combinatory anti-tumor therapy include, for example: aminoglutethimide, amsacrine, anastrozole, asparaginase, bcg, bicalutamide, bleomycin, buserelin, busulfan, campothecin, capecitabine, carboplatin, carmustine, chlorambucil, cisplatin, cladribine, clodronate, colchicine, cyclophosphamide, cyproterone, cytarabine, dacarbazine, dactinomycin, daunorubicin, dienestrol, diethylstilbestrol, docetaxel, doxorubicin, epirubicin, estradiol, estramustine, etoposide, exemestane, filgrastim, fludarabine, fludrocortisone, fluorouracil, fluoxymesterone, flutamide, gemcitabine, genistein, goserelin, hydroxyurea, idarubicin, ifosfamide, imatinib, interferon,

irinotecan, ironotecan, letrozole, leucovorin, leuprolide, levamisole, lomustine, mechlorethamine, medroxyprogesterone, megestrol, melphalan, mercaptopurine, mesna, methotrexate, mitomycin, mitotane, mitoxantrone, nilutamide, nocodazole, octreotide, oxaliplatin, paclitaxel, pamidronate, pentostatin, plicamycin, porfimer, procarbazine, raltitrexed, rituximab, streptozocin, suramin, tamoxifen, temozolomide, teniposide, testosterone, thioguanine, thiotepa, titanocene dichloride, topotecan, trastuzumab, tretinoin, vinblastine, vincristine, vindesine, and vinorelbine.

Certain chemotherapeutic anti-tumor compounds may be categorized by their mechanism of action into, for example, following groups: anti-metabolites/anti-cancer agents, such as pyrimidine analogs (5-fluorouracil, floxuridine, capecitabine, gemcitabine and cytarabine) and purine analogs, folate antagonists and related inhibitors (mercaptopurine, thioguanine, pentostatin and 2-chlorodeoxyadenosine (cladribine)); antiproliferative/antimitotic agents including natural products such as vinca alkaloids (vinblastine, vincristine, and vinorelbine), microtubule disruptors such as taxane (paclitaxel, docetaxel), vincristin, vinblastin, nocodazole, epothilones and navelbine, epidipodophyllotoxins (etoposide, teniposide), DNA damaging agents (actinomycin, amsacrine, anthracyclines, bleomycin, busulfan, camptothecin, carboplatin, chlorambucil, cisplatin, cyclophosphamide, cytoxan, dactinomycin, daunorubicin, doxorubicin, epirubicin, hexamethylmelamineoxaliplatin, iphosphamide, melphalan, merchlorehtamine, mitomycin, mitoxantrone, nitrosourea, plicamycin, procarbazine, taxol, taxotere, teniposide, triethylenethiophosphoramide and etoposide (VP16)); antibiotics such as dactinomycin (actinomycin D), daunorubicin, doxorubicin (adriamycin), idarubicin, anthracyclines, mitoxantrone, bleomycins, plicamycin (mithramycin) and mitomycin; enzymes (L-asparaginase which systemically metabolizes L-asparagine and deprives cells which do not have the capacity to synthesize their own asparagine); antiplatelet agents; antiproliferative/antimitotic alkylating agents such as nitrogen mustards (mechlorethamine, cyclophosphamide and analogs, melphalan, chlorambucil), ethylenimines and methylmelamines (hexamethylmelamine and thiotepa), alkyl sulfonates-busulfan, nitrosoureas (carmustine (BCNU) and analogs, streptozocin), trazenes--dacarbazinine (DTIC); antiproliferative/antimitotic antimetabolites such as folic acid analogs (methotrexate); platinum coordination complexes (cisplatin, carboplatin), procarbazine, hydroxyurea, mitotane, aminoglutethimide; hormones, hormone analogs (estrogen, tamoxifen, goserelin, bicalutamide, nilutamide) and aromatase inhibitors (letrozole, anastrozole); anticoagulants (heparin, synthetic heparin salts and other inhibitors of thrombin); fibrinolytic agents (such as tissue plasminogen activator, streptokinase and urokinase), aspirin, dipyridamole, ticlopidine, clopidogrel,

abciximab; antimigratory agents; antisecretory agents (breveldin); immunosuppressives (cyclosporine, tacrolimus (FK-506), sirolimus (rapamycin), azathioprine, mycophenolate mofetil); anti-angiogenic compounds (TNP-470, genistein) and growth factor inhibitors (*e.g.*, VEGF inhibitors, fibroblast growth factor (FGF) inhibitors); angiotensin receptor blocker; nitric oxide donors; anti-sense oligonucleotides; antibodies (trastuzumab); cell cycle inhibitors and differentiation inducers (tretinoin); mTOR inhibitors, topoisomerase inhibitors (doxorubicin (adriamycin), amsacrine, camptothecin, daunorubicin, dactinomycin, eniposide, epirubicin, etoposide, idarubicin and mitoxantrone, topotecan, irinotecan), corticosteroids (cortisone, dexamethasone, hydrocortisone, methylpednisolone, prednisone, and prenisolone); growth factor signal transduction kinase inhibitors; mitochondrial dysfunction inducers and caspase activators; and chromatin disruptors.

Depending on the nature of the combinatory therapy, administration of the polypeptide therapeutic agents may be continued while the other therapy is being administered and/or thereafter. Administration of the polypeptide therapeutic agents may be made in a single dose, or in multiple doses. In some instances, administration of the polypeptide therapeutic agents is commenced at least several days prior to the conventional therapy, while in other instances, administration is begun either immediately before or at the time of the administration of the conventional therapy.

The PD-1 binding polypeptides are further attached to a label that is able to be detected (*e.g.*, the label can be a radioisotope, fluorescent compound, enzyme or enzyme co-factor). The active moiety may be a radioactive agent, such as: radioactive heavy metals such as iron chelates, radioactive chelates of gadolinium or manganese, positron emitters of oxygen, nitrogen, iron, carbon, or gallium, ^{43}K, ^{52}Fe, ^{57}Co, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{123}I, ^{125}I, ^{131}I, ^{132}I, or ^{99}Tc. A binding agent affixed to such a moiety may be used as an imaging agent and is administered in an amount effective for diagnostic use in a mammal such as a human and the localization and accumulation of the imaging agent is then detected. The localization and accumulation of the imaging agent may be detected by radioscintigraphy, nuclear magnetic resonance imaging, computed tomography or positron emission tomography. Immunoscintigraphy using PD-1 binding polypeptides directed at PD-1 may be used to detect and/or diagnose cancers and vasculature. For example, any of the binding polypeptide against a PD-1 marker labeled with ^{99}Technetium, ^{111}Indium, or ^{125}Iodine may be effectively used for such imaging. As will be evident to the skilled artisan, the amount of radioisotope to be administered is dependent upon the radioisotope. Those having ordinary skill in the art can readily formulate the amount of the imaging agent to be administered based upon the specific activity and energy of a given

radionuclide used as the active moiety. Typically 0.1-100 millicuries per dose of imaging agent, preferably 1-10 millicuries, most often 2-5 millicuries are administered. Thus, compositions according to the present invention useful as imaging agents comprising a targeting moiety conjugated to a radioactive moiety comprise 0.1-100 millicuries, in some embodiments preferably 1-10 millicuries, in some embodiments preferably 2-5 millicuries, in some embodiments more preferably 1-5 millicuries.

The PD-1 binding polypeptides can also be used to deliver additional therapeutic agents (including but not limited to drug compounds, chemotherapeutic compounds, and radiotherapeutic compounds) to a cell or tissue expressing PD-1. In one example, the PD-1 binding polypeptide is fused to a chemotherapeutic agent for targeted delivery of the chemotherapeutic agent to a tumor cell or tissue expressing PD-1.

The PD-1 binding polypeptides are useful in a variety of applications, including research, diagnostic and therapeutic applications. For instance, they can be used to isolate and/or purify receptor or portions thereof, and to study receptor structure (*e.g.*, conformation) and function.

The present disclosure also provides a method of detecting the susceptibility of a mammal to certain diseases. To illustrate, the method can be used to detect the susceptibility of a mammal to diseases which progress based on the amount of PD-1 present on cells and/or the number of PD-1-positive cells in a mammal.

Polypeptide sequences are indicated using standard one- or three-letter abbreviations. Unless otherwise indicated, each polypeptide sequence has amino termini at the left and a carboxy termini at the right; each single-stranded nucleic acid sequence, and the top strand of each double-stranded nucleic acid sequence, has a 5' termini at the left and a 3' termini at the right. A particular polypeptide sequence also can be described by explaining how it differs from a reference sequence.

The following terms, unless otherwise indicated, shall be understood to have the following meanings:

The terms "PD-1 inhibitor" and "PD-1 antagonist" are used interchangeably. Each is a molecule that detectably inhibits at least one function of PD-1. Conversely, a "PD-1 agonist" is a molecule that detectably increases at least one function of PD-1. The inhibition caused by a PD-1 inhibitor need not be complete so long as it is detectable using an assay. Any assay of a function of PD-1 can be used, examples of which are provided herein. Examples of functions of PD-1 that can be inhibited by a PD-1 inhibitor, or increased by a PD-1 agonist, include cancer cell growth or apoptosis (programmed cell death), and so on. Examples of types of PD-

1 inhibitors and PD-1 agonists include, but are not limited to, PD-1 binding polypeptides such as antigen binding proteins (*e.g.*, PD-1 inhibiting antigen binding proteins), antibodies, antibody fragments, and antibody derivatives.

The terms "peptide," "polypeptide" and "protein" each refers to a molecule comprising two or more amino acid residues joined to each other by peptide bonds. These terms encompass, *e.g.*, native and artificial proteins, protein fragments and polypeptide analogs (such as muteins, variants, and fusion proteins) of a protein sequence as well as post-translationally, or otherwise covalently or non-covalently, modified proteins. A peptide, polypeptide, or protein may be monomeric or polymeric.

A "variant" of a polypeptide (for example, an antibody) comprises an amino acid sequence wherein one or more amino acid residues are inserted into, deleted from and/or substituted into the amino acid sequence relative to another polypeptide sequence. Disclosed variants include, for example, fusion proteins.

A "derivative" of a polypeptide is a polypeptide (*e.g.*, an antibody) that has been chemically modified, *e.g.*, via conjugation to another chemical moiety (such as, for example, polyethylene glycol or albumin, *e.g.*, human serum albumin), phosphorylation, and glycosylation. Unless otherwise indicated, the term "antibody" includes, in addition to antibodies comprising two full-length heavy chains and two full-length light chains, derivatives, variants, fragments, and muteins thereof, examples of which are described below.

An "antigen binding protein" is a protein comprising a portion that binds to an antigen and, optionally, a scaffold or framework portion that allows the antigen binding portion to adopt a conformation that promotes binding of the antigen binding protein to the antigen. Examples of antigen binding proteins include antibodies, antibody fragments (*e.g.*, an antigen binding portion of an antibody), antibody derivatives, and antibody analogs. The antigen binding protein can comprise, for example, an alternative protein scaffold or artificial scaffold with grafted CDRs or CDR derivatives. Such scaffolds include, but are not limited to, antibody-derived scaffolds comprising mutations introduced to, for example, stabilize the three-dimensional structure of the antigen binding protein as well as wholly synthetic scaffolds comprising, for example, a biocompatible polymer. See, for example, Korndorfer et al., 2003, *Proteins: Structure, Function, and Bioinformatics*, Volume 53, Issue 1:121-129; Roque et al., 2004, *Biotechnol. Prog.* 20:639-654. In addition, peptide antibody mimetics ("PAMs") can be used, as well as scaffolds based on antibody mimetics utilizing fibronection components as a scaffold.

An antigen binding protein can have, for example, the structure of a naturally occurring immunoglobulin. An "immunoglobulin" is a tetrameric molecule. In a naturally occurring immunoglobulin, each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa or lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Preferably, the anti-PD-1 antibodies disclosed herein are characterized by their variable domain region sequences in the heavy V_H and light V_L amino acid sequences. The preferred antibody is A6 which is a kappa IgG antibody. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)). The variable regions of each light/heavy chain pair form the antibody binding site such that an intact immunoglobulin has two binding sites.

An antigen binding protein "specifically binds" to an antigen (*e.g.*, human PD-1) if it binds to the antigen with a dissociation constant of 1 nanomolar or less.

An "antigen binding domain, "antigen binding region," or "antigen binding site" is a portion of an antigen binding protein that contains amino acid residues (or other moieties) that interact with an antigen and contribute to the antigen binding protein's specificity and affinity for the antigen. For an antibody that specifically binds to its antigen, this will include at least part of at least one of its CDR domains.

An "epitope" is the portion of a molecule that is bound by an antigen binding protein (*e.g.*, by an antibody). An epitope can comprise non-contiguous portions of the molecule (*e.g.*, in a polypeptide, amino acid residues that are not contiguous in the polypeptide's primary sequence but that, in the context of the polypeptide's tertiary and quaternary structure, are near enough to each other to be bound by an antigen binding protein).

The "percent homology" of two polynucleotide or two polypeptide sequences is determined by comparing the sequences using the GAP computer program (a part of the GCG Wisconsin Package, version 10.3 (Accelrys, San Diego, Calif.)) using its default parameters.

A "host cell" is a cell that can be used to express a nucleic acid. A host cell can be a prokaryote, for example, *E. coli*, or it can be a eukaryote, for example, a single-celled

eukaryote (*e.g.*, a yeast or other fungus), a plant cell (*e.g.*, a tobacco or tomato plant cell), an animal cell (*e.g.*, a human cell, a monkey cell, a hamster cell, a rat cell, a mouse cell, or an insect cell) or a hybridoma. Examples of host cells include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman et al., 1981, *Cell* 23:175), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells or their derivatives such as Veggie CHO and related cell lines which grow in serum-free media (Rasmussen et al., 1998, *Cytotechnology* 28:31) or CHO strain DX-B11, which is deficient in DHFR (Urlaub et al., 1980, *Proc. Natl. Acad. Sci. USA* 77:4216-20), HeLa cells, BHK (ATCC CRL 10) cell lines, the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) (McMahan et al., 1991, *EMBO J.* 10:2821), human embryonic kidney cells such as 293,293 EBNA or MSR 293, human epidermal A431 cells, human Colo205 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HL-60, U937, HaK or Jurkat cells. Typically, a host cell is a cultured cell that can be transformed or transfected with a polypeptide-encoding nucleic acid, which can then be expressed in the host cell. The phrase "recombinant host cell" can be used to denote a host cell that has been transformed or transfected with a nucleic acid to be expressed. A host cell also can be a cell that comprises the nucleic acid but does not express it at a desired level unless a regulatory sequence is introduced into the host cell such that it becomes operably linked with the nucleic acid. It is understood that the term host cell refers not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to, e.g., mutation or environmental influence, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

Antigen binding proteins (*e.g.*, antibodies, antibody fragments, antibody derivatives, antibody muteins, and antibody variants) are polypeptides that bind to PD-1, (preferably, human PD-1). Antigen binding proteins include antigen binding proteins that inhibit a biological activity of PD-1.

The term "Fc polypeptide" includes native and mutein forms of polypeptides derived from the Fc region of an antibody. Truncated forms of such polypeptides containing the hinge region that promotes dimerization also are included. Fusion proteins comprising Fc moieties (and oligomers formed therefrom) offer the advantage of facile purification by affinity chromatography over Protein A or Protein G columns.

Antigen-binding fragments of antigen binding proteins of the invention may be produced by conventional techniques. Examples of such fragments include, but are not limited to, Fab and $F(ab')_2$ fragments.

The present disclosure provides monoclonal antibodies that bind to PD-1. Monoclonal antibodies may be produced using any technique known in the art, *e.g.*, by immortalizing spleen cells harvested from the transgenic animal after completion of the immunization schedule. The spleen cells can be immortalized using any technique known in the art, *e.g.*, by fusing them with myeloma cells to produce hybridomas. Myeloma cells for use in hybridoma-producing fusion procedures preferably are non-antibody-producing, have high fusion efficiency, and enzyme deficiencies that render them incapable of growing in certain selective media which support the growth of only the desired fused cells (hybridomas). Examples of suitable cell lines for use in mouse fusions include Sp-20, P3-X63/Ag8, P3-X63-Ag8.653, NS1/1.Ag 4 1, Sp210-Ag14, FO, NSO/U, MPC-11, MPC11-X45-GTG 1.7 and S194/5XX0 Bul; examples of cell lines used in rat fusions include R210.RCY3, Y3-Ag 1.2.3, IR983F and 48210. Other cell lines useful for cell fusions are U-266, GM1500-GRG2, LICR-LON-HMy2 and UC729-6.

Antigen binding proteins directed against PD-1 can be used, for example, in assays to detect the presence of PD-1 polypeptides, either *in vitro* or *in vivo*. The antigen binding proteins also may be employed in purifying PD-1 proteins by immunoaffinity chromatography. Blocking antigen binding proteins can be used in the methods disclosed herein. Such antigen binding proteins that function as PD-1 antagonists may be employed in treating any PD-1-induced condition, including but not limited to various cancers.

Antigen binding proteins may be employed in an *in vitro* procedure, or administered *in vivo* to inhibit PD-1-induced biological activity. Disorders caused or exacerbated (directly or indirectly) by the proteolytic activation of PD-1, examples of which are provided herein, thus may be treated. In one embodiment, the present invention provides a therapeutic method comprising *in vivo* administration of a PD-1 blocking antigen binding protein to a mammal in need thereof in an amount effective for reducing an PD-1-induced biological activity.

Antigen binding proteins include fully human monoclonal antibodies that inhibit a biological activity of PD-1.

Antigen binding proteins may be prepared by any of a number of conventional techniques. For example, they may be purified from cells that naturally express them (*e.g.*, an antibody can be purified from a hybridoma that produces it), or produced in recombinant expression systems, using any technique known in the art. See, for example, Monoclonal

Antibodies, Hybridomas: A New Dimension in Biological Analyses, Kennet et al. (eds.), Plenum Press, New York (1980); and Antibodies: A Laboratory Manual, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1988).

Any expression system known in the art can be used to make the recombinant polypeptides of the invention. In general, host cells are transformed with a recombinant expression vector that comprises DNA encoding a desired polypeptide. Among the host cells that may be employed are prokaryotes, yeast or higher eukaryotic cells. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or *bacilli*. Higher eukaryotic cells include insect cells and established cell lines of mammalian origin. Examples of suitable mammalian host cell lines include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman et al., 1981, *Cell* 23:175), L cells, 293 cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells, HeLa cells, BHK (ATCC CRL 10) cell lines, and the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) as described by McMahan et al., 1991, *EMBO J.* 10: 2821. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (Cloning Vectors: A Laboratory Manual, Elsevier, N.Y., 1985).

The transformed cells can be cultured under conditions that promote expression of the polypeptide, and the polypeptide recovered by conventional protein purification procedures. One such purification procedure includes the use of affinity chromatography, *e.g.*, over a matrix having all or a portion (*e.g.*, the extracellular domain) of PD-1 bound thereto. Polypeptides contemplated for use herein include substantially homogeneous recombinant mammalian anti-PD-1 antibody polypeptides substantially free of contaminating endogenous materials.

Antigen binding proteins may be prepared, and screened for desired properties, by any of a number of known techniques. Certain of the techniques involve isolating a nucleic acid encoding a polypeptide chain (or portion thereof) of an antigen binding protein of interest (*e.g.*, an anti-PD-1 antibody), and manipulating the nucleic acid through recombinant DNA technology. The nucleic acid may be fused to another nucleic acid of interest, or altered (*e.g.*, by mutagenesis or other conventional techniques) to add, delete, or substitute one or more amino acid residues, for example.

Single chain antibodies may be formed by linking heavy and light chain variable domain (Fv region) fragments via an amino acid bridge (short peptide linker), resulting in a single polypeptide chain. Such single-chain Fvs (scFvs) have been prepared by fusing DNA encoding a peptide linker between DNAs encoding the two variable domain polypeptides (V_L

and V_H). The resulting polypeptides can fold back on themselves to form antigen-binding monomers, or they can form multimers (*e.g.*, dimers, trimers, or tetramers), depending on the length of a flexible linker between the two variable domains (Kortt et al., 1997, *Prot. Eng.* 10:423; Kortt et al., 2001, *Biomol. Eng.* 18:95-108). By combining different V_L and V_H-comprising polypeptides, one can form multimeric scFvs that bind to different epitopes (Kriangkum et al., 2001, *Biomol. Eng.* 18:31-40). Techniques developed for the production of single chain antibodies include those described in U.S. Patent 4,946,778; Bird, 1988, *Science* 242:423; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879; Ward et al., 1989, *Nature* 334:544, de Graaf et al., 2002, *Methods Mol. Biol.* 178:379-87.

Techniques are known for deriving an antibody of a different subclass or isotype from an antibody of interest, *i.e.*, subclass switching. Thus, IgG antibodies may be derived from an IgM antibody, for example, and vice versa. Such techniques allow the preparation of new antibodies that possess the antigen-binding properties of a given antibody (the parent antibody), but also exhibit biological properties associated with an antibody isotype or subclass different from that of the parent antibody. Recombinant DNA techniques may be employed. Cloned DNA encoding particular antibody polypeptides may be employed in such procedures, *e.g.*, DNA encoding the constant domain of an antibody of the desired isotype (Lantto et al., 2002, *Methods Mol. Biol.* 178:303-16). Moreover, if an IgG4 is desired, it may also be desired to introduce a point mutation (CPSCP->CPPCP) in the hinge region (Bloom et al., 1997, *Protein Science* 6:407) to alleviate a tendency to form intra-H chain disulfide bonds that can lead to heterogeneity in the IgG4 antibodies.

In particular embodiments, antigen binding proteins of the present invention have a binding affinity (K_a) for PD-1 of at least 10^6. In other embodiments, the antigen binding proteins exhibit a K_a of at least 10^7, at least 10^8, at least 10^9, or at least 10^{10}. In another embodiment, the antigen binding protein exhibits a K_a substantially the same as that of an antibody described herein in the Examples.

In another embodiment, the present disclosure provides an antigen binding protein that has a low dissociation rate from PD-1. In one embodiment, the antigen binding protein has a K_{off} of 1×10^{-4} to $^{-1}$ or lower. In another embodiment, the K_{off} is 5×10^{-5} to $^{-1}$ or lower. In another embodiment, the K_{off} is substantially the same as an antibody described herein. In another embodiment, the antigen binding protein binds to PD-1 with substantially the same K_{off} as an antibody described herein.

In another aspect, the present disclosure provides an antigen binding protein that inhibits an activity of PD-1. In one embodiment, the antigen binding protein has an IC_{50} of

1000 nM or lower. In another embodiment, the IC_{50} is 100 nM or lower; in another embodiment, the IC_{50} is 10 nM or lower. In another embodiment, the IC_{50} is substantially the same as that of an antibody described herein in the Examples. In another embodiment, the antigen binding protein inhibits an activity of PD-1 with substantially the same IC_{50} as an antibody described herein.

In another aspect, the present disclosure provides an antigen binding protein that binds to human PD-1 expressed on the surface of a cell and, when so bound, inhibits PD-1 signaling activity in the cell without causing a significant reduction in the amount of PD-1 on the surface of the cell. Any method for determining or estimating the amount of PD-1 on the surface and/or in the interior of the cell can be used. In other embodiments, binding of the antigen binding protein to the PD-1-expressing cell causes less than about 75%, 50%, 40%, 30%, 20%, 15%, 10%, 5%, 1%, or 0.1% of the cell-surface PD-1 to be internalized.

In another aspect, the present disclosure provides an antigen binding protein having a half-life of at least one day *in vitro* or *in vivo* (*e.g.*, when administered to a human subject). In one embodiment, the antigen binding protein has a half-life of at least three days. In another embodiment, the antigen binding protein has a half-life of four days or longer. In another embodiment, the antigen binding protein has a half-life of eight days or longer. In another embodiment, the antigen binding protein is derivatized or modified such that it has a longer half-life as compared to the underivatized or unmodified antigen binding protein. In another embodiment, the antigen binding protein contains one or more point mutations to increase serum half life, such as described in WO00/09560, incorporated by reference herein.

The present disclosure further provides multi-specific antigen binding proteins, for example, bispecific antigen binding protein, *e.g.*, antigen binding protein that bind to two different epitopes of PD-1, or to an epitope of PD-1 and an epitope of another molecule, via two different antigen binding sites or regions. Moreover, bispecific antigen binding protein as disclosed herein can comprise a PD-1 binding site from one of the herein-described antibodies and a second PD-1 binding region from another of the herein-described antibodies, including those described herein by reference to other publications. Alternatively, a bispecific antigen binding protein may comprise an antigen binding site from one of the herein described antibodies and a second antigen binding site from another PD-1 antibody that is known in the art, or from an antibody that is prepared by known methods or the methods described herein.

Numerous methods of preparing bispecific antibodies are known in the art. Such methods include the use of hybrid-hybridomas as described by Milstein et al., 1983, *Nature* 305:537, and chemical coupling of antibody fragments (Brennan et al., 1985, *Science* 229:81;

Glennie et al., 1987, *J. Immunol.* 139:2367; U.S. Patent 6,010,902). Moreover, bispecific antibodies can be produced via recombinant means, for example by using leucine zipper moieties (*i.e.*, from the *Fos* and *Jun* proteins, which preferentially form heterodimers; Kostelny et al., 1992, *J. Immunol.* 148:1547) or other lock and key interactive domain structures as described in U.S. Patent 5,582,996. Additional useful techniques include those described in U.S. Patents 5,959,083; and 5,807,706.

In another aspect, the antigen binding protein comprises a derivative of an antibody. The derivatized antibody can comprise any molecule or substance that imparts a desired property to the antibody, such as increased half-life in a particular use. The derivatized antibody can comprise, for example, a detectable (or labeling) moiety (*e.g.*, a radioactive, colorimetric, antigenic or enzymatic molecule, a detectable bead (such as a magnetic or electrodense (*e.g.*, gold) bead), or a molecule that binds to another molecule (*e.g.*, biotin or streptavidin), a therapeutic or diagnostic moiety (*e.g.*, a radioactive, cytotoxic, or pharmaceutically active moiety), or a molecule that increases the suitability of the antibody for a particular use (*e.g.*, administration to a subject, such as a human subject, or other *in vivo* or *in vitro* uses). Examples of molecules that can be used to derivatize an antibody include albumin (*e.g.*, human serum albumin) and polyethylene glycol (PEG). Albumin-linked and PEGylated derivatives of antibodies can be prepared using techniques well known in the art. In one embodiment, the antibody is conjugated or otherwise linked to transthyretin (TTR) or a TTR variant. The TTR or TTR variant can be chemically modified with, for example, a chemical selected from the group consisting of dextran, poly(n-vinyl pyurrolidone), polyethylene glycols, propropylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols and polyvinyl alcohols.

Indications

The present disclosure provides methods of treating a subject. To treat, prevent, cure, relieve, or ameliorate ("treat") a disease, disorder, condition, or illness ("a condition"). Among the conditions to be treated are conditions characterized by inappropriate expression or activity of PD-1. In some such conditions, the expression or activity level is too high, and the treatment comprises administering a PD-1 antagonist. The disorders or conditions are cancer-related. In particular, those cancers include, but are not limited to, lung, ovarian and colon carcinoma and various myelomas.

Specific medical conditions and diseases that are treatable or preventable with the antigen binding proteins of this disclosure include various cancers.

Therapeutic Methods and Administration of Antigen Binding Proteins

Certain methods provided herein comprise administering a PD-1 binding antigen binding protein to a subject, thereby reducing a PD-1-induced biological response that plays a role in a particular condition. In particular embodiments, methods of the invention involve contacting endogenous PD-1 with a PD-1 binding antigen binding protein, *e.g.*, via administration to a subject or in an *ex vivo* procedure.

The term "treatment" encompasses alleviation or prevention of at least one symptom or other aspect of a disorder, or reduction of disease severity, and the like. An antigen binding protein need not effect a complete cure, or eradicate every symptom or manifestation of a disease, to constitute a viable therapeutic agent. As is recognized in the pertinent field, drugs employed as therapeutic agents may reduce the severity of a given disease state, but need not abolish every manifestation of the disease to be regarded as useful therapeutic agents. Similarly, a prophylactically administered treatment need not be completely effective in preventing the onset of a condition in order to constitute a viable prophylactic agent. Simply reducing the impact of a disease (for example, by reducing the number or severity of its symptoms, or by increasing the effectiveness of another treatment, or by producing another beneficial effect), or reducing the likelihood that the disease will occur or worsen in a subject, is sufficient. One embodiment of the invention is directed to a method comprising administering to a patient a PD-1 antagonist in an amount and for a time sufficient to induce a sustained improvement over baseline of an indicator that reflects the severity of the particular disorder.

As is understood in the pertinent field, pharmaceutical compositions comprising the antibodies and fragments thereof of the disclosure are administered to a subject in a manner appropriate to the indication. Pharmaceutical compositions may be administered by any suitable technique, including but not limited to, parenterally, topically, or by inhalation. If injected, the pharmaceutical composition can be administered, for example, via intra-articular, intravenous, intramuscular, intralesional, intraperitoneal or subcutaneous routes, by bolus injection, or continuous infusion. Localized administration, *e.g.* at a site of disease or injury is contemplated, as are transdermal delivery and sustained release from implants. Delivery by inhalation includes, for example, nasal or oral inhalation, use of a nebulizer, inhalation of the antagonist in aerosol form, and the like. Other alternatives include eyedrops; oral preparations including pills, syrups, lozenges or chewing gum; and topical preparations such as lotions, gels, sprays, and ointments.

Use of antigen binding proteins in *ex vivo* procedures also is contemplated. For example, a patient's blood or other bodily fluid may be contacted with an antigen binding

protein that binds PD-1 *ex vivo*. The antigen binding protein may be bound to a suitable insoluble matrix or solid support material.

Advantageously, antigen binding proteins are administered in the form of a composition comprising one or more additional components such as a physiologically acceptable carrier, excipient or diluent. Optionally, the composition additionally comprises one or more physiologically active agents, for example, a second inflammation- or immune-inhibiting substance, an anti-angiogenic substance, an analgesic substance, etc., non-exclusive examples of which are provided herein. In various particular embodiments, the composition comprises one, two, three, four, five, or six physiologically active agents in addition to a PD-1 binding antigen binding protein

Combination Therapy

The present disclosure provides a method of treating a subject with a PD-1 inhibiting antigen binding protein and one or more other treatments. Such a combination therapy achieves synergy or an additive effect by, for example, attacking multiple sites or molecular targets in a tumor. Types of combination therapies that can be used include inhibiting or activating (as appropriate) multiple nodes in a single disease-related pathway, multiple pathways in a target cell, and multiple cell types within a target tissue.

A combination therapy method comprises administering to the subject two, three, four, five, six, or more of the PD-1 agonists or antagonists described herein. In another embodiment, the method comprises administering to the subject two or more treatments that together inhibit or activate (directly or indirectly) PD-1-mediated signal transduction. Examples of such methods include using combinations of two or more PD-1 inhibiting antigen binding proteins, of a PD-1 inhibiting antigen binding protein and one or more other therapeutic moiety having anti-cancer properties (for example, cytotoxic agents, and/or immunomodulators), or of a PD-1 inhibiting antigen binding protein and one or more other treatments (*e.g.*, surgery, or radiation). Furthermore, one or more anti-PD-1 antibodies or antibody derivatives can be used in combination with one or more molecules or other treatments, wherein the other molecule(s) and/or treatment(s) do not directly bind to or affect PD-1, but which combination is effective for treating or preventing the condition being treated. In one embodiment, one or more of the molecule(s) and/or treatment(s) treats or prevents a condition that is caused by one or more of the other molecule(s) or treatment(s) in the course of therapy, *e.g.*, nausea, fatigue, alopecia, cachexia, insomnia, etc. In every case where a combination of molecules and/or other treatments is used, the individual molecule(s) and/or treatment(s) can be administered in any order, over any length of time, which is effective, *e.g.*, simultaneously, consecutively, or

alternately. In one embodiment, the method of treatment comprises completing a first course of treatment with one molecule or other treatment before beginning a second course of treatment. The length of time between the end of the first course of treatment and beginning of the second course of treatment can be any length of time that allows the total course of therapy to be effective, *e.g.*, seconds, minutes, hours, days, weeks, months, or even years.

Example 1

The ability of anti-PD-1 antibodies to modulate immune responsiveness was assessed using a mixed lymphocyte reaction (MLR). With this assay, the effects of an anti-PD-1 antibody (RG1H10) on cell activation and the production of both IL-2 and interferon γ were measured. The MLR was performed by culturing 10^5 purified human CD4+ cells from one donor with 10^4 monocyte derived dendritic cells prepared from another donor. To prepare the dendritic cells, purified monocytes were cultured with GM-CSF (1,000 U/ml) and IL-4 (500 U/ml) for seven days. Anti-PD-1 or control antibodies were added to the allogeneic MLR cultures at 10 μg/ml unless stated otherwise. Parallel plates were set up to allow collection of supernatants at day 2 or 3 and at day 5 to measure IL-2 and IFNγ respectively using a commercial ELISA kit (Biolegend). The remaining cells of the day 5 culture were assayed for CD25 expression as a measure of cell activation.

The results for cell activation are shown in Figure 1. With all anti-PD-1 antibodies there was an increase in cell activation. In Figure 1, the data are expressed as a percentage of test value with of the respect to that obtained in the absence of any added antibody. The ordinate value is %CD25+, as this refers to the cell activation presented as a percentage increase over medium control. In this way, the percent increase in cell activation was realized. Supernatants were collected from the cultures on day 3 to measure IL-2 (Figure 2) or on day 5 to measure interferon γ (Figure 3). In Figure 2, the value for the ordinate is IFNγ pg/ml. In Figure 3, the ordinate is IL-2 pg/ml. Production of both cytokines was enhanced by the addition of the anti-PD-1 antibodies.

Example 2

This example illustrates *in vitro* data for cellular binding EC50 measurements and cell binding specificity. More specifically, this example shows the binding characteristics for anti-PD-1 antibodies (as indicated in Figure 4) in terms of specific cell binding and the concentration at which 50% binding saturation (EC50) is reached. In this example, the anti-PD-1 antibodies are compared to the commercially available therapeutic anti-PD-1 antibody, BMS 5C4. The experimental procedure was as follows: CHO-PD-1 cells and CHO-PD-L1 cells were obtained by stably transfecting CHO-K1 cells with PD-1 cDNA ORF clone

(Origene RG210364) and PD-L1 cDNA ORF clone (Origene RG213071) respectively. After 2 weeks of Neomycin selection, clones were isolated by Fluorescence-activated cell sorting (FACS) and further expanded. ES-2 cell line was established from an ovarian carcinoma (ATCC CRL-1978) and does not express detectable levels of PD-1. For cell binding assays, 50,000 CHO-PD-1 cells, CHO-PD-L1 cells, and ES-2 cells were aliquoted into the wells of a 96-well, v-bottom plate in 100 µl FACS Buffer (PBS + 2% FBS). A twelve point, 3x dilution curve of each anti-PD-1 antibody was made in FACS Buffer starting at 50 µg/ml ($3.33x10^{-7}$ M). Cells were spun down, washed twice with FACS Buffer, and then resuspended in 25 µl of antibody solution in triplicate. After 0.5 hr incubation, cells were washed 1x with FACS Buffer and resuspended in 50 µl PE-conjugated, goat anti-human IgG (γ-chain specific) secondary antibody (Southern Biotech Cat #2040-09). Cells were further incubated for 0.5 hr and then washed 1x with FACS Buffer. Cells were resuspended in 25 µl FACS Buffer and the median fluorescence intensity in the FL2-H channel was determined using the Intellicyt HTFC flow cytometer. Data was analyzed and plotted in Graph Pad Prism using non-linear regression fit.

The antibodies used were the disclosed GA2, RG1B3, RG1H10, and RG2A7, RG2H10, SH-A4, RG4A6 and RG6B5 as compared to prior disclosed antibodies 5C4 (Bristol-Myers-Squibb/Medarex) that was obtained via in-house production from prior-disclosed antibody sequences (U.S. Patent Application US 2009/0217401; the disclosure of which is incorporated by reference herein),

Results: As shown in Figure 4 and Table 1, the cell binding EC50 for anti-PD-1 antibodies on CHO-PD-1 cells was determined to be ranging from 36 picomolar (pM) to 2802 pM. Data points on the graph are shown as the median fluorescence intensity (MFI) of positively labeled cells depending on the antibody concentration +/- Std Error. No non-specific binding was observed to cells not expressing PD-1 (CHO-PD-L1 cells and ES-2 cells).

Table 1:

	BMS 5C4	PD1 H7	PD1 GA2	PD1 RG1B3	PD1 RG1H10	PD1 RG2A7	PD1 RG2H10	PD1 SHA4	PD1 RG4A6	PD1 RG6B5
EC50 (pM)	529.9	222.5	201.5	183.4	35.8	403.0	2802.0	114.8	778.9	240.1

Example 3

This example provides an analysis of the inhibition of the binding of recombinant PD-1 to recombinant PD-L1 by anti-PD-1 antibodies (Figure 5). Briefly, an ELISA plate was coated with PD-L1-His, blocked with casein in PBS, then pre-incubated mixture of anti-PD-1 antibodies (scFv) and recombinant human PD-1 protein as added. Remaining binding of PD-1 protein to PD-L1 protein was measured.

The antibodies used were the disclosed GA1, GA2, GB1, GB6, GH1, A2, C7, H7, SH-A4, SH-A9, RG1B3, RG1H10, and RG1H11 as compared to prior disclosed antibodies 5C4 (Bristol-Myers-Squibb/Medarex) that was obtained via in-house production from prior-disclosed antibody sequences (U.S. Patent Application US 2009/0217401; the disclosure of which is incorporated by reference herein).

Results: The anti-PD-1 antibodies possess the ability to block the interaction between recombinant human PD-1 and PD-L1 proteins.

Example 4

This example provide a comparison of affinity measurements of the identified anti-PD-1 antibody RG1H10 as an immunoglobulin gamma 1 (IgG1) and immunoglobulin gamma 4 IgG4) with a stabilizing mutation in the antibody hinge region at residue 228 changing a serine to a proline (S228P) using BIAcore methodology. Briefly, a CM5 chip was coated with anti-human Fc antibody, the anti-PD1 mAb was captured, then serially-diluted recombinant human PD1 (His-tagged) protein was used in the analysis.

Result: The human anti-PD-1 antibody RG1H10 possesses excellent kinetic properties for its recognition and binding to human PD-1 protein either as an IgG1 or an IgG4 (S228P) as shown in Table 2.

mAb	**ka 1/Ms)**	**kd (1/s)**	**Kd (M)**	**Activated PBMC Binding; EC_{50} (M)**
RG1H10 (IgG1)	7.5E05	1E-03	1.3E-09	6.7E-10
RG1H10 (IgG4 S228P)	8.8E05	1.4E-03	1.6E-09	4.7E-11

Example 5

This example illustrates *in vitro* data for cellular binding EC50 measurements. More specifically, this example shows the binding characteristics for anti-PD-1 antibody RG1H10 as IgG1 or IgG4 (S228P) in terms of cell binding and the concentration at which 50% binding saturation (EC50) is reached. The experimental procedure is as follows. Peripheral blood mononuclear cells (PBMC) were added to the wells of a 24 well plate and stimulated anti-CD3 (3 ng/ml). After three days of culture the cells were harvested and added to the wells of wells of a 96-well, v-bottom plate in 100 µl FACS Buffer (PBS + 2% FBS). A twelve point, 3x dilution curve of each anti-PD-1 antibody was made in FACS Buffer starting at 10 µg/ml (0.6 $x10^{-7}$ M). Cells were spun down, washed twice with FACS Buffer, and then resuspended in 25

μl of antibody solution in triplicate. After 0.5 hr incubation, cells were washed 1x with FACS Buffer and resuspended in 50 μl PE-conjugated, goat anti-human IgG (γ-chain specific) secondary antibody (Southern Biotech Cat #2040-09). Cells were further incubated for 0.5 hr and then washed 1x with FACS Buffer. Cells were resuspended in 25 μl FACS Buffer and the median fluorescence intensity in the FL2-H channel was determined using the Intellicyt HTFC flow cytometer. Data was analyzed and plotted in Graph Pad Prism using non-linear regression fit.

Results: As shown in Table 3, the cell binding EC50 for anti-PD-1 antibodies on activated peripheral blood mononuclear cells (PBMCs) was determined to be 47 picomolar (pM) for the IgG4 (S228P) isotype and 670 pM for the IgG1 version.

Table 3

mAb	**Activated PBMC Binding; EC_{50} (M)**
RG1H10 (IgG1)	6.7E-10
RG1H10 (IgG4 S228P)	4.7E-11

Example 6

This example provides an analysis of the ability of RG1H10 to specifically bind to recombinant PD-1 but not to other related proteins (Figure 6). Briefly, an ELISA plate was coated with the appropriate recombinant proteins, namely human PD-1, cynomolgus PD-1, human CTLA-4, human CD28, and human ICOS. blocked with casein in PBS, then incubated with the anti-PD-1 antibody(RG1H10). Binding of RG1H10 was measured.

Results: The anti-PD-1 antibody RG1H10 possess excellent specific for PD-1 (either human or cynomolgus) but does not recognize proteins related to PD-1.

Example 7

The ability of the two isotypes of RG1H10, namely IgG1 and IgG4 (S228P) to modulate immune responsiveness was assessed using a mixed lymphocyte reaction (MLR). With this assay, the effects anti-PD-1 antibodies on cell activation and the production of both IL-2 and interferon γ were measured. The MLR was performed by culturing 10^5 purified human CD4+ cells from one donor with 10^4 monocyte derived dendritic cells prepared from another donor. To prepare the dendritic cells, purified monocytes were cultured with GM-CSF (1,000 U/ml) and IL-4 (500 U/ml) for seven days. Anti-PD-1 or control antibodies were added to the allogeneic MLR cultures at 10 μg/ml unless stated otherwise. Parallel plates were set up

to allow collection of supernatants at day 2 or 3 and at day 5 to measure IL-2 and IFNγ respectively using a commercial ELISA kit (Biolegend). The remaining cells of the day 5 culture were assayed for CD25 expression as a measure of cell activation.

The results for cell activation are shown in Figures 7, 8, and 9. With both anti-PD-1 antibody isotypes there was an increase in cell activation (Figure 7). The data are expressed as a percentage of test value with of the respect to that obtained in the absence of any added antibody. In this way, the percent increase in cell activation was realized. Supernatants were collected from the cultures on day 3 to measure IL-2 (Figure 8) or on day 5 to measure interferon γ (Figure 9). Production of both cytokines was similarly enhanced by the addition of the anti-PD-1 antibodies.

Example 8

The heavy chain sequence was further optimized through an affinity maturation process. Specifically, antibody RG1H10 (heavy chain SEQ ID NO. 23) was affinity matured into 12 additional heavy chain sequences (SEQ ID NOs. 37-49) listed on the Sequence Listing table below, together with light chain SEQ ID NO. 24.

Example 9

This example shows a PD-1 blocking ELISA assay using the indicated antibodies in Figure 5. ELISA Plates were coated with PD-L1-His, blocked with Casein in PBS, then added pre-incubated mixture of scFv and PD1/Fc (12.5 µL 10µg/ml PD1/Fc+ 12.5 µl phage soup). Incubated 1h, Washed 3 times with PBS. Added Anti-human Fc-HRP: 1:200 diluted in casein. Incubated 30 min, wash 3 times with PBS. Used TMB as substrate and 2M H_2SO_4 to stop the reaction. Read the O.D. 450nm. All antibodies tested except GA2 and H7 blocked PD-1 receptor.

Example 10

This example shows a PD-1 blocking assay using the indicated antibodies in Figure 5. ELISA Plates were coated with PD-L1-His, blocked with Casein in PBS, then added pre-incubated mixture of scFv and PD1/Fc(12.5 µL 0.7 µg/ml PD1/Fc+ 12.5 µl phage soup). Incubated 1h, Washed 3 times with PBS. Added Anti-human Fc-HRP: 1:500 diluted in casein. Incubated 30 min, wash 3 times with PBS. Used TMB as substrate and 2M H_2SO_4 to stop the reaction. Read the O.D. 450nm.

Example 11

This example shows the cross reactivity, or lack of cross reactivity as between human and murine anti-PD-1 antibodies disclosed herein in Figure 10. Loaded 50 µl 1 µg/ml human PD-L1/His or mouse PD1/His (control: PBS) on Ni-NTA plate, incubated 1h, and washed 3

times with PBS. Added IgGs that diluted in Casein and incubated 30 min. Wash 3 time with PBS. Added Goat anti-human Fc-HRP: 1:300 diluted in casein. Incubated 30 min, wash 3 times with PBS. Used TMB as substrate and 2M H_2SO_4 to stop the reaction. Read the O.D. 450nm.

Example 12

This example shows an epitope comparison between antibodies 5C4 and RG1H10. IgG5C4 was coated on AR2G sensor. Ran the base line with PBS. PD1/His was captured on the sensor . Then the sensor was dipped sequentially into the wells with PBS and RG1H10.

Example 12

This example shows a comparison of the affinity of the disclosed anti-PD-1 antibodies. Anti-human Fc antibody was immobilized on CM5 sensor chip with approximately 1000 RU. Antibodies (about 10 μg/ml) were captured for 60 s at a flow rate 10 μl/min.PD1/His was serially diluted in running buffer (HBS-EP). All measurements were conducted with a flow rate of 30 μL/min. Surfaces were regenerated with 3M $MgCl_2$ for 60 s. A 1:1 (Langmuir) binding model was used to fit the data.

Table 4

name	ka (1/Ms)	kd (1/s)	Rmax (RU)	KA (1/M)	KD (M)	Chi2
5C4	**2.16E5**	**1.7E-3**	**102**	**1.27E8**	**7.87E-9**	**0.145**
RG1H10	**1.3E6**	**1.04E-3**	**81.6**	**1.25E9**	**8.02E-10**	**1.52**

Example 13

This example illustrates an experiment showing target (PD-1) specificity of the RG1H10 antibody against three anti-PD-1 competitor antibodies. Using an in vitro ELISA assay with either anti-human IgG4 heavy chain or anti-human lambda light chain detection reagents, we can confirm that RG1H10 binds specifically to recombinant human and cynomolgus PD-1 but not mouse PD-1. The EC_{50} values for RG1H10 binding to human and cynomolgous PD-1 were 0.391 nM and 0.840 nM respectively, essentially identical to three reference antibodies tested, including competitor 1 (human EC_{50} 0.419 nM, cyno EC_{50} 1.02 nM), competitor 2 (human EC_{50} 0.495 nM, cyno EC_{50} 0.773 nM) and competitor 3 (human EC_{50} 0.390 nM, cyno EC_{50} 1.295 nM). In addition, RG1H10 does not bind to other structurally related family members, including human CTLA4, human CD28 and human ICOS. These results suggest that RG1H10 exhibits unique target specificity toward PD-1.

Example 14

This example illustrates an experiment showing target (PD-1) affinity of the RG1H10 antibody against three anti-PD-1 competitor antibodies using Biacore affinity characterization and cell-based binding studies. For the Biacore affinity measurements, anti-PD-1 antibodies were captured onto a protein-A immobilized CM5 sensor chip, after which monomeric recombinant human PD-1 was flowed over the chip. Under these conditions, RG1H10 exhibited an affinity (KD) of 3.2 nM, compared to competitor 1 (7.0 nM), competitor 2 (3 nM) and competitor 3 (42 nM). These studies suggest that RG1H10 exhibits equivalent target affinity to competitor molecules 1 and 2 and approximately 10-fold higher affinity over competitor 3.

Furthermore, we used a human Jurkat T cell line stably expressing high levels of human PD-1 to measure binding of RG1H10 and three reference antibodies to human PD-1 expressed on the T cell surface. Primary antibody binding to Jurkat PD-1 cells was detected by flow cytometry using either anti-heavy chain or light chain antibodies directly conjugated to fluorochromes (identical binding curves were obtained using either detection reagent). In this assay, RG1H10 bound to membrane expressed PD-1 with an EC_{50} value of 25.76 nM, compared to competitor 1 (5.18 nM), competitor 2 (4.57 nM) and competitor 3 (8.83 nM). While performing these studies, we also observed variability in the maximum number of PD-1 receptor molecules bound by various anti-PD1 antibodies, whereby RG1H10 achieved a C_{max} of 1103 mean fluorescence intensity (MFI) units, equivalent to that observed for competitor 2 (C_{max} of 1110 MFI), higher than that observed for competitor 3 (C_{max} of 789 MFI) and almost 2-fold lower than that observed for competitor 1 (C_{max} of 1906 MFI). In summary, RG1H10 binds to membrane expressed human PD-1 with similar potency to all reference antibodies tested.

Sequence Listing

	Heavy chain variable domain region	Light chain variable domain region
GA1	QVQLQQSGPGLVRPSQTLSLSCDISGDSVSSNSATW NWIRQSPSRGLEWLGRTFYRSKWYHDYALSVKSRIT INPDTSKNQFSLQLNSVSPGDTAVYFCVREDIDGRL DYWGQGTLVTVSS SEQ ID NO. 1	LPVLTQPASVSGSPGQSITISCTGTSSDVGGY NYVSWYQQHPGKAPKLMIYEVSKRPSGVP DRFSGSKSGNTASLTVSGLQAEDEADYYCSA WDDSLNADVFGGGTKLTVL SEQ ID NO. 2
GA2	QVQLVQSGSELKKPGASVKISCKASGYIFSDNGVNW VRQAPGQGLEWMGWINTKIGNPTYAQGFTGRFVF SLDTSVSTTYLQISSLQAGDTAVYYCAREHDYYYGM DVWGQGTTVTVSS SEQ ID NO. 3	QPVLTQPPSASGTPGQRVTISCSGSSSNIGT NTVNWYQQVPGTAPKLLIHGNDQRPSGVP DRFSGSKSDTSASLAITGLQSDDDADYYCSA WDDSLNADVFGGGTKLTVL SEQ ID NO. 4

GB1	MAEVQLLESGAEVKKPGASVKVSCKASGYTFTSHY MHWVRQAPGQGLEWMGVINPSGGSTSYAQKFQ GRVTMTRDTSTSTVYMDLSSLRSEDTAVYYCARRSE AYYHGMDVWGQGTTVTVSS SEQ ID NO. 5	QAVLTQPPSASATPGQRVTISCSGSDSNIGT NYVYWYQQFPGTAPQPLIYRDNQRPSGVP DRFSGSKSGTSASLAISGLRSEDEATYFCSTW DDSLNGWVFGGGTKLTVL SEQ ID NO. 6
GB6	EVQLVQSGSELKKPGASVKISCKASGYIFSDNGVNW VRQAPGQGLEWMGWINTKIGNPTYAQGFTGRFVF SLDTSISTTYLQISSLQAGDTAVYYCAREHDYYYGMD VWGQGTTVTVSS SEQ ID NO. 7	QPVLTQPRSVSGSPGQSITTSCTGTSSDVGG YNYVSWYQQHPGKAPKLMIYEVSNRPSGV SNRFSGSKSGNTASLTISGLQAEDEADYYCS SYTSSSTLEVFGTGTKVTVL SEQ ID NO. 8
GH1	QVQLVESGGGLVQPGGSLRLSCEATGFTFSRYWMH WVRQAPGKGLEWVSSISSSSSYIYYADSVKGRFTISR DNAKNSLYLQMNSLRAEDTAVYYCARDTLEYYGSGI LENAMGYYGMDVWGQGTTVTVSS SEQ ID NO. 9	SYELMQPPSVSGAPGQRVTISCTGSSSNIGA AYDVHWYQQLPGKAPKLVMFANSNRPSG VPDRFSGSKSGTSASLAITGLQAEDEADYYC QSYDISLRAYVFGTGTKLTVL SEQ ID NO. 10
A2	EVQLVESGGGLVRPGGSLRLACAASGFSFSDYYMT WIRQAPGRGLEWIAYISDSGQTVHYADSVKGRFTIS RDNTKNSLFLQVNTLRAEDTAVYYCAREDLLGYYLQ SWGQGTLVTVSS SEQ ID NO. 11	SYELMQPPSASGTPGQRVTISCSGSSSNIGT NTVNWYQHLPGTAPKLLIYSNNQRPSGVP DRFSGSKSGTSASLAISGLQSEDEADYYCAT WDDSPNGWVFGGGTKLTVL SEQ ID NO. 12
C7	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYWMS WVRQAPGKGLEWVANIKQDGSEKYYVDSVKGRFTI SRDNAKNSLYLQMNSLRAEDTAVYYCAREGEHDAF DIWGQGTMVTVSS SEQ ID NO. 13	QAVLTQPPSVSAAPGQRVTISCSGSNSNIAD TYVSWYQQLPGTAPRLLIYDNDQRPSGIPD RFSGSKSGTSATLGITGLQTGDEADYYCGT WDSSLSGVFGTGTKVTVL SEQ ID NO. 14
H7	QVQLVQSGSELKKPGASVKISCKASGYIFSDNGVNW VRQAPGQGLEWMGWINTKIGNPTYAQGFTGRFVF SLDTSVSTTYLQISSLQAGDTAVYYCAREHDYYYGM DVWGQGTTVTVSS SEQ ID NO. 15	QSVLTQPASVSGSPGQSVTISCTGSSSDVGA YNFVSWYRQYPGKAPKLLIYEVNKRPSDVP DRFSGSKFGNTASLTVSGLQADDEADYYCSS YAGSTDVFGTGTKVTVL SEQ ID NO. 16
SH-A4	QVQLVQSGSELKKPGASVKISCKASGYIFSDNGVNW VRQAPGQGLEWMGWINTKIGNPTYAQGFTGRFVF SLDTSVSTTYLQISSLQAGDTAVYYCAREHDYYYGM DVWGQGTTVTVSS SEQ ID NO. 17	LPVLTQPPSVSGTPGQRVTISCSGSSSNIGSN TVNWYQQLPGTAPKLLIYTNNQRPSGVPDR FSGSKSGTSASLAISGLQSEDEADYYCAAWD ESLNGDVFGTGTKVTVL SEQ ID NO. 18
SH-A9	QVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMH WVRQAPGQRLEWMGWINAGNGNTKYSQKFQGR VTITRDTSASTAYMELSSLRSEDTAVYYCAKVSAGTE SWFDPWGQGTLVTVSS SEQ ID NO. 19	AIRMTQSPSFLSASVGDRVTITCRTSQNIYN YLNWYQQKPGKAPELLIFVASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYFCLQDHSY PYTFGQGTKVEIK SEQ ID NO. 20
RG1B3	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISW VRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVT MTTDTSTSTAYMELRSLRSDDTAVYYCARGLYGDED YWGQGTLVTVSS SEQ ID NO. 21	LPVLTQPPSVSEVPGQRVTISCSGGISNIGSN AVNWYQHFPGKAPKLLIYYNDLLPSGVSDR FSASKSGTSASLAISGLRSEDEADYYCAAWD DNLSAYVFATGTKVTVL SEQ ID NO. 22
RG1H10	QVQLVQSGSELKKPGASVKISCKASGYIFSDNGVNW VRQAPGQGLEWMGWINTKIGNPTYAQGFTGRFVF SLDTSISTTYLQISSLQAGDTAVYYCAREHDYYYGMD VWGQGTTVTVSS SEQ ID NO. 23	QSALTQPPSASGSPGQSVTISCTGTSSDVGG YNYVSWYQHHPGKAPKLMIYEVSKRPSGVP DRFSGSKSAITASLTISGLLTEDEADYYCSAW DDSLNADVFGGGTKVTVL SEQ ID NO. 24
RG1H11	QMQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIG WVRQMPGKGLEWMGIIYPGDSDTRYSPSFQGQVT ISADKSISTAYLQWSSLKASDTAMYYCASGVTRKRYS SSWPPFDYWGQGTLVTVSS SEQ ID NO. 25	DIQMTQSPSSLSASVGDRVTITCRASQSISTY LNWYQQKPGKAPKVLITDASSLETGVPSRFS GSGSGTDFTFTISSLQPEDTATYFCQQYDDL PPTFGQGTKLEIK SEQ ID NO. 26

RG2H7	QVQLQQWGAGLLKSSETLSLSCAVYGGTFRDDHW SWIRQPPGKGLEWIGESHHTGRTIYNPSLRSRVTMS IDTSKNEFSLILRSVTAADTATYFCARGNNYVWGNQ EDFWGQGTLVTVSS SEQ ID NO. 27	QAGLTQPRSVSGSPGQSVTISCTGTSSDVG GYNYVSWYQQHPGKAPKLMIYDVTKRPSG VSNRFSGSKSGNTASLTISGLQAEDEADYYC SSYTSSSTYVFGTGTKVTVL SEQ ID NO. 28
RG2H10	QVQLQQSGPGLVRPSQTLSLSCDISGDSVSSNSATW NWIRQSPSRGLEWLGRTFYRSKWYHDYALSVKSRIT INPDTSKNQFSLQLNSVSPGDTAVYFCVREDIDGRL DYWGQGTLVTVSS SEQ ID NO. 29	QPVLTQPASVSGSPGQSITISCTGTSSDVGG YNYVSWYQQHPGKAPKLMIYDVSNRPSGV SNRFSGSKSGNTASLAITGLQSDDDADYYCS AWDDSLNADVFGGGTKLTVL SEQ ID NO. 30
RG3E12	EVQLVESGGALVQPGGSLRLSCAVSGFTFSDHYMD WVRQAPGKGLEWVARSRNKGNSYTTEYAASVRGR FTISRDDSKNSLYLQMNSLKTEDTAVYYCVRVGVVP ALDGMDVWGQGTTVTVSS SEQ ID NO. 31	QAGLTQPPSVSKGLRQTATLTCTGNSNNIG DQGAAWLQQHQGHPPRLLSYRNNNRPSGI SERLSASRSGNIASLTITGLQPEDEADYYCSA WDSSLSVWVFGGGTKLTVL SEQ ID NO. 32
RG4A6	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMH WVRQAPGKGLEWVSSISSSSSYIYYADSVKGRFTISR DNAKNSLYLQMNSLRAEDTAVYYCARDTLEYYGSGI LENAMGYYGMDVWGQGTTVTVSS SEQ ID NO. 33	QAVLTQPPSASGTPGQRVTISCSGSSSNIGS NTVNWYQQLPGTAPKLLIYSNNQRPSGVP DRFSGSKSGTSASLAISGLQSEDEADYYCAA WDDSLNGYVFGTGTKLTVL SEQ ID NO. 34
RG5D9	EVQLLESGGGVVQTGRSLRLSCSDSGSTFRSQAMH WVRQTPGKGLEWLAVTSHDGSKTYYADSVKGRFTI SRDNSKNTLYLQMNSLRGEDTAVYYCARGGRGYTY DHSFFDYWGQGTLVTVSS SEQ ID NO. 35	AIRMTQSPSTLSASVGDRVTITCRASENIRNL LAWYQQKPGKAPELLIHGASTLGTGVPSRF SGGGSGTEFTLTISSLQPDDFATYYCQQYES YFNTFGQGTKVEIK SEQ ID NO. 36
RG1H10-H2A-22-1S	QVQLVQSGSELKKPGASVKISCKASGYIFSDNGVNW VRQAPGQGLEWMGWINTKDGNPTYAQGFTGRFV FSLDTSISTTYLQISSLQAGDTAVYYCAREHDYYYGM DVWGQGTTVTVSS SEQ ID NO. 37	QSALTQPPSASGSPGQSVTISCTGTSSDVGG YNYVSWYQHHPGKAPKLMIYEVSKRPSGVP DRFSGSKSAITASLTISGLLTEDEADYYCSAW DDSLNADVFGGGTKVTVL SEQ ID NO. 24
RG1H10-H2A-27-2S	QVQLVQSGSELKKPGASVKISCKASGYIFSDNGVNW VRQAPGQGLEWMGWINTKTGNPTYAQGFTGRFV FSLDTSISTTYLQISSLQAGDTAVYYCAREHDYYYGM DVWGQGTTVTVSS SEQ ID NO. 38	QSALTQPPSASGSPGQSVTISCTGTSSDVGG YNYVSWYQHHPGKAPKLMIYEVSKRPSGVP DRFSGSKSAITASLTISGLLTEDEADYYCSAW DDSLNADVFGGGTKVTVL SEQ ID NO. 24
RG1H10-3C	QVQLVQSGSELKKPGASVKISCKASGYTFSDNGVN WVRQAPGQGLEWMGWINTKDGNPTYAQGFTGR FVFSLDTSISTTYLQISSLQAGDTAVYYCAREHDYYYG MDVWGQGTTVTVSS SEQ ID NO. 39	QSALTQPPSASGSPGQSVTISCTGTSSDVGG YNYVSWYQHHPGKAPKLMIYEVSKRPSGVP DRFSGSKSAITASLTISGLLTEDEADYYCSAW DDSLNADVFGGGTKVTVL SEQ ID NO. 24
RG1H10-16C	QVQLVQSGSELKKPGASVKISCKASGYTFSDNGVN WVRQAPGQGLEWMGWINTKTGNPTYAQGFTGR FVFSLDTSISTTYLQISSLQAGDTAVYYCAREHDYYYG MDVWGQGTTVTVSS SEQ ID NO. 40	QSALTQPPSASGSPGQSVTISCTGTSSDVGG YNYVSWYQHHPGKAPKLMIYEVSKRPSGVP DRFSGSKSAITASLTISGLLTEDEADYYCSAW DDSLNADVFGGGTKVTVL SEQ ID NO. 24
RG1H10-17C	QVQLVQSGSELKKPGASVKISCKASGYKFSDNGVN WVRQAPGQGLEWMGWINTKSGNPTYAQGFTGRF VFSLDTSISTTYLQISSLQAGDTAVYYCAREHDYYYG MDVWGQGTTVTVSS SEQ ID NO. 41	QSALTQPPSASGSPGQSVTISCTGTSSDVGG YNYVSWYQHHPGKAPKLMIYEVSKRPSGVP DRFSGSKSAITASLTISGLLTEDEADYYCSAW DDSLNADVFGGGTKVTVL SEQ ID NO. 24
RG1H10-19C	QVQLVQSGSELKKPGASVKISCKASGYKFSDNGVN WVRQAPGQGLEWMGWINTKTGNPTYAQGFTGR FVFSLDTSISTTYLQISSLQAGDTAVYYCAREHDYYYG MDVWGQGTTVTVSS SEQ ID NO. 42	QSALTQPPSASGSPGQSVTISCTGTSSDVGG YNYVSWYQHHPGKAPKLMIYEVSKRPSGVP DRFSGSKSAITASLTISGLLTEDEADYYCSAW DDSLNADVFGGGTKVTVL SEQ ID NO. 24
RG1H10-21C	QVQLVQSGSELKKPGASVKISCKASGYTFSDNGVN WVRQAPGQGLEWMGWINTKSGNPTYAQGFTGRF VFSLDTSISTTYLQISSLQAGDTAVYYCAREHDYYYG MDVWGQGTTVTVSS SEQ ID NO. 43	QSALTQPPSASGSPGQSVTISCTGTSSDVGG YNYVSWYQHHPGKAPKLMIYEVSKRPSGVP DRFSGSKSAITASLTISGLLTEDEADYYCSAW DDSLNADVFGGGTKVTVL SEQ ID NO. 24

	QVQLVQSGSELKKPGASVKISCKASGYKFSDNGVN	QSALTQPPSASGSPGQSVTISCTGTSSDVGG
	WVRQAPGQGLEWMGWINTKDGNPTYAQGFTGR	YNYVSWYQHHPGKAPKLMIYEVSKRPSGVP
RG1H10-	FVFSLDTSISTTYLQISSLQAGDTAVYYCAREHDYYYG	DRFSGSKSAITASLTISGLLTEDEADYYCSAW
23C2	MDVWGQGTTVTVSS SEQ ID NO. 44	DDSLNADVFGGGTKVTVL SEQ ID NO. 24

We claim:

1. A fully human antibody of an IgG class that binds to a PD-1 epitope with a binding affinity of at least 10^{-6}M, that has a heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, and combinations thereof.

2. The fully human antibody of claim 1, wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2 (called GA1 herein), SEQ ID NO. 3/SEQ ID NO. 4 (called GA2 herein), SEQ ID NO. 5/SEQ ID NO. 6 (called GB1 herein), SEQ ID NO. 7/SEQ ID NO. 8 (called GB6 herein), SEQ ID NO. 9/SEQ ID NO. 10 (called GH1 herein), SEQ ID NO. 11/SEQ ID NO. 12 (called A2 herein), SEQ ID NO. 13/SEQ ID NO. 14 (called C7 herein), SEQ ID NO. 15/SEQ ID NO. 16 (called H7 herein), SEQ ID NO. 17/SEQ ID NO. 18 (called SH-A4 herein), SEQ ID NO. 19/SEQ ID NO. 20 (called SH-A9 herein), SEQ ID NO. 21/SEQ ID NO. 22 (called RG1B3 herein), SEQ ID NO. 23/SEQ ID NO. 24 (called RG1H10 herein), SEQ ID NO. 25/SEQ ID NO. 26 (called RG1H11 herein), SEQ ID NO. 27/SEQ ID NO. 28 (called RG2H7 herein), SEQ ID NO. 29/SEQ ID NO. 30 (called RG2H10 herein), SEQ ID NO. 31/SEQ ID NO. 32 (called RG3E12 herein), SEQ ID NO. 33/SEQ ID NO. 34 (called RG4A6 herein), SEQ ID NO. 35/SEQ ID NO. 36 (called RG5D9 herein), SEQ ID NO. 37/SEQ ID NO. 24 (called RG1H10-H2A-22-15 herein), SEQ ID NO. 38/SEQ ID NO. 24 (called RG1H10-H2A-27-25 herein), SEQ ID NO. 39/SEQ ID NO. 24 (called RG1H10-3C herein), SEQ ID NO. 40/SEQ ID NO. 24 (called RG1H10-16C herein), SEQ ID NO. 41/SEQ ID NO. 24 (called RG1H10-17C herein), SEQ ID NO. 42/SEQ ID NO. 24 (called RG1H10-19C herein), SEQ ID NO. 43/SEQ ID NO. 24 (called RG1H10-21C herein), SEQ ID NO. 44/SEQ ID NO. 24 (called RG1H10-23C2 herein), and combinations thereof.

3. A Fab fully human antibody fragment, having a variable domain region from a heavy chain and a variable domain region from a light chain, wherein the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, and combinations thereof.

4. The fully human antibody Fab fragment of claim 3, wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 24, SEQ ID NO. 38/SEQ ID NO. 24, SEQ ID NO. 39/SEQ ID NO. 24, SEQ ID NO. 40/SEQ ID NO. 24, SEQ ID NO. 41/SEQ ID NO. 24, SEQ ID NO. 42/SEQ ID NO. 24, SEQ ID NO. 43/SEQ ID NO. 24, SEQ ID NO. 44/SEQ ID NO. 24, and combinations thereof.

5. A single chain human antibody, having a variable domain region from a heavy chain and a variable domain region from a light chain and a peptide linker connection the heavy chain and light chain variable domain regions, wherein the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID

NO. 40, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, and combinations thereof.

6. The fully human single chain antibody of claim 5, wherein the single chain fully human antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 24, SEQ ID NO. 38/SEQ ID NO. 24, SEQ ID NO. 39/SEQ ID NO. 24, SEQ ID NO. 40/SEQ ID NO. 24, SEQ ID NO. 41/SEQ ID NO. 24, SEQ ID NO. 42/SEQ ID NO. 24, SEQ ID NO. 43/SEQ ID NO. 24, SEQ ID NO. 44/SEQ ID NO. 24, and combinations thereof.

7. The fully human single chain antibody of claim 5, wherein the fully human single chain antibody has both a heavy chain variable domain region and a light chain variable domain region, wherein the single chain fully human antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 24, SEQ ID NO. 38/SEQ ID NO. 24, SEQ ID NO. 39/SEQ ID NO. 24, SEQ ID NO. 40/SEQ ID NO. 24, SEQ ID NO. 41/SEQ ID NO. 24, SEQ ID NO. 42/SEQ ID NO. 24, SEQ ID NO. 43/SEQ ID NO. 24, SEQ ID NO. 44/SEQ ID NO. 24, and combinations thereof.

8. A method for treating a broad spectrum of mammalian cancers or treating inflammatory diseases or autoimmune diseases, comprising administering an effective amount of an anti-PD-1 polypeptide, wherein the anti-PD-1 polypeptide is selected from the group consisting of a fully human antibody of an IgG class that binds to a PD-1 epitope with a binding affinity of at least 10^{-6} M, a Fab fully human antibody fragment, having a variable domain region from a heavy chain and a variable domain region from a light chain, a single chain human antibody, having a variable domain region from a heavy chain and a variable domain region from a light chain and a peptide linker connection the heavy chain and light chain variable domain regions, and combinations thereof;

wherein the fully human antibody has a heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, and combinations thereof;

wherein the Fab fully human antibody fragment has the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, and combinations thereof, and that has the light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, and combinations thereof; and

wherein the single chain human antibody has the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, and combinations thereof, and that has the light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, and combinations thereof.

9. The method for treating a broad spectrum of mammalian cancers of claim 8, wherein the fully human antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 24, SEQ ID NO. 38/SEQ ID NO. 24, SEQ ID NO. 39/SEQ ID NO. 24, SEQ ID NO. 40/SEQ ID NO. 24, SEQ ID NO. 41/SEQ ID NO. 24, SEQ ID NO. 42/SEQ ID NO. 24, SEQ ID NO. 43/SEQ ID NO. 24, SEQ ID NO. 44/SEQ ID NO. 24, and combinations thereof.

10. The method for treating a broad spectrum of mammalian cancers of claim 8, wherein the fully human antibody Fab fragment has both a heavy chain variable domain region and a light chain variable domain region wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ

ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 24, SEQ ID NO. 38/SEQ ID NO. 24, SEQ ID NO. 39/SEQ ID NO. 24, SEQ ID NO. 40/SEQ ID NO. 24, SEQ ID NO. 41/SEQ ID NO. 24, SEQ ID NO. 42/SEQ ID NO. 24, SEQ ID NO. 43/SEQ ID NO. 24, SEQ ID NO. 44/SEQ ID NO. 24, and combinations thereof.

11. The method for treating a broad spectrum of mammalian cancers of claim 8, wherein the fully human single chain antibody has both a heavy chain variable domain region and a light chain variable domain region, wherein the single chain fully human antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 24, SEQ ID NO. 38/SEQ ID NO. 24, SEQ ID NO. 39/SEQ ID NO. 24, SEQ ID NO. 40/SEQ ID NO. 24, SEQ ID NO. 41/SEQ ID NO. 24, SEQ ID NO. 42/SEQ ID NO. 24, SEQ ID NO. 43/SEQ ID NO. 24, SEQ ID NO. 44/SEQ ID NO. 24, and combinations thereof.

12. The method for treating a broad spectrum of mammalian cancers or inflammatory diseases or autoimmune diseases of claim 8, wherein the broad spectrum of mammalian cancers to be treated is selected from the group consisting of ovarian, colon, breast, lung cancers, myelomas, neuroblastic-derived CNS tumors, monocytic leukemias, B-cell derived leukemias, T-cell derived leukemias, B-cell derived lymphomas, T-cell derived lymphomas, mast cell derived tumors, and combinations thereof.

13. The method for treating a broad spectrum of mammalian cancers or inflammatory diseases or autoimmune diseases of claim 8, wherein the broad the autoimmune disease or inflammatory disease is selected from the group consisting of intestinal mucosal inflammation, wasting disease associated with colitis, multiple sclerosis, systemic lupus erythematosus, viral infections, rheumatoid arthritis, osteoarthritis, psoriasis, Cohn's disease, and inflammatory bowel disease.

Figure 1

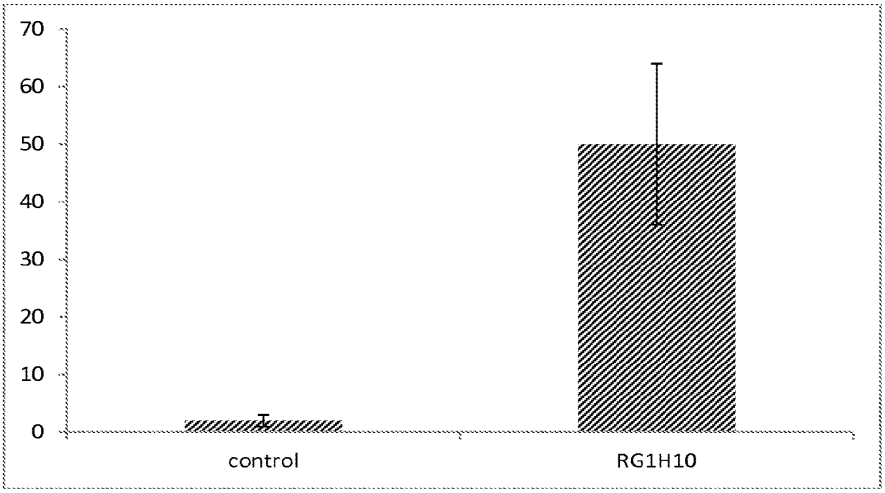


Figure 2

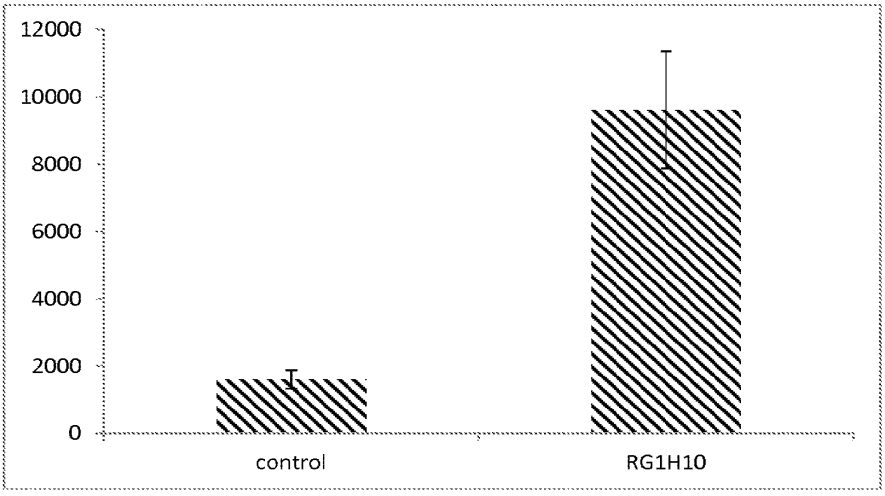


Figure 3

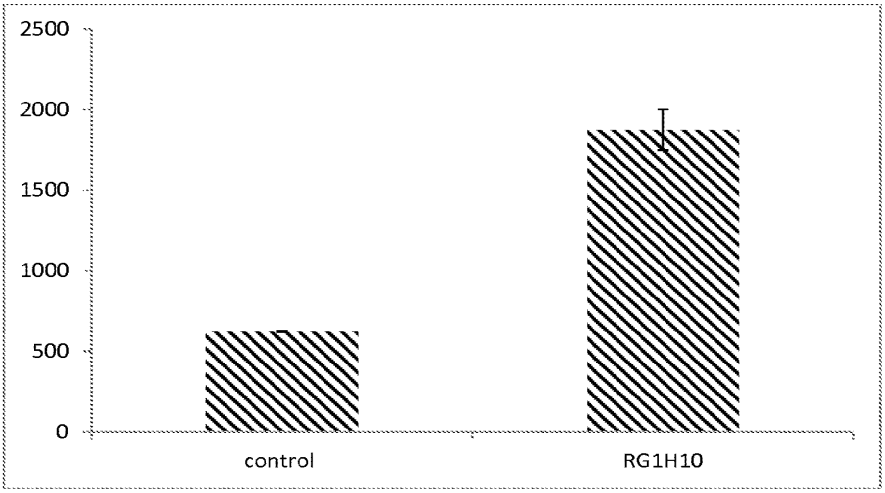


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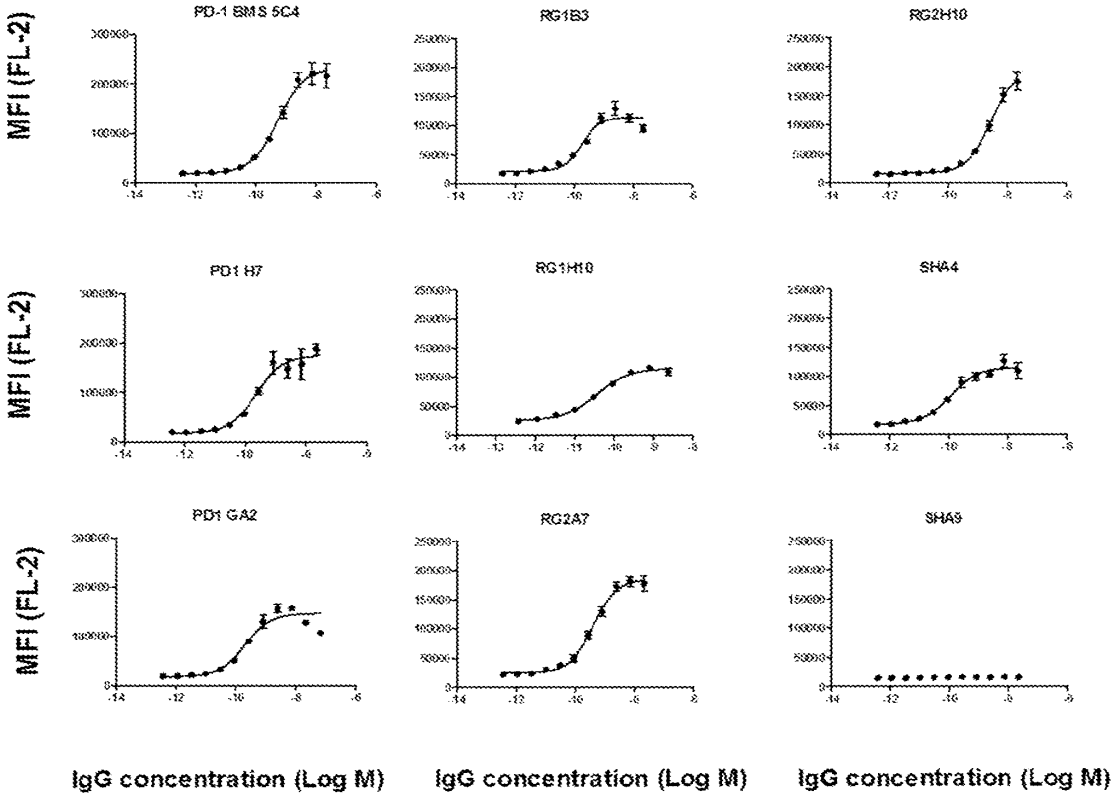
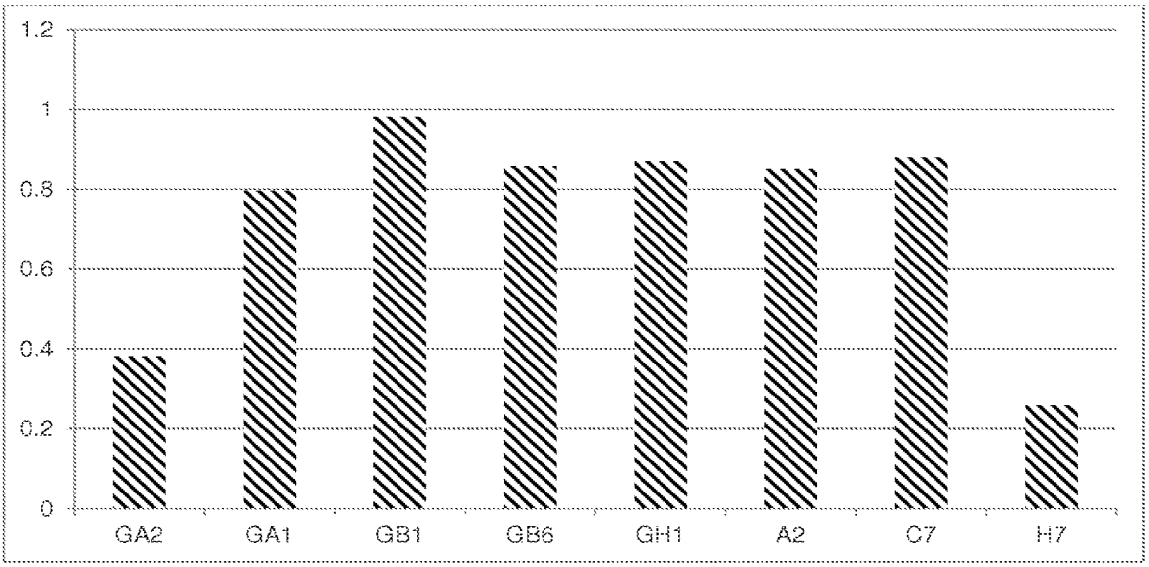

PD-1 BMS 5C4
RG1B3
RG2H10
PD1 H7
RG1H10
SHA4
PD1 GA2
RG2A7
SHA9
MFI (FL-2)
MFI (FL-2)
MFI (FL-2)
IgG concentration (Log M)
IgG concentration (Log M)
IgG concentration (Log M)

Figure 5


1.2
1
0.8
0.6
0.4
0.2
0
GA2
GA1
GB1
GB6
GH1
A2
C7
H7

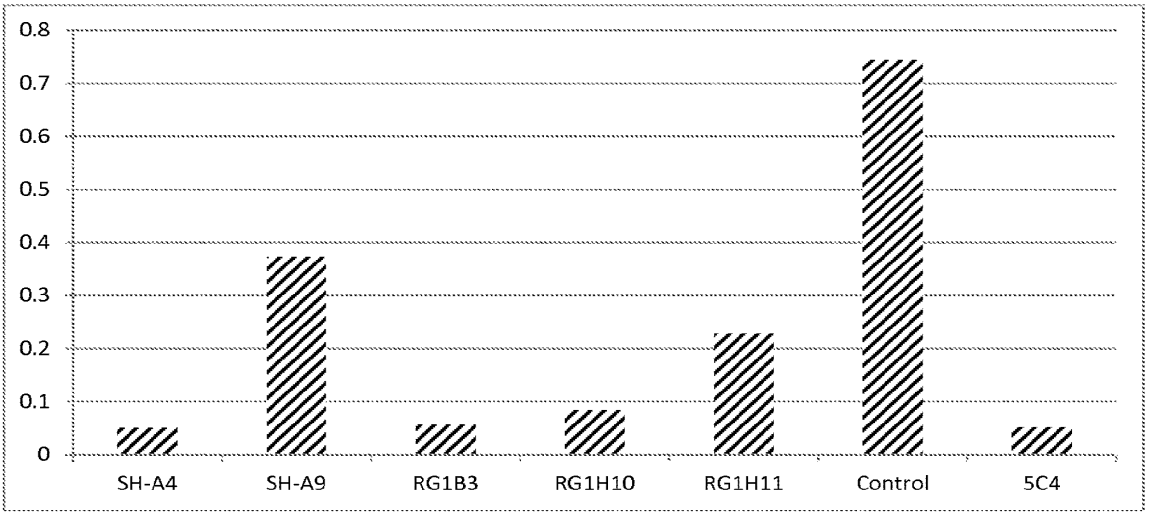

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
SH-A4
SH-A9
RG1B3
RG1H10
RG1H11
Control
5C4

Figure 6

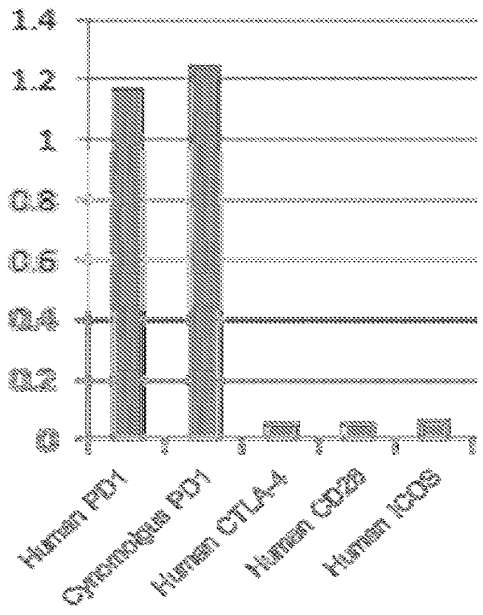

1.4
1.2
1
0.8
0.6
0.4
0.2
0
Human PD1
Cynomolgus PD1
Human CTLA-4
Human CD28
Human ICOS

Figure 7

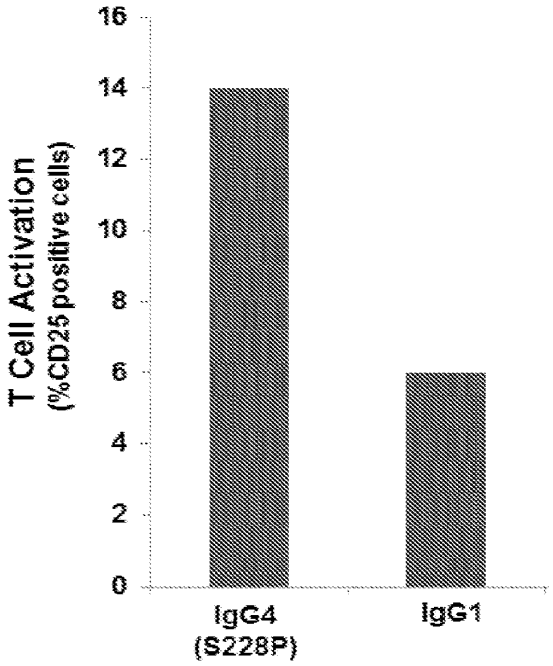

T Cell Activation
(%CD25 positive cells)
16
14
12
10
8
6
4
2
0
IgG4
(S228P)
IgG1

Figure 8

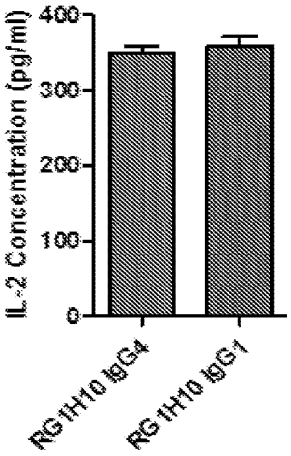


Figure 9

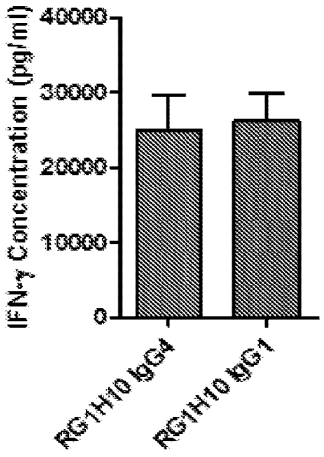


Figure 10

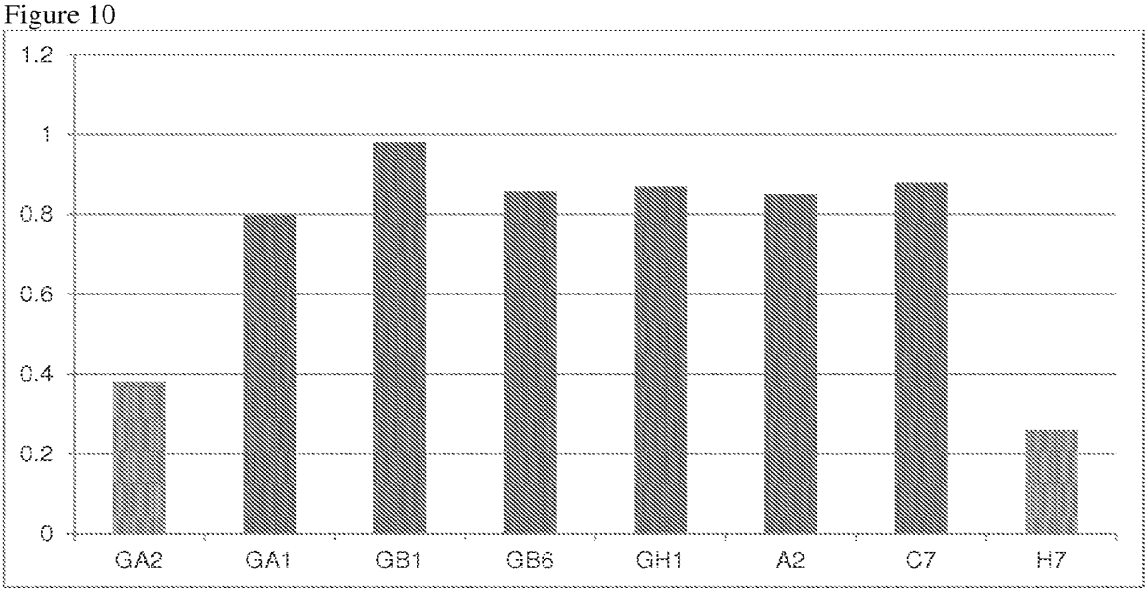


Figure 11

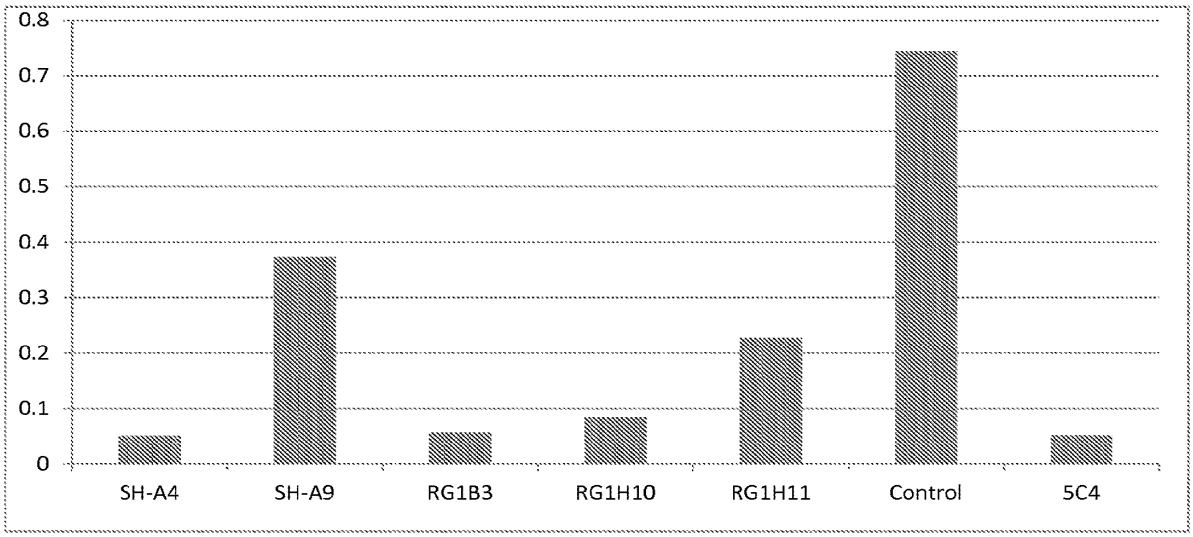
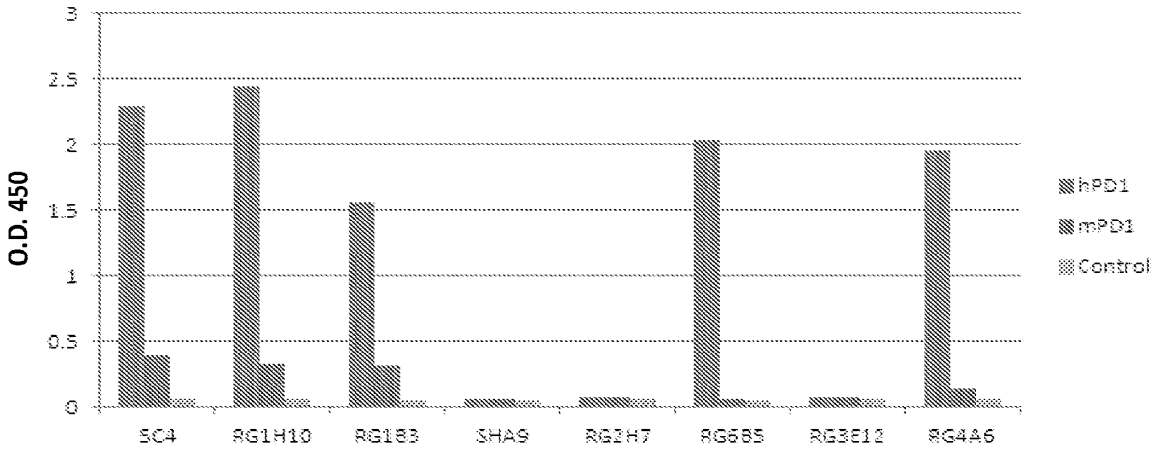

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
SH-A4
SH-A9
RG1B3
RG1H10
RG1H11
Control
5C4

Figure 12


O.D. 450
3
2.5
2
1.5
1
0.5
0
5C4
RG1H10
RG1B3
SHA9
RG2H7
RG6B5
RG3E12
RG4A6
hPD1
mPD1
Control

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国 际 局

(43) 国际公布日
2015 年 6 月 18 日 (18.06.2015)

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(54) Title: PD-1 ANTIBODY, ANTIGEN-BINDING FRAGMENT THEREOF, AND MEDICAL APPLICATION THEREOF

(54) 发明名称 : PD-1 抗体、其抗原结合片段及其医药用途

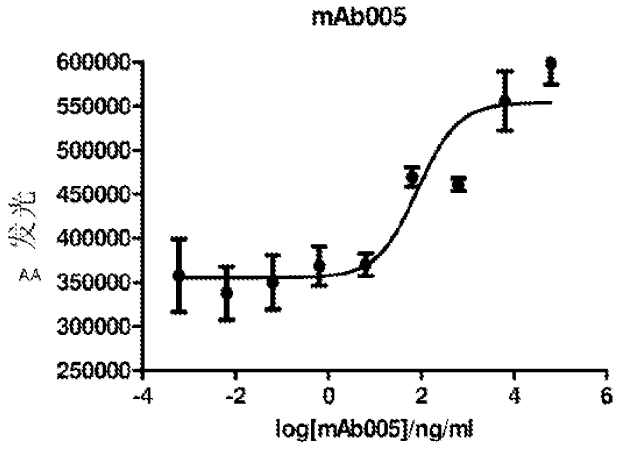


图 1 / Fig. 1

(57) Abstract: The present invention provides a human PD-1 antibody, an antigen-binding fragment thereof, and a medical application thereof, and further provides a chimeric antibody comprising a complementarity-determining region (CDR) of the antibody, a humanized antibody, a pharmaceutical composition comprising the human PD-1 antibody and the antigen-binding fragment thereof, and a purpose of the antibody in preparing medicine for treating diseases or symptoms.

(57) 摘要: 本发明提供了人 PD-1 抗体、其抗原结合片段及其医药用途，还提供了包含所述抗体 CDR 区的嵌合抗体、人源化抗体、包含人 PD-1 抗体及其抗原结合片段的药物组合物，以及所述抗体在制备用于治疗疾病或病症的药物中的用途。

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GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW。

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本国际公布:

— 包括国际检索报告(条约第 21 条(3))。

— 包括说明书序列表部分(细则 5.2(a))。

PD-1 抗体、其抗原结合片段及其医药用途

技术领域

本发明涉及一种 PD-1 抗体，PD-1 的抗原结合片段，包含所述 PD-1 抗体 CDR 区的嵌合抗体、人源化抗体，以及包含 PD-1 抗体及其抗原结合片段的药物组合物，以及其作为抗癌药物的用途。

背景技术

肿瘤免疫治疗是肿瘤治疗领域一个长时间的热点，其中 T 细胞的肿瘤免疫治疗又处于其核心位置。肿瘤免疫治疗是充分利用、调动肿瘤患者体内的杀伤性 T 细胞，对肿瘤进行杀伤作用，它可能是最有效的也是最安全的治疗肿瘤的途径。与此同时，肿瘤逃逸是肿瘤免疫治疗面临的一个巨大障碍，肿瘤细胞利用其自身对免疫系统的抑制作用促进了肿瘤的快速生长。

肿瘤的免疫逃逸机制与机体对肿瘤的免疫应答之间存在着极为复杂的关系。肿瘤免疫治疗早期肿瘤特异性的杀伤性 T 细胞是有其生物活性的，但随着肿瘤生长后期失去了杀伤的功能。所以肿瘤免疫治疗是为了最大限度的提高患者自身对肿瘤的免疫系统反应，它不但要在体内激活原有的免疫系统反应，更要维持免疫系统反应的持续时间和反应强度，才是免疫治疗肿瘤的关键。

人体内 T 细胞的活化采取了两条信号通路的系统，除了需要通过抗原呈递细胞递呈 MHC-抗原肽给 T 细胞提供第一信号外，还需要一系列协同刺激分子提供第二信号，进而才能使 T 细胞产生正常的免疫应答。这个双信号通路系统对体内免疫系统的平衡起到着至关重要的作用，它严格调控着机体对自身和非自身抗原产生不同的免疫应答。如果缺少协同刺激分子提供的第二信号，将会导致 T 细胞的无应答或持续特异性免疫应答，从而产生耐受。因此，第二信号通路在机体免疫应答的整个过程中起着非常关键的调节作用。

程序性死亡分子 1 (programmed death-1 , PD-1)是 1992 年发现的表达在 T 细胞表面的一个蛋白受体，参与到细胞的凋亡过程之中。PD-1 属于 CD28 家族，与细胞毒性 T 淋巴细胞抗原 4(cytotoxic T lymphocyte antigen 4, CTLA -4)具有 23%的氨基酸同源性，但其表达却与 CTLA 不同，主要表达在活化的 T 细胞、B 细胞和髓系细胞上。PD-1 有两个配体，分别为 PD-L1 和 PD-L2。PD-L1 的主要表达于 T 细胞、B 细胞、巨噬细胞和树突状细胞(dendritic cell, DC)上，在活化后细胞上的表达能够进行上调。而 PD-L2 的表达相对较局限，主要表达在抗原呈递细胞上，如活化的巨噬细胞和树突状细胞。

新的研究发现乳腺癌、肺癌、胃癌、肠癌、肾癌、黑素瘤等人类肿瘤组织中检测到高 PD-L1 蛋白的表达，且 PD-L1 的表达水平和患者的临床及预后紧密相关。

由于 PD-L1 起到着第二信号通路抑制 T 细胞增殖的作用，所以阻断 PD-L1 / PD-1 之间结合成为了肿瘤免疫治疗领域一个非常有潜力的新兴靶点。

目前有多家跨国制药公司在研发针对 PD-1 的单克隆抗体，它通过阻断 PD-L1 / PD-1 之间结合，最大限度的提高患者自身对肿瘤的免疫系统反应，从而达到对肿瘤细胞进行杀伤的目的。如 WO2009114335。BMS 公司和 Merck 公司的 PD-1 单克隆抗体在临床结果中，在非小细胞肺癌，黑素瘤和肾癌中都看到了一定的响应率，而且响应率和肿瘤 PD-L1 的表达有很高的关联性。这表明 PD-1 抗体对肿瘤有着积极疗效。

本发明提供有着高亲和力，高选择性，高生物活性的 PD-1 抗体。

发明内容

本发明提供一种 PD-1 抗体或其抗原结合片段，其包含：

抗体轻链可变区，所述的抗体轻链可变区至少 1 个选自如以下序列所示的 LCDR：SEQ ID NO:6，SEQ ID NO:7 或 SEQ ID NO: 8；和

抗体重链可变区，所述的抗体重链可变区至少 1 个选自如以下序列所示的 HCDR：SEQ ID NO:3，SEQ ID NO:4 或 SEQ ID NO:5。

在本发明一个优选的实施方案中，本发明提供一种 PD-1 抗体或其抗原结合片段，其中所述的抗体轻链可变区包含如 SEQ ID NO:6 所示的 LCDR1 。

在本发明一个优选的实施方案中，本发明提供一种 PD-1 抗体或其抗原结合片段，其中所述的抗体轻链可变区包含如 SEQ ID NO:7 所示的 LCDR2。

在本发明一个优选的实施方案中，本发明提供一种 PD-1 抗体或其抗原结合片段，其中所述的抗体轻链可变区包含如 SEQ ID NO:8 LCDR3 区。

在本发明一个优选的实施方案中，本发明提供一种 PD-1 抗体或其抗原结合片段，其中所述的抗体重链可变区包含如 SEQ ID NO:3 所示的 HCDR1。

在本发明一个优选的实施方案中，本发明提供一种 PD-1 抗体或其抗原结合片段，其中所述的抗体重链可变区包含如 SEQ ID NO:4 所示的 HCDR2。

在本发明一个优选的实施方案中，本发明提供一种 PD-1 抗体或其抗原结合片段，其中所述的抗体重链可变区包含如 SEQ ID NO:5 所示的 HCDR3。

在本发明一个优选的实施方案中，本发明提供一种 PD-1 抗体或其抗原结合片段，其所述的抗体轻链可变区包分别如 SEQ ID NO:6、SEQ ID NO:7 和 SEQ ID NO: 8 所示的 LCDR1、 LCDR2 和 LCDR3。

在本发明一个优选的实施方案中，本发明提供一种 PD-1 抗体或其抗原结合片段， 其所述的抗体重链可变区包含分别如 SEQ ID NO:3、SEQ ID NO:4 和 SEQ ID NO:5 所示的 HCDR1、 HCDR2 和 HCDR3。

在本发明一个优选的实施方案中，本发明提供一种 PD-1 抗体或其抗原结合片

段，其中所述的抗体轻链可变区包含分别如 SEQ ID NO:6、SEQ ID NO:7 和 SEQ ID NO: 8 所示的 LCDR1、 LCDR2 和 LCDR3；且其中所述的抗体重链可变区包含分别如 SEQ ID NO:3、SEQ ID NO:4 和 SEQ ID NO:5 所示的 HCDR1、 HCDR2 和 HCDR3。

在本发明一个优选的实施方案中，根据本发明提供的 PD-1 抗体或其抗原结合片段，其中所述的抗体为鼠源抗体或其片段。

在本发明一个优选的实施方案中，根据本发明提供的鼠源抗体或其片段，其抗体轻链可变区进一步包含鼠源 κ、λ 链或其变体的轻链 FR 区。

在本发明一个优选的实施方案中，根据本发明提供的鼠源抗体或其片段，其进一步包含鼠源 κ、λ 链或其变体的轻链恒定区。

在本发明一个优选的实施方案中，根据本发明提供的鼠源抗体或其片段，其抗体重链可变区进一步包含鼠源 IgG1, IgG2, IgG3, IgG4 或其变体的重链 FR 区。

在本发明一个优选的实施方案中，根据本发明提供的鼠源抗体或其片段，其进一步包含鼠源 IgG1, IgG2, IgG3, IgG4 或其变体的重链恒定区。

在本发明一个优选的实施方案中，根据本发明提供的 PD-1 抗体或其抗原结合片段，其中所述的抗体为嵌合抗体或其片段。

在本发明一个优选的实施方案中，根据本发明提供的 PD-1 嵌合抗体或其片段，其中所述的嵌合抗体轻链可变区序列为：SEQ ID NO:10。

在本发明一个优选的实施方案中，根据本发明提供的 PD-1 嵌合抗体或其片段，其中所述的嵌合抗体重链可变区序列为：SEQ ID NO:9。

在本发明一个优选的实施方案中，根据本发明提供的 PD-1 嵌合抗体或其片段，其进一步包含人源 κ、λ 链或其变体的轻链恒定区。

在本发明一个优选的实施方案中，根据本发明提供的 PD-1 嵌合抗体或其片段，其进一步包含人源 IgG1, IgG2, IgG3 或 IgG4 或其变体的重链恒定区，优选包含人源 IgG2 或 IgG4 重链恒定区，或者使用氨基酸突变后无 ADCC（antibody-dependent cell-mediated cytotoxicity，抗体依赖的细胞介导的细胞毒作用）毒性的 IgG1。

在本发明一个优选的实施方案中，根据本发明提供的 PD-1 抗体或其抗原结合片段，其中所述的抗体为人源化抗体或其片段。

在本发明一个优选的实施方案中，根据本发明提供的 PD-1 人源化抗体或其片段，其人源化抗体轻链可变区进一步包含人源 κ、λ 链或其变体的轻链 FR 区。

在本发明一个优选的实施方案中，根据本发明提供的 PD-1 人源化抗体或其片段，其中所述人源化抗体轻链可变区上的轻链 FR 区序列，来源于如 SEQ ID NO:14 所示的人种系轻链 IGKV1-39 和 JK4 的组合序列；其包含人种系轻链 IGKV1-39 的 FR1，FR2，FR3 区和 JK4 的 FR4 区。

在本发明一个优选的实施方案中，根据本发明提供的 PD-1 人源化抗体或其片段，其中所述人源化抗体轻链序列为如 SEQ ID NO:12 所示的序列，或其变体。

在本发明一个优选的实施方案中，根据本发明提供的 PD-1 人源化抗体或其片段，其中所述人源化抗体轻链可变区变体优选在轻链可变区有 0-10 的氨基酸变化；所述人源化抗体轻链可变区上的可变区序列变体的氨基酸变化优选为 A43S。

在本发明一个优选的实施方案中，根据本发明提供的 PD-1 人源化抗体或其片段，其进一步包含人源 κ、λ 链或其变体的轻链恒定区。

在本发明一个优选的实施方案中，根据本发明提供的 PD-1 人源化抗体或其片段，其人源化抗体重链可变区进一步包含人源 IgG1, IgG2, IgG3, IgG4 或其变体的重链 FR 区。

在本发明一个优选的实施方案中，根据本发明提供的 PD-1 人源化抗体或其片段，其中所述人源化抗体重链可变区上的重链 FR 区序列，来源于人种系重链 IgHV3-7 和 JH6 的组合序列 ：SEQ ID NO:13；包含人种系重链 IgHV3-7 的 FR1，FR2，FR3 区和 JH6 的 FR4 区。

在本发明一个优选的实施方案中，根据本发明提供的 PD-1 人源化抗体或其片段，其中所述人源化抗体重链序列为如 SEQ ID NO:11 所示的序列，或其变体；所述变体优选在重链可变区有 0-10 的氨基酸变化；更优选为 G44R 的氨基酸变化。

在本发明一个优选的实施方案中，根据本发明提供的 PD-1 人源化抗体或其片段，其进一步包含人源 IgG1, IgG2, IgG3 或 IgG4 或其变体的重链恒定区，优选包含人源 IgG2 或 IgG4 重链恒定区。因为 IgG2 或 IgG4 没有 ADCC 毒性，或者使用氨基酸突变后无 ADCC（antibody-dependent cell-mediated cytotoxicity，抗体依赖的细胞介导的细胞毒作用）毒性的 IgG1。所述的变体优选 ADCC 效应功能降低或缺失的重链恒定区突变，更优选 IgG1 的 N297A，L234A，L235A； IgG2/4chimera，IgG4 的 F235E,或 L234A/E235A 突变。

在本发明一个优选的实施方案中，根据本发明提供的 PD-1 抗体或其抗原结合片段，其中所述的抗原结合片段为 Fab、Fv、sFv、$F(ab')_2$。

本发明进一步提供一种编码如上所述的 PD-1 抗体或其抗原结合片段的 DNA 分子。

本发明进一步提供一种含有如上所述的 DNA 分子的表达载体。

本发明进一步提供一种用如上所述的表达载体转化的宿主细胞。

在本发明一个优选的实施方案中，根据本发明提供的宿主细胞，其中所述的宿主细胞为细菌，优选为大肠杆菌。

在本发明一个优选的实施方案中，本发明提供的宿主细胞为酵母菌，优选为毕赤酵母。

本发明进一步提供一种药物组合物，其含有根据本发明所述的 PD-1 抗体或其抗原结合片段和可药用的赋形剂、稀释或载体。

本发明进一步提供一种根据本发明所述的 PD-1 抗体或其抗原结合片段、或包含其的的药物组合物，在制备用于治疗 PD-1 介导的疾病或病症的药物中的用途；

其中所述的疾病优选为癌症；更优选为表达 PD-L1 的癌症；所述的癌症最优选为乳腺癌、肺癌、胃癌、肠癌、肾癌、黑素瘤；最优选为非小细胞肺癌、黑素瘤和肾癌。

本发明进一步提供一种治疗和预防 PD-1 介导的疾病或病症的方法，该方法包括给予所需患者治疗有效量的根据本发明所述的 PD-1 抗体或其抗原结合片段、或包含其的的药物组合物；其中所述的疾病优选为癌症；更优选为表达 PD-L1 的癌症；所述的癌症最优选为乳腺癌、肺癌、胃癌、肠癌、肾癌、黑素瘤、非小细胞肺癌；最优选为非小细胞肺癌、黑素瘤和肾癌。

附图说明：

图 1 ：人外周血单个核细胞增殖试验。显示待测PD-1抗体mAb005可以有效的刺激人外周血单个核细胞的增殖，EC50为83 ng/ml。

图 2： 人外周血单个核细胞细胞因子IFN-γ分泌试验。显示待测PD-1抗体mAb005可以在刺激PBMC增殖的同时，有效的刺激其细胞因子IFN-γ的分泌，EC50为13 ng/ml。

图3：本发明PD-1抗体H005-1对胶质瘤细胞生长的抑制效果。

图4：治疗后的肿瘤体积变化图。

图5：治疗后的小鼠体重变化图。

发明详述

一、术语

为了更容易理解本发明，以下具体定义了某些技术和科学术语。除显而易见在本文件中的它处另有明确定义，否则本文使用的所有其它技术和科学术语都具有本发明所属领域的一般技术人员通常理解的含义。

本发明所用氨基酸三字母代码和单字母代码如 J.biol.chem,243,p3558(1968)中所述。

本发明所述的抗体指免疫球蛋白，是由两条相同的重链和两条相同的轻链通过链间二硫键连接而成的四肽链结构。免疫球蛋白重链恒定区的氨基酸组成和排列顺序不同，故其抗原性也不同。据此，可将免疫球蛋白分为五类，或称为免疫球蛋白的同种型，即 IgM，IgD，IgG，IgA 和 IgE，其相应的重链分别为 μ 链，δ 链 γ，α 链，ε 链。同一类 Ig 根据其铰链区氨基酸组成和重链二硫键的数目和位置的差别，又可分为不同的亚类，如 IgG 可分为 IgG1，IgG2，IgG3，IgG4。轻链通过恒定区的不同分为 κ 链或 λ 链。五类 Ig 中第每类 Ig 都可以有 κ 链或 λ 链。

在本发明中，本发明所述的抗体轻链可变区可进一步包含轻链恒定区，所述的轻链恒定区包含人源或鼠源的 κ、λ 链或其变体。

在本发明中，本发明所述的抗体重链可变区可进一步包含重链恒定区，所述

的重链恒定区包含人源或鼠源的 IgG1, 2, 3, 4 或其变体。

抗体重链和轻链靠近 N 端的约 110 个氨基酸的序列变化很大，为可变区（V 区）；靠近 C 端的其余氨基酸序列相对稳定，为恒定区（C 区）。可变区包括 3 个高变区（HVR）和 4 个序列相对保守的骨架区（FR）。3 个高变区决定抗体的特异性，又称为互补性决定区（CDR）。每条轻链可变区（LCVR）和重链可变区（HCVR）由 3 个 CDR 区 4 个 FR 区组成，从氨基端到羧基端依次排列的顺序为：FR1，CDR1，FR2，CDR2，FR3，CDR3，FR4。轻链的 3 个 CDR 区指 LCDR1，LCDR2，和 LCDR3；重链的 3 个 CDR 区指 HCDR1，HCDR2 和 HCDR3。发明所述的抗体或抗原结合片段的 LCVR 区和 HCVR 区的 CDR 氨基酸残基在数量和位置符合已知的 Kabat 编号规则（LCDR1-3，HCDE2-3），或者符合 kabat 和 chothia 的编号规则（HCDR1）。

术语“鼠源抗体”在本发明中为根据本领域知识和技能制备的对人 PD-1 的单克隆抗体。制备时用 PD-1 抗原注射试验对象，然后分离表达具有所需序列或功能特性的抗体的杂交瘤。在本发明一个优选的实施方案中，所述的鼠源 PD-1 抗体或其抗原结合片段，可进一步包含鼠源 κ、λ 链或其变体的轻链恒定区，或进一步包含鼠源 IgG1, IgG2, IgG3 或 IgG4 或其变体的重链恒定区。

术语“嵌合抗体（chimeric antibody）”，是将鼠源性抗体的可变区与人抗体的恒定区融合而成的抗体，可以减轻鼠源性抗体诱发的免疫应答反应。建立嵌合抗体，要选建立分泌鼠源性特异性单抗的杂交瘤，然后从小鼠杂交瘤细胞中克隆可变区基因，再要据需要克隆人抗体的恒定区基因，将小鼠可变区基因与人恒定区基因连接成嵌合基因后插入人载体中，最后在真核工业系统或原核工业系统中表达嵌合抗体分子。在本发明一个优选的实施方案中，所述的 PD-1 嵌合抗体的抗体轻链可变区进一步包含鼠源 κ、λ 链或其变体的轻链 FR 区，抗体轻链可变区序列如 SEQ ID NO:10 所示。所述的 PD-1 嵌合抗体的抗体重链可变区进一步包含鼠源 IgG1, IgG2, IgG3, IgG4 或其变体的重链 FR 区，抗体重链可变区序列如 SEQ ID NO:10 所示。人抗体的恒定区可选自人源 IgG1, IgG2, IgG3 或 IgG4 或其变体的重链恒定区，优选包含人源 IgG2 或 IgG4 重链恒定区，或者使用氨基酸突变后无 ADCC（antibody-dependent cell-mediated cytotoxicity，抗体依赖的细胞介导的细胞毒作用）毒性的 IgG1。

术语“人源化抗体（humanized antibody）”，也称为 CDR 移植抗体（CDR-grafted antibody），是指将小鼠的 CDR 序列移植到人的抗体可变区框架，即不同类型的人种系抗体构架序列中产生的抗体。可以克服嵌合抗体由于携带大量小鼠蛋白成分，从而诱导的强烈的抗体可变抗体反应。此类构架序列可以从包括种系抗体基因序列的公共 DNA 数据库或公开的参考文献获得。如人重链和轻链可变区基因的种系 DNA 序列可以在“VBase”人种系序列数据库（在因特网 www.mrccpe.com.ac.uk/vbase 可获得），以及在 Kabat，E.A.等人，1991 Sequences of Proteins of Immunological Interest，第 5 版中找到。在本发明一个优选的实施方

案中，所述的 PD-1 人源化抗体小鼠的 CDR 序列选自 SEQ ID NO:3，4，5，6，7，8。人的抗体可变区框架经过设计选择，其中所述抗体轻链可变区上的轻链 FR 区序列，来源于人种系轻链 IGKV1-39 和 JK4 的组合序列 ：SEQ ID NO:14，包含人种系轻链 IGKV1-39 的 FR1，FR2，FR3 区和 JK4 的 FR4 区；其中所述抗体重链可变区上的重链 FR 区序列，来源于人种系重链 IgHV3-7 和 JH6 的组合序列 ：SEQ ID NO:13；包含人种系重链 IgHV3-7 的 FR1，FR2，FR3 区和 JH6 的 FR4 区。为避免免疫原性下降的同时，引起的活性下降，可对所述的人抗体可变区可进行最少反向突变，以保持活性。

本发明中所述的“抗原结合片段”，指具有抗原结合活性的 Fab 片段，Fab‘片段，F（ab’）2 片段，以及与人 PD-1 结合的 Fv 片段 sFv 片段；包含本发明所述抗体的选自 SEQ ID NO:3 至 SEQ ID NO:8 中的一个或多个 CDR 区。Fv 片段含有抗体重链可变区和轻链可变区，但没有恒定区，并具有全部抗原结合位点的最小抗体片段。一般地，Fv 抗体还包含在 VH 和 VL 结构域之间的多肽接头，且能够形成抗原结合所需的结构。也可以用不同的连接物将两个抗体可变区连接成一条多肽链，称为单链抗体（single chain antibody）或单链 Fv（sFv）。本发明的术语“与 PD-1 结合”，指能与人 PD-1 相互作用。本发明的术语“抗原结合位点”指抗原上不连续的，由本发明抗体或抗原结合片段识别的三维空间位点。

本发明中所述的“ADCC”，即 antibody-dependent cell-mediated cytotoxicity，抗体依赖的细胞介导的细胞毒作用，是指表达 Fc 受体的细胞通过识别抗体的 Fc 段直接杀伤被抗体包被的靶细胞。可通过对 IgG 上 Fc 段的修饰，降低或消除抗体的 ADCC 效应功能。所述的修饰指在抗体的重链恒定区进行突变，如选自 IgG1 的 N297A，L234A，L235A； IgG2/4chimera，IgG4 的 F235E,或 L234A/E235A 突变。

本发明中所述的融合蛋白是一种通过 DNA 重组，得到的两个基因共表达的蛋白产物。重组 PD-1 胞外区 Fc 融合蛋白通过 DNA 重组，把 PD-1 胞外区和人抗体 Fc 片段共表达的融合蛋白。所述的 PD-1 胞外区，是指 PD-1 蛋白表达在细胞膜以外的部分，序列见下面 SEQID NO.1 划线区。

生产和纯化抗体和抗原结合片段的方法在现有技术中熟知和能找到，如冷泉港的抗体实验技术指南，5-8 章和 15 章。如，小鼠可以用人 PD-1 或其片段免疫，所得到的抗体能被复性，纯化，并且可以用常规的方法进行氨基酸测序。抗原结合片段同样可以用常规方法制备。发明所述的抗体或抗原结合片段用基因工程方法在非人源的 CDR 区加上一个或多个人 FR 区。人 FR 种系序列可以从 ImMunoGeneTics(IMGT)的网站 http://imgt.cines.fr 得到，或者从免疫球蛋白杂志，2001ISBN012441351 上获得。具体的，本发明所述的抗体或抗原结合片段所用的轻链 FR 种系包括 A3 和 O2。本发明所述的抗体或抗原结合片段所用的具体的种系重链 FR 包括 VH3-21 和 VH3-23。

本发明工程化的抗体或抗原结合片段可用常规方法制备和纯化。比如，编码

重链（SEQID NO：11）和轻链（SEQID NO：12）的CDNA序列，可以克隆并重组至GS表达载体。重组的免疫球蛋白表达载体可以稳定地转染CHO细胞。作为一种更推荐的现有技术，哺乳动物类表达系统会导致抗体的糖基化，特别是在FC区的高度保守N端。通过表达与人PCSK9特异性结合的抗体得到稳定的克隆。阳性的克隆在生物反应器的无血清培养基中扩大培养以生产抗体。分泌了抗体的培养液可以用常规技术纯化。比如，用含调整过的缓冲液的A或G Sepharose FF柱进行过柱。洗去非特异性结合的组分。再用PH梯度法洗脱结合的抗体，用SDS-PAGE检测抗体片段，收集。抗体可用常规方法进行过滤浓缩。可溶的混合物和多聚体，也可以用常规方法去除，比如分子筛，离子交换。得到的产物需立即冷冻，如-70℃，或者冻干。

本发明的抗体指单克隆抗体。本发明所述的单克隆抗体或mAb，指由单一的克隆细胞株得到的抗体，所述的细胞株不限于真核的，原核的或噬菌体的克隆细胞株。单克隆抗体或抗原结合片段可以用如杂交瘤技术、重组技术、噬菌体展示技术，合成技术（如CDR-grafting），或其它现有技术进行重组得到。

“给予”和“处理”当应用于动物、人、实验受试者、细胞、组织、器官或生物流体时，是指外源性药物，治疗剂、诊断剂或组合物与动物、人、受试者、细胞、组织、器官或生物流体的接触。“给予”和“处理”可以指例如治疗、药物代谢动力学、诊断、研究和实验方法。细胞的处理包括试剂与细胞的接触，以及试剂与流体的接触，其中所述流体与细胞接触。“给予”和“处理”还意指通过试剂、诊断、结合组合物或通过另一种细胞体外和离体处理例如细胞。“处理”当应用于人、兽医学或研究受试者时，是指治疗处理、预防或预防性措施，研究和诊断应用。

“治疗”意指给予患者内用或外用治疗剂，诸如包含本发明的任一种结合化合物的组合物，所述患者具有一种或多种疾病症状，而已知所述治疗剂对这些症状具有治疗作用。通常，在受治疗患者或群体中以有效缓解一种或多种疾病症状的量给予治疗剂，无论是通过诱导这类症状退化还是抑制这类症状发展到任何临床右测量的程度。有效缓解任何具体疾病症状的治疗剂的量（也称作“治疗有效量”）可根据多种因素变化，例如患者的疾病状态、年龄和体重，以及药物在患者产生需要疗效的能力。通过医生或其它专业卫生保健人士通常用于评价该症状的严重性或进展状况的任何临床检测方法，可评价疾病症状是否已被减轻。尽本发明的实施方案（例如治疗方法或制品）在缓解每个患都有的目标疾病症状方面可能无效，但是根据本领域已知的任何统计学检验方法如Student t检验、卡方检验、依据Mann和Whitney的U检验、Kruskal-Wallis检验（H检验）、Jonckheere-Terpstra检验和Wilcoxon检验确定，其在统计学显著数目的患者中应当减轻目标疾病症状。

“保守修饰”或“保守置换或取代”是指具有类似特征（例如电荷、侧链大小、疏水性/亲水性、主链构象和刚性等）的其它氨基酸置换蛋白中的氨基酸，使

得可频繁进行改变而不改变蛋白的生物学活性。本领域技术人员知晓，一般而言，多肽的非必需区域中的单个氨基酸置换基本上不改变生物学活性（参见例如 Watson 等（1987）Molecular Biology of the Gene,The Benjamin/Cummings Pub.Co., 第 224 页，（第 4 版））。另外，结构或功能类似的氨基酸的置换不大可能破坏生物学活性。

整个说明书和权利要求书中使用的术语“基本上由……组成”或其变形表示包括所有所述元件或元件组，并且任选包括与所述元件类似或不同性质的其它元件，所述其它元件非显著改变指定给药方案、方法或组合物的基本性质或新性质。作为非限制性例子，基本上由所提及的氨基酸序列组成的结合化合物还可以包括一种或多种氨基酸，其不显著影响结合化合物的性质。

“有效量”包含足以改善或预防医学病症的症状或病症的量。有效量还意指足以允许或促进诊断的量。用于特定患者或兽医学受试者的有效量可依据以下因素而变化：如待治疗的病症、患者的总体健康情况、给药的方法途径和剂量以及副作用严重性。有效量可以是避免显著副作用或毒性作用的最大剂量或给药方案。

“外源性”指要据背景在生物、细胞或人体外产生的物质。“内源性”指根据背景在细胞，生物或人体内产生的物质。

“同源性”是指两个多核苷酸序列之间或两个多肽之间的序列相似性。当两个比较序列中的位置均被相同碱基或氨基酸单体亚基占据时，例如如果两个 DNA 分子的每一个位置都被腺嘌呤占据时，那么所述分子在该位置是同源的。两个序列之间的同源怀百分率是两个序列共有的匹配或同源位置数除以比较的位置数×100 的函数。例如，在序列最佳比对时，如果两个序列中的 10 个位置有 6 个匹配或同源，那么两个序列为 60%同源。一般而言，当比对两个序列而得到最大的同源性百分率时进行比较。

本文使用的表述“细胞”、“细胞系”和“细胞培养物”可互换使用，并且所有这类名称都包括后代。因此，单词“转化体”和“转化细胞”包括原代受试细胞和由其衍生的培养物，而不考虑转移数目。还应当理解的是，由于故意或非有意的突变，所有后代在 DNA 含量方面不可能精确相同。包括具有与最初转化细胞中筛选的相同的功能或生物学活性的突变后代。在意指不同名称的情况下，其由上下文清楚可见。

本文使用的“聚合酶链式反应”或“PCR”是指其中微量的特定部分的核酸、RNA 和/或 DNA 如在例如美国专利号 4,683,195 中所述扩增的程序或技术。一般来说，需要获得来自目标区域末端或之外的序列信息，使得可以设计寡核苷酸引物；这些引物在序列方面与待扩增模板的对应链相同或相似。2 个引物的 5’末端核苷酸可以与待扩增材料的末端一致。PCR 可用于扩增特定的 RNA 序列、来自总基因组 DNA 的特定 DNA 序列和由总细胞 RNA 转录的 cDNA、噬菌体或质粒序列等。一般参见 Mullis 等(1987) Cold Spring Harbor Symp. Ouant. Biol. 51:263; Erlich 编辑，

(1989) PCR TECHNOLOGY (Stockton Press, N.Y.)。本文使用的 PCR 被视为用于扩增核酸测试样品的核酸聚合酶反应法的一个实例，但不是唯一的实例，所述方法包括使用作为引物的已知核酸和核酸聚合酶，以扩增或产生核酸的特定部分。

"任选"或"任选地"意味着随后所描述地事件或环境可以但不必发生，该说明包括该事件或环境发生或不发生地场合。例如，"任选包含 1-3 个抗体重链可变区"意味着特定序列的抗体重链可变区可以但不必须存在。

"药物组合物"表示含有一种或多种本文所述化合物或其生理学上/可药用的盐或前体药物与其他化学组分的混合物，以及其他组分例如生理学/可药用的载体和赋形剂。药物组合物的目的是促进对生物体的给药，利于活性成分的吸收进而发挥生物活性。

具体实施方式

以下结合实施例进一步描述本发明，但这些实施例并非限制着本发明的范围。本发明实施例中未注明具体条件的实验方法，通常按照常规条件，如冷泉港的抗体技术实验手册，分子克隆手册；或按照原料或商品制造厂商所建议的条件。未注明具体来源的试剂，为市场购买的常规试剂。

实施例 1：抗体的制备

生成针对人PD-1 的鼠源单克隆抗体，用纯化的重组PD-1胞外区Fc融合蛋白（PD-1 Fc）(SEQID NO:1)；或PD-1 (SEQID NO:2)转染的CHO 细胞作为抗原，免疫Balb/C小鼠和SJL小鼠。人PD-1抗原购自ORIGENE ,货号SC117011， NCBI Reference Sequence: NM_005018.1。

PD-1 Fc，重组 PD-1 胞外区 Fc 融合蛋白 (SEQ ID NO：1)：

MDMRVPAQLLGLLLLWFPGSRCPGWFLDSPDRPWNPPTFSPALLVVTEGDNATF
TCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDF
HMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPR
PAGQFQTLVDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK。

PD-1，转染细胞的 PD-1 抗原(SEQ ID NO：2)：

MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFT
CSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFH
MSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRP
AGQFQTLVVGVVGGLLGSLVLLVWVLAVICSRAARGTIGARRTGQPLKEDPSAV
PVFSVDYGELDFQWREKTPEPPVPCVPEQTEYATIVFPSGMGTSSPARRGSADGP
RSAQPLRPEDGHCSWPL。

对于使用PD-1胞外区Fc融合蛋白的免疫，使用的纯化抗原分别为高剂量（50ug）和低剂量(10ug)， PD-1 转染的CHO 细胞免疫为0.5-1×10^7个细胞。用完全弗氏佐剂分别于第0,14,35天进行3次免疫，通过眼窝后放血监测免疫应答。通过ELISA 筛选血浆，获取有抗PD-1 人免疫球蛋白滴度的小鼠。于第56天，对有最高的抗PD-1 人免疫球蛋白滴度的小鼠进行加强免疫。3天后，处死小鼠并取出脾来进行融合。融合后的杂交瘤细胞进行抗体筛选，得到鼠单抗mAb005。鼠单抗mAb005的重链和轻链可变区序列如下：

mAb005 HCVR

EVMLVESGGGLVKPGGSLKLSCAASGFTFSSYMMSWVRQTPEKRLEWVATISG GGANTYYPDSVKGRFTISRDNAKNTLYLQMSSLRSEDTALYYCARQLYYFDYW GQGTTLTVSS SEQID NO：9

mAb005 LCVR

DIQMTQSPASQSASLGEGVTITCLASQTIGTWLTWYQQKPGKSPQLLIYTATSLA DGVPSRFSGSGSGTKFSFKISSLQAEDFVTYYCQQVYSIPWTFGGGTKLEIK SEQID NO：10

其含有下列CDR序列：

名称	序列	编号
HCDR1	SYMMS	SEQID NO：3
HCDR2	TISGGGANTYYPDSVKG	SEQID NO：4
HCDR3	QLYYFDY	SEQID NO：5
LCDR1	LASQTIGTWLT	SEQID NO：6
LCDR2	TATSLAD	SEQID NO：7
LCDR3	QQVYSIPWT	SEQID NO：8

实施例2：抗体的筛选

PD-1抗体体外ELISA结合实验：

PD-1抗体是通过和PD-1胞外区的结合，从而阻断PD-1和其配体的信号通路。体外的ELISA实验被用来检测PD-1抗体的结合特性。生物素标记的PD-1胞外区FC融合蛋白(PD-1 FC)通过和中和亲和素的结合，包被到96孔板中，抗体加入后信号的强弱被用于判断抗体和PD-1的结合特性。

用PBS缓冲液将用于和生物素结合的中和亲和素稀释至1μg/ml，以100μl/孔的体积加于96孔板中，于4℃放置16h-20h。将96孔板中PBS缓冲液吸掉，用PBST（PH7.4 PBS 含0.05% tweeen20）缓冲液洗板1次后，加入120μl/孔 PBST/1% milk，室温孵育1h进行封闭。移去封闭液，用PBST缓冲液洗板1次后，加入用PBST/1% milk稀释的1μg/ml的生物素标记 PD1-FC，置室温孵育1h。移去封闭液，用PBST缓冲液洗板3次后，加入用 PBST/1% milk稀释至合适浓度的待测PD-1抗体，置室

温孵育1.5h。移去反应体系，用PBST洗板3次后，以100μl/孔加入用 PBST/1% milk稀释HRP标记的抗鼠抗体二抗（The Jackson Laboratory），室温孵育1h。用PBST洗板3次后，加入100μl/孔TMB，于室温孵育5-10min。加入100μl/孔 1M H_2SO_4终止反应。用 NOVOStar 酶标仪在 450nm 处读取吸收值，计算ELISA结合EC_{50}值。

待测抗体	ELISA, EC50,nM	
	human PD-1	cyno PD-1
mAb005	0.25	0.27

结合结果显示抗体mAb005对人源PD-1 Fc（human PD-1）和食蟹猴PD-1 Fc（cyno PD-1）都有着非常高的结合活性。

PD-1抗体体外PD-1配体结合阻断实验：

肿瘤细胞表面的PD-L1通过和T细胞表面的PD-1的结合，从而对T细胞的增殖起到抑制的效果。PD-1的抗体能通过和PD-1的结合，而阻断PD-L1/PD-1的信号通路，进而刺激T细胞的增殖。PD-1/PD-L1的结合阻断实验用于检测PD-1抗体对于信号通路的阻断活性。

本实验中，将胞外区与FC融合的PD-1蛋白（PD-1-FC）包被96孔板后，加入待测的PD-1抗体，进行孵育反应；稍后再加入生物素标记的PD-L1，孵育反应。洗板后，检测生物素标记的PD-L1结合量，计算得到PD-1抗体对配体PD-L1结合阻断的IC_{50}值。

用PH 9.6 CB 缓冲液（1.59g Na_2CO_3和2.93g $NaHCO_3$溶于 1L 蒸馏水）将PD-1-FC 稀释至1μg/ml，以100μl/孔的体积加于96孔板中，于4℃放置16h-20h。将96孔板中PBS缓冲液吸掉，用 PBST（PH7.4 PBS含0.05% tweeen20）缓冲液洗板1次后，加入120μl/孔PBST/1% milk，室温孵育1h进行封闭。移去封闭液，用PBST缓冲液洗板1次后，加入90μl 用样品稀释液（PH7.4 PBS含5%BSA，0.05% Tween20）稀释至合适浓度的待测PD-1抗体，置4℃预孵育1h。以10μl/孔的体积加入10×浓度的生物素标记 PD-L1（北京义翘神州生物技术有限公司）（10μg/ml），在振荡器上振荡、混匀后，置37℃孵育1h。移去反应体系，用PBST洗板6次后，加入100ul/孔用 PBST 缓冲液1:400稀释的 Streptavidin－Peroxidase Polymer，室温振荡孵育50分钟。用 PBST洗板6次后，加入100μl/孔TMB，于室温孵育5-10min。加入100μl/孔 1M H_2SO_4终止反应。用NOVOStar 酶标仪在450nm处读取吸收值，计算PD-1抗体对配体PD-L1结合阻断的IC_{50}值。

待测抗体	LBB assay
	IC50,nM
mAb005	1.13

实验结果显示抗体mAb005能非常有效的阻断PD-L1与PD-1之间的结合。

实施例3：PD-1抗体体外结合选择性实验

为检测PD-1抗体对于PD-1同一家族其他蛋白的特异结合活性，human CTLA4, human CD28被用于进行结合检测。同时，为了检测PD-1抗体对于人/猴以外的不同种属的差异性，小鼠的PD-1也进行了结合检测。

用PBS缓冲液将选择性结合蛋白：human PD-1, human ICOS，human CTLA4, human CD28, mouse PD-1，（北京义翘神州生物技术有限公司），稀释至 1μg/ml，以 100μl/孔的体积加于96孔板中，于4℃放置16h-20h。将96孔板中PBS缓冲液吸掉，用 PBST（PH7.4 PBS含0.05% tweeen20）缓冲液洗板1次后，加入120μl/孔 PBST/1% milk，室温孵育1h进行封闭。移去封闭液，用PBST缓冲液洗板3次后，加入待测PD-1抗体，置室温孵育1.5h。移去反应体系，用PBST洗板3次后，以100μl/孔加入用PBST/1% milk稀释HRP标记的抗鼠抗体二抗（The Jackson Laboratory），室温孵育1小时。用PBST洗板3次后，加入 100μl/孔TMB，于室温孵育5-10min。加入100μl/孔 1M H_2SO_4终止反应。用 NOVOStar酶标仪在450nm处读取吸收值。

待测抗体	human PD1-FC	mouse PD1-Fc	human ICOS/Fc	human CTLA4	human CD28
mAb005	2.64	0.07	0.15	0.17	0.12

结果显示，抗体mAb005对PD-1家族的其他蛋白没有特异的结合活性。同时，mAb对鼠源的PD-1也没有种属交叉活性。

实施例4：PD-1抗体体外细胞结合实验

FACS （fluorescence-activated cell sorter 荧光活性细胞分选）是一个用于检测蛋白和细胞结合的试验方法。本测试用于检测本发明的PD-1抗体和细胞表面表达的天然PD-1结合活性。本测试所用的细胞为PD-1高表达的CHO细胞（见实施例1中，PD-1 (SEQID NO:2)转染的CHO 细胞）。

将PD-1高表达的CHO细胞以1000 rpm的转速离心5分钟，收集沉淀并用10-15 ml的预冷的流式缓冲液悬浮，细胞计数。用50ml的离心管中以1000 rpm的转速离心5分钟收集细胞，丢掉上清，沉淀用预冷封闭缓冲液重悬，密度为0.5-1.0×10^7 细胞/毫升。于4℃ 孵育30分钟后，重悬以每孔100μl加入到96孔板。96孔板在1500rpm的转速下离心5分钟后，弃上清。向每个孔加入100μl一抗，将细胞重悬，4℃ 避光孵育60分钟。离心弃上清，加入100μl的 1:400稀释的FITC标记二抗（BD Biosciences）。将细胞重悬，4℃避光孵育60分钟。用流式缓冲液洗两次细胞，并用1%的多聚甲醛重悬细胞进行固定，进行流式检测。

待测抗体	MFI			
	50nM	5nM	0.5nM	0.05nM
mAb005	468	319	71.2	14

结果显示抗体mAb005同样可以结合细胞表面的PD-1。

实施例5：体外结合亲和力和动力学实验

Biacore方法是一个公认的客观检测蛋白相互间亲和力和动力学的检测方法。我们通过Biacore（GE）分析本发明待测PD-1 抗体表征亲和力及结合动力学。

利用由 Biacore 提供的试剂盒，采用标准氨基偶联法将本发明待测PD-1抗体共价连接至CM5（GE）芯片上。然后将将稀释于同样缓冲液中的一系列浓度梯度的PD-1 His蛋白(北京义翘神州生物技术有限公司)于前后各个循环进样，进样后均以试剂盒内配再生试剂再生。追踪抗原-抗体结合动力学3分钟并追踪解离动力学10分钟。使用 GE 的 BIAevaluation软件以1:1（Langmuir）结合模型分析所得数据，以此法测定的ka (kon)、kd (koff)和KD值显示于下表。

待测抗体	ka (1/Ms)	kd (1/s)	KD (M)
mAb005	1.057E+5	3.769E-4	3.566E-9

结果显示抗体mAb005对PD-1的结合达到了Kd 3.57 nM。

实施例6：体外细胞学实验

新鲜人外周血单个核细胞（PBMC）在抗体作用下的增殖试验，用来对抗体mAb005进行细胞活性的检测。

新鲜人PBMC调整细胞密度为2×10^6/ml，每孔2ml接种于6孔板，37 ℃, 5%CO_2培养箱中放置6小时，将悬浮细胞吸走，向贴壁细胞中加入2ml含有100ng/ml GM-CSF（粒细胞集落刺激生物因子） 和 100 ng/ml IL-4 的 RPMI1640培养基，培养2天后每孔再加入1ml含有100ng/ml GM-CSF 和100 ng/ml IL-4的RPMI1640培养基，继续培养2天后，每孔加入100ng/ml TNF-α （肿瘤坏死因子-a），继续培养2天得到成熟树突细胞。将上述树突细胞及同种异体的T 细胞分别离心重悬成浓度为1×10^6/ml 和1×10^5/ml，于96孔板中每孔各加入100μl，抗体用PBS按一定倍数稀释成不同浓度梯度96孔板中每孔加入20μl，37 ℃，5% CO_2 培养箱中培养5天，取100μl用CellTiter-Glo® Luminescent Cell Viability Assay试剂盒检测细胞增殖， 结果见图1，显示待测PD-1抗体mAb005可以有效的刺激人外周血单个核细胞的增殖，EC50为83 ng/ml。剩余样品检测细胞因子IFN-γ的分泌，结果见图2，显示待测PD-1抗体mAb005可以在刺激PBMC增殖的同时，有效的刺激其细胞因子IFN-γ的分泌，EC50为13 ng/ml。

实施例7：小鼠抗体人源化实验

参照mAb005的抗体轻链可变区序列（mAb005 LCVR，SEQID NO：10）和重链可变区序列（mAb005 HCVR，SEQID NO：9）， 在Germline数据库中选取与其非CDR区匹配最好的人源化模板。其中抗体重链的模版为IgHV3-7/JH6，选择人种系轻链IGKV1-39的FR1，FR2，FR3，和JK4的FR4区，序列为SEQ ID NO：13；轻链的模版为IGKV1-39/JK4，选择人种系轻链IGKV1-39的FR1，FR2，FR3，和JK4的FR4，序列为SEQ ID NO：14。

人种系重链模版（SEQ ID NO：13）：

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQDGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARWGQGTTVTVSS；

人种系轻链模板（SEQ ID NO：14）：

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCFGGGTKVEIK。

将鼠源抗体CDR区移植到选择的人源化模板上，替换人源模板的CDR区，再与IgG4恒定区重组，得到人源化的抗体H005-1。然后，以鼠源抗体的三维结构为基础，对包埋残基、与CDR区有直接相互作用的残基，以及对VL和VH的构象有重要影响的残基进行回复突变，得到人源化之后的抗体H005-2，H005-3，H005-4，序列见下表。

抗体表达

H005-1 HC

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYMMSWVRQAPGKGLEWVATISGGGANTYYPDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARQLYYFDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQID NO：11

H005-1 LC

DIQMTQSPSSLSASVGDRVTITCLASQTIGTWLTWYQQKPGKAPKLLIYTATSLADGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQVYSIPWTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQID NO：12

鼠源CDR区移植的人源化抗体H005-1的HC序列为（SEQID NO：11），人源化抗体LC序列为（SEQID NO：12）。对可能影响抗体活性的位点进行点突变，其序列为：

	HC	LC
H005-1	SEQID NO：11	SEQID NO：12
H005-2	SEQID NO：11, G44R	SEQID NO：12
H005-3	SEQID NO：11	SEQID NO：12 ,A43S
H005-4	SEQID NO：11 ,G44R	SEQID NO：12 ,A43S

根据各人源化抗体轻链和重链的氨基酸序列(SEQ NO 11, SEQ NO 12及其变体)合成cDNA，用BamHI和XhoI消化后，将cDNA片段通过BamHI / XhoI酶切位点插入到pcDNA3.1表达载体（Life Technologies Cat. No. V790-20 ）中。将表达载体和转染试剂PEI（Polysciences, Inc. Cat. No. 23966）以1:2 的比例转染HEK293细胞（Life Technologies Cat. No. 11625019），并置于CO_2孵育箱中孵育4-5天。表达的抗体通过离心回收后，按常规方法进行抗体纯化，得到本发明的人源化抗体。

实施例8：人源化抗体活性数据

对人源化的抗体在体外进行了ELISA结合实验（方法步骤同实施例2），配体结合阻断实验（方法步骤同实施例2），亲和力动力学实验（方法步骤同实施例5），结果显示于下表：

待测抗体	ELISA, EC50,nM	LBB assay, IC50,nM	KD(M)
H005-1	0.11	1.27	2.79E-09
H005-2	0.14	1.27	2.98E-09
H005-3	0.15	1.33	2.45E-09
H005-4	0.14	1.36	3.89E-09

试验结果显示人源化的抗体 H005-1, H005-2, H005-3, H005-4 都保持了和 PD-1 的结合活性，它们的亲和力动力学 KD 分别为 2.79，2.98,2.45,3.89 nM。同时所有人源化抗体也能有效的对 PD-L1/PD-1 通路产生阻断活性。

实施例 9 ： PD-1 抗体对肿瘤细胞生长的抑制

一、实验材料：

U87MG 细胞（胶质瘤细胞）：购自中科院细胞库，货号 TCHu138；

PBMC 细胞(外周血单个核细胞)来自上海血液中心；

CD3: 购自 Miltenyi Biotec 货号 130-093-387；

CD28: 购自 Miltenyi Biotec 货号 130-093-375；

Cell Counting Kit-8：购自同仁化学，货号 CK04 ；

mIgG(阴性对照)：购自 SANTA CRUZ 货号：sc-2025；使用浓度为 1660ng/ml。

二、实验方法：

1. U87MG 细胞培养在含有 10% FBS、1% P/S 的 EMEM 培养基中，96 孔板中每孔加入 1×10^4 个细胞。

2. 抗体 H005-1 用 PBS 按一定倍数稀释成不同浓度梯度(如图 3 横坐标所示)，96 孔板中每孔加入 10ul，37℃、5%CO_2 培养箱中培养 4 小时。

3. 细胞贴壁后每孔加入 80ul PBMC 细胞悬液，细胞密度为 2×10^4 个/孔，每孔分别加入 10ul CD3 和 CD28 抗体，CD3 和 CD28 抗体终浓度均为 500ng/ml。

4. 37℃，5% CO_2 培养箱中培养 72 小时，每孔加入 10ul CCK8 显色，2 小时后检测 OD450。

三、实验结果：

结果如图 3 所示，与 mIgG(阴性对照)相比，不同浓度 PD-1 抗体（H005-1）对 U87MG 细胞生长有明显的抑制效果，最高浓度抑制率约为 30%。

实施例 10：H005-1对结核菌素刺激的PBMC增殖活性

对人源化的抗体 H005-1 在体外对结核菌素刺激的 PBMC 的增殖活性进行检测。

取 15ml 新鲜 PBMC 细胞，约 3×10^7 个，加入 20μl 结核菌素(上海碧优生物科技，cat#97-8800)，37℃、5% CO_2 培养箱培养 5 天。第 6 天，取上述培养的细胞离心，重悬至新鲜的培养基中，调整密度为 5×10^5 个/ml。在 96 孔细胞培养板中加入 190μl 重悬后的细胞，将人源化抗体 H005-1 加入上述 96 孔细胞培养板的对应孔中，每孔 10μl，对照组和空白组分别加入 10μl PBS。细胞培养板置于 37℃，5% CO_2 培养箱孵育 72 小时后检测 PBMC 细胞增殖(Promega，cat#G7571)和 IFN-γ 的分泌(Neo Bioscience，cat#EHC102g)。结果如下：

受试样品对结核菌素刺激的 PBMC 增殖和 IFN-γ 分泌的激活作用

Sample	T cell proliferation EC50(ng/ml)	IFN-λ EC50(ng/ml)
H005-1	15.95±17.15	56.87±48.53

注：n=4

实验结果显示人源化的抗体 H005-1 可以很好的激活外源结核菌素刺激下的 PBMC 的增殖以及 IFN-γ 的分泌。

实施例 11：H005-1对U-87MG皮下移植瘤抑制实验

将 U87 细胞（5×10^6 个）100 ul 接种于 SCID-Beige 小鼠右肋部皮下，待 7-10 d 肿瘤长至 80-100 mm^3 后，去除体重、肿瘤过大和过小的，按肿瘤体积将小鼠随机分为 H005-1 10 mg/kg 组和 Human IgG 10 mg/kg 组，每组 7 只（D0）。将经 CD3 抗体刺激 3 天的两种 PBMC 以 1:1 比例混合，以 5×10^5 cells/60 ul 量注射到肿瘤组织中，并开始皮下注射抗体，7 天一次，共给药 3 次。每周测 2 次瘤体积，称鼠重，记录数据。肿瘤体积（V）计算公式为：V＝1/2×a×b2； 其中 a、b 分别表示长、宽。

结果见图 4：治疗后的肿瘤体积变化图，和图 5：治疗后的鼠重变化图，显示抗体 H005-1 可以很好的抑制肿瘤 U87MG 的生长，而且对小鼠的体重没有任何的影响。

权利要求书：

1、一种 PD-1 抗体或其抗原结合片段，其包含：

抗体轻链可变区，所述的抗体轻链可变区包含至少 1 个选自如以下序列所示的 LCDR：SEQ ID NO:6，SEQ ID NO:7 或 SEQ ID NO: 8；和

抗体重链可变区，所述的抗体重链可变区包含至少 1 个选自如以下序列所述的 HCDR：SEQ ID NO:3，SEQ ID NO:4 或 SEQ ID NO:5。

2、如权利要求 1 所述的 PD-1 抗体或其抗原结合片段，其中所述的抗体轻链可变区包含如 SEQ ID NO:6 所示的 LCDR1。

3、如权利要求 1 所述的 PD-1 抗体或其抗原结合片段，其中所述的抗体轻链可变区包含如 SEQ ID NO:7 所示的 LCDR2。

4、如权利要求 1 所述的 PD-1 抗体或其抗原结合片段，其中所述的抗体轻链可变区包含如 SEQ ID NO:8 所示的 LCDR3。

5、如权利要求 1 所述的 PD-1 抗体或其抗原结合片段，其中所述的抗体重链可变区包含如 SEQ ID NO:3 所示的 HCDR1。

6、如权利要求 1 所述的 PD-1 抗体或其抗原结合片段，其中所述的抗体重链可变区包含如 SEQ ID NO:4 所示的 HCDR2。

7、如权利要求 1 所述的 PD-1 抗体或其抗原结合片段，其中所述的抗体重链可变区包含如 SEQ ID NO:5 所示的 HCDR3。

8、如权利要求 1 所述的 PD-1 抗体或其抗原结合片段， 其中所述的抗体轻链可变区包含分别如 SEQ ID NO:6、SEQ ID NO:7 和 SEQ ID NO: 8 所示的 LCDR1、LCDR2 和 LCDR3。

9、如权利要求 1 所述的 PD-1 抗体或其抗原结合片段， 其中所述的抗体重链可变区包含分别如 SEQ ID NO:3、SEQ ID NO:4 和 SEQ ID NO:5 所示的 HCDR1、HCDR2 和 HCDR3。

10、如权利要求 1 所述的 PD-1 抗体或其抗原结合片段，其中所述的抗体轻链可变区包含分别如 SEQ ID NO:6、SEQ ID NO:7 和 SEQ ID NO: 8 所示的 LCDR1、LCDR2 和 LCDR3；且其中所述的抗体重链可变区包含分别如 SEQ ID NO:3、SEQ ID NO:4 和 SEQ ID NO:5 所示的 HCDR1， HCDR2 和 HCDR3。

11、如权利要求 1-10 任一项所述的 PD-1 抗体或其抗原结合片段，其中所述的抗体或其抗原结合片段为鼠源抗体或其片段。

12、如权利要求 1-10 任一项所述的 PD-1 抗体或其抗原结合片段，其中所述的抗体或其抗原结合片段为嵌合抗体或其片段。

13、如权利要求 12 所述的 PD-1 抗体或其抗原结合片段，其中所述的嵌合抗体轻链可变区序列为：SEQ ID NO: 10。

14、如权利要求 12 所述的 PD-1 抗体或其抗原结合片段，其中所述的嵌合抗体重链可变区序列为：SEQ ID NO:9。

15、如权利要求 1-10 任一项所述的 PD-1 抗体或其抗原结合片段，其中所述的抗体或其抗原结合片段为人源化抗体或其片段。

16、如权利要求 15 所述的 PD-1 抗体或其抗原结合片段，其中所述人源化抗体轻链可变区上的轻链 FR 区序列，来源于如 SEQ ID NO:14 所示的人种系轻链 IGKV1-39 和 JK4 的组合序列；其包含人种系轻链 IGKV1-39 的 FR1，FR2，FR3 区和 JK4 的 FR4 区。

17、如权利要求 15 所述的 PD-1 抗体或其抗原结合片段，其中所述人源化抗体轻链序列为如 SEQ ID NO:12 所示的序列或其变体；所述的变体优选在轻链可变区有 0-10 的氨基酸变化；更优选为 A43S 的氨基酸变化。

18、如权利要求 15 所述的 PD-1 抗体或其抗原结合片段，其中所述人源化抗体重链可变区进一步包含人源 IgG1, IgG2, IgG3 或 IgG4 或其变体的重链 FR 区，优选包含人源 IgG2 或 IgG4 重链 FR 区。

19、如权利要求 15 所述的 PD-1 抗体或其抗原结合片段，其中所述人源化抗体重链可变区上的重链 FR 区序列，来源于如 SEQ ID NO:13 所示的人种系重链 IgHV3-7 和 JH6 的组合序列；其包含人种系重链 IgHV3-7 的 FR1，FR2，FR3 区和 JH6 的 FR4 区。

20、如权利要求 15 所述的 PD-1 抗体或其抗原结合片段，其中所述人源化抗体重链序列为如 SEQ ID NO:11 所示的序列或其变体；所述变体优选在重链可变区有 0-10 的氨基酸变化；更优选为 G44R 的氨基酸变化。

21、一种编码如权利要求 1-20 任一项所述的抗体的 DNA 分子。

22、一种含有如权利要求 21 所述的 DNA 分子的表达载体。

23、一种用如权利要求 22 所述的表达载体转化的宿主细胞。

24、如权利要求 23 所述的宿主细胞，其中所述的宿主细胞为细菌，优选为大肠杆菌。

25、如权利要求 23 所述的宿主细胞，其中所述的宿主细胞为酵母菌，优选为毕赤酵母。

26、一种药物组合物，其含有如权利要求 1 至 20 任一项所述的 PD-1 抗体或其抗原结合片段和可药用的赋形剂、稀释剂或载体。

27、如权利要求 1 至 20 任一项所述的 PD-1 抗体或其抗原结合片段、如权利要求 26 所述的药物组合物，在制备用于治疗 PD-1 介导的疾病或病症的药物中的用途，其中所述的疾病或病症优选为癌症；更优选为表达 PD-L1 的癌症；最优选为乳腺癌、肺癌、胃癌、肠癌、肾癌、黑素瘤、非小细胞肺癌；最优选为非小细胞肺癌、黑素瘤和肾癌。

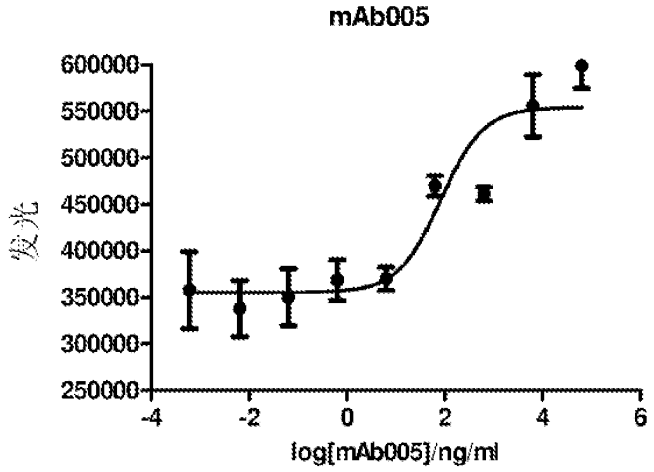

mAb005
600000
550000
500000
450000
400000
350000
300000
250000
发光
-4
-2
0
2
4
6
log[mAb005]/ng/ml

图 1

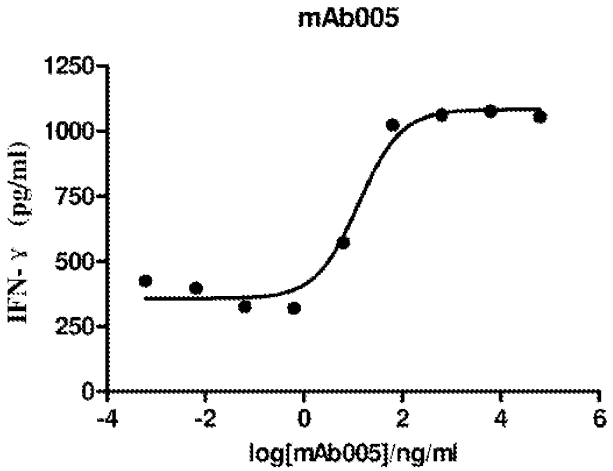

mAb005
1250
1000
750
500
250
0
IFN-γ (pg/ml)
-4
-2
0
2
4
6
log[mAb005]/ng/ml

图 2

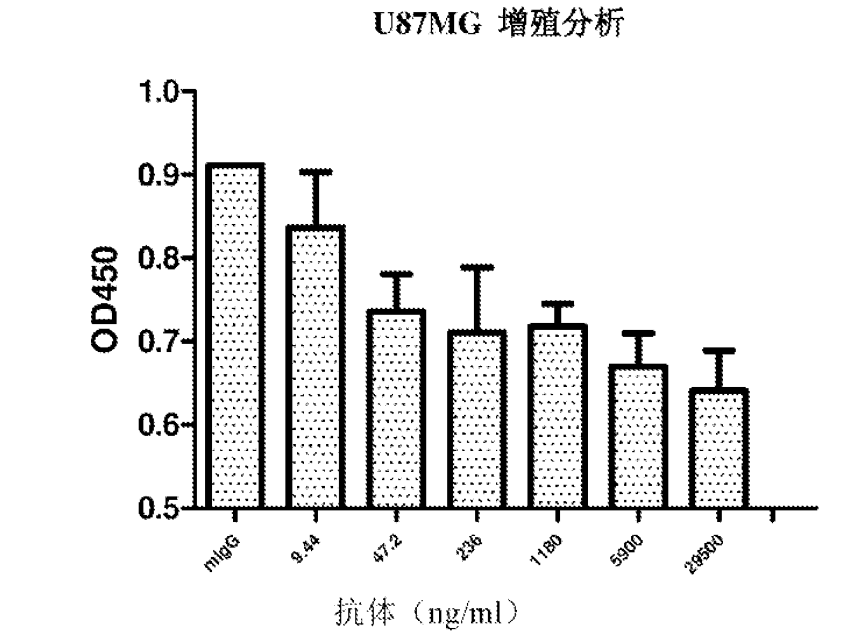

U87MG 增殖分析
OD450
1.0
0.9
0.8
0.7
0.6
0.5
mIgG
9.44
47.2
236
1180
5900
29500
抗体（ng/ml）

图 3

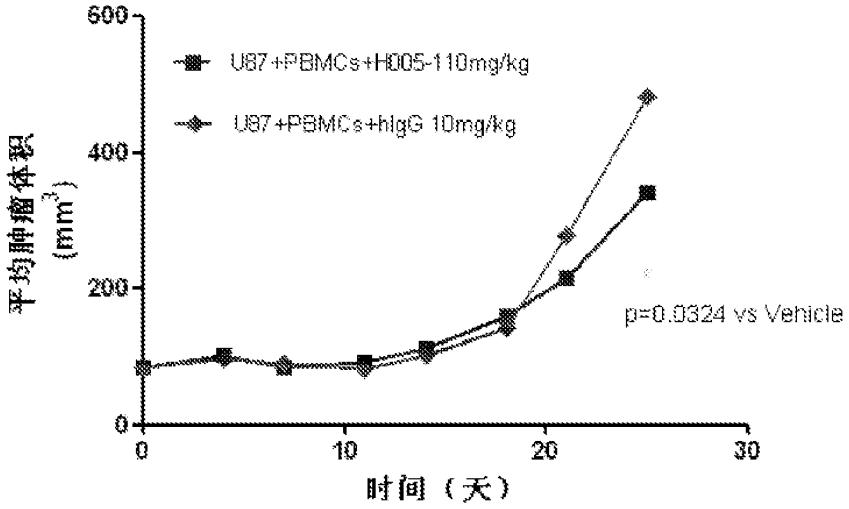

600
400
200
0
U87+PBMCs+H005-110mg/kg
U87+PBMCs+hIgG 10mg/kg
平均肿瘤体积
(mm3)
p=0.0324 vs Vehicle
0
10
20
30
时间（天）

图 4

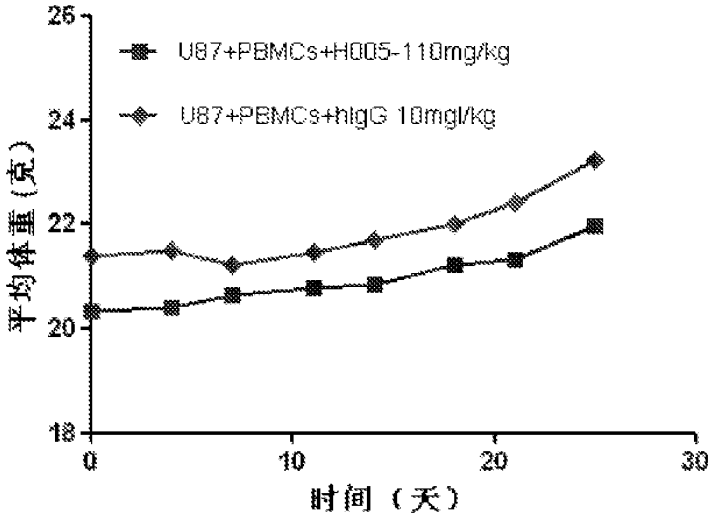

U87+PBMCs+H005-110mg/kg
U87+PBMCs+hIgG 10mgl/kg
平均体重（克）
时间（天）
26
24
22
20
18
0
10
20
30

图 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2014/091090

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/28 (2006.01) i; C12N 15/13 (2006.01) i; C12N 15/70 (2006.01) i; C12N 15/81 (2006.01) i; C12N 1/21 (2006.01) i; C12N 1/19 (2006.01) i; A61K 39/395 (2006.01) i; A61P 35/00 (2006.01) i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K; C12N; A61K; A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CPRSABS; CNABS; CNKI; CNTXT and Key Words: HENGRUI MEDICINE; YUAN, Jijun; QU, Xiangdong; PD-1, antibody, mAb005 etc.

VEN; MEDLINE; EPTXT; USTXT; WOTXT and Key Words: YUAN, Jijun; QU, Xiangdong; programmed death-1, PD-1, mAb005, antibod+ etc.

Genbank; EMBL; STN and searched sequences: SEQ ID NOs: 3-14 etc.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 102892785 A (UCB PHARMA SA), 23 January 2013 (23.01.2013), see embodiments 1-4	1-27
A	CN 1259961 A (GENENTECH INC.), 12 July 2000 (12.07.2000), see the whole document	1-27

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02 February 2014 (02.02.2014)	**11 February 2015 (11.02.2015)**
Name and mailing address of the ISA/CN: State Intellectual Property Office of the P. R. China No. 6, Xitucheng Road, Jimenqiao Haidian District, Beijing 100088, China Facsimile No.: (86-10) 62019451	Authorized officer **WANG, Jinfeng** Telephone No.: (86-10) **62412282**

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2014/091090

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
CN 102892785 A	23 January 2013	TW 201134488 A	16 October 2011
		JP 2013521769 A	13 June 2013
		AR 080501 A1	11 April 2012
		EP 2545076 A1	16 January 2013
		US 2011229461 A1	22 September 2011
		WO 2011110621 A1	15 September 2011
CN 1259961 A	12 July 2000	SI 0971959 T1	30 June 2006
		ZA 9802907 A	09 October 1998
		DE 69842174 D1	21 April 2011
		TR 9902818 T2	22 May 2000
		EP 2336190 A3	05 October 2011
		EP 0971959 A1	19 January 2000
		CN 101210051 A	02 July 2008
		HK 1114621 A1	13 September 2013
		CN 101665536 A	10 March 2010
		EP 1695985 A3	22 November 2006
		SI 1695985 T1	30 June 2011
		DK 1787999 T3	15 November 2010
		DE 69832970 D1	02 February 2006
		CN 101210051 B	26 December 2012
		ES 2361267 T3	15 June 2011
		HK 1114622 A1	01 April 2011
		CN 101665536 B	03 July 2013
		EP 1695985 A2	30 August 2006
		AT 501170 T	15 March 2011
		SI 1787999 T1	31 December 2010
		CA 2286397 A1	15 October 1998
		PT 971959 T	31 May 2006
		PT 973804 E	30 March 2007
		SI 0973804 T1	30 June 2007
		NZ 500077 A	26 October 2001
		CN 101838328 A	22 September 2010
		SI 2301580 T1	31 May 2012
		BR 9809388 B1	04 June 2013
		IL 132239 D0	19 March 2001
		AU 740738 B2	15 November 2001
		DK 2301580 T3	14 May 2012
		AU 7102398 A	30 October 1998
		EP 2301580 B1	18 January 2012
		PT 1787999 E	11 November 2010
		ZA 9802908 A	21 January 1999
		DE 69841815 D1	16 September 2010
		AT 314395 T	15 January 2006
		IL 132240 A	05 September 2006
		EP 2301580 A9	14 September 2011
		CN 101210050 A	02 July 2008
		AT 541586 T	15 February 2012
		PT 1695985 E	06 June 2011
		ES 2349559 T3	05 January 2011
		WO 9845332 A3	03 December 1998

Form PCT/ISA/210 (patent family annex) (July 2009)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2014/091090

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
		PT 971959 E	31 May 2006
		HK 1025338 A1	12 May 2006
		ES 2256935 T3	16 July 2006
		CN 101210050 B	08 December 2010
		DE 69832970 T2	10 August 2006

Form PCT/ISA/210 (patent family annex) (July 2009)

国际检索报告

国际申请号
PCT/CN2014/091090

A. 主题的分类

C07K 16/28(2006.01)i; C12N 15/13(2006.01)i; C12N 15/70(2006.01)i; C12N 15/81(2006.01)i; C12N 1/21(2006.01)i; C12N 1/19(2006.01)i; A61K 39/395(2006.01)i; A61P 35/00(2006.01)i

按照国际专利分类(IPC)或者同时按照国家分类和IPC两种分类

B. 检索领域

检索的最低限度文献(标明分类系统和分类号)

C07K; C12N; A61K; A61P

包含在检索领域中的除最低限度文献以外的检索文献

在国际检索时查阅的电子数据库(数据库的名称，和使用的检索词（如使用））

CPRSABS; CNABS; CNKI; CNTXT和关键词：恒瑞医药, 袁纪军, 屈向东, PD-1, 抗体, mAb005等; VEN; MEDLINE; EPTXT; USTXT; WOTXT和关键词: yuan jijun, qu xiangdong, programmed death-1, PD-1, mAb005, antibod+等; Genbank; EMBL; STN和检索的序列: SEQ ID NOs:3-14等。

C. 相关文件

类 型*	引用文件，必要时，指明相关段落	相关的权利要求
X	CN 102892785 A (EOB医药有限公司) 2013年 1月 23日 (2013-01-23) 见实施例1-4	1-27
A	CN 1259961 A (基因技术股份有限公司) 2000年 7月 12日 (2000-07-12) 见全文	1-27

☐ 其余文件在C栏的续页中列出。

☑ 见同族专利附件。

* 引用文件的具体类型：

"A" 认为不特别相关的表示了现有技术一般状态的文件

"E" 在国际申请日的当天或之后公布的在先申请或专利

"L" 可能对优先权要求构成怀疑的文件，或为确定另一篇引用文件的公布日而引用的或者因其他特殊理由而引用的文件（如具体说明的）

"O" 涉及口头公开、使用、展览或其他方式公开的文件

"P" 公布日先于国际申请日但迟于所要求的优先权日的文件

"T" 在申请日或优先权日之后公布、与申请不相抵触，但为了理解发明之理论或原理的在后文件

"X" 特别相关的文件，单独考虑该文件，认定要求保护的发明不是新颖的或不具有创造性

"Y" 特别相关的文件，当该文件与另一篇或者多篇该类文件结合并且这种结合对于本领域技术人员为显而易见时，要求保护的发明不具有创造性

"&" 同族专利的文件

国际检索实际完成的日期	国际检索报告邮寄日期
2014年 2月 02日	2015年 2月 11日
ISA/CN的名称和邮寄地址 中华人民共和国国家知识产权局(ISA/CN) 北京市海淀区蓟门桥西土城路6号 100088 中国 传真号 (86-10)62019451	受权官员 王金凤 电话号码 (86-10)62412282

表 PCT/ISA/210 (第2页) (2009年7月)

国际检索报告
关于同族专利的信息

国际申请号
PCT/CN2014/091090

检索报告引用的专利文件			公布日（年/月/日）	同族专利			公布日（年/月/日）
CN	102892785	A	2013年 1月 23日	TW	201134488	A	2011年 10月 16日
				JP	2013521769	A	2013年 6月 13日
				AR	080501	A1	2012年 4月 11日
				EP	2545076	A1	2013年 1月 16日
				US	2011229461	A1	2011年 9月 22日
				WO	2011110621	A1	2011年 9月 15日
CN	1259061	A	2000年 7月 12日	SI	0971959	T1	2006年 6月 30日
				ZA	9802967	A	1998年 10月 09日
				DE	69842174	D1	2011年 4月 21日
				TR	9902818	T2	2000年 5月 22日
				EP	2336190	A3	2011年 10月 05日
				EP	0971959	A1	2000年 1月 19日
				CN	101210051	A	2008年 7月 02日
				HK	1114621	A1	2013年 9月 13日
				CN	101665536	A	2010年 3月 10日
				EP	1696985	A3	2006年 11月 22日
				SI	1695985	T1	2011年 6月 30日
				DK	1787999	T3	2010年 11月 15日
				DE	69832970	D1	2006年 2月 02日
				CN	101210051	B	2012年 12月 26日
				ES	2361267	T3	2011年 6月 15日
				HK	1114622	A1	2011年 4月 01日
				CN	101665536	B	2013年 7月 03日
				EP	1695985	A2	2006年 8月 30日
				AT	501170	T	2011年 3月 15日
				SI	1787999	T1	2010年 12月 31日
				CA	2286397	A1	1998年 10月 15日
				PT	971959	T	2006年 5月 31日
				PT	973804	E	2007年 3月 30日
				SI	0973804	T1	2007年 6月 30日
				NZ	500077	A	2001年 10月 26日
				CN	101838328	A	2010年 9月 22日
				SI	2301580	T1	2012年 5月 31日
				BR	9809388	B1	2013年 6月 04日
				IL	132239	D0	2001年 3月 19日
				AU	740738	B2	2001年 11月 15日
				DK	2301580	T3	2012年 5月 14日
				AU	7102398	A	1998年 10月 30日
				EP	2301580	B1	2012年 1月 18日
				PT	1787999	E	2010年 11月 11日
				ZA	9802908	A	1999年 1月 21日
				DE	69841815	D1	2010年 9月 16日
				AT	314395	T	2006年 1月 15日
				IL	132240	A	2006年 9月 05日
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(54) **Title**: HUMAN ANTIBODIES TO PD-1

(57) **Abstract**: The present invention provides antibodies that bind to the T-cell co-inhibitor programmed death- 1 (PD-1) protein, and methods of use. In various embodiments of the invention, the antibodies are fully human antibodies that bind to PD-1. In certain embodiments, the present invention provides multi-specific antigen-binding molecules comprising a first binding specificity that binds to PD-1 and a second binding specificity that binds to an autoimmune tissue antigen, another T- cell co-inhibitor, an Fc receptor, or a T-cell receptor. In some embodiments, the antibodies of the invention are useful for inhibiting or neutralizing PD-1 activity, thus providing a means of treating a disease or disorder such as cancer or a chronic viral infection. In other embodiments, the antibodies are useful for enhancing or stimulating PD-1 activity, thus providing a means of treating, for example, an autoimmune disease or disorder.

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HUMAN ANTIBODIES TO PD-1

FIELD OF THE INVENTION

[001] The present invention is related to human antibodies and antigen-binding fragments of human antibodies that specifically bind to the immunomodulatory receptor programmed death-1 (PD-1), and therapeutic and diagnostic methods of using those antibodies.

STATEMENT OF RELATED ART

[002] Programmed death-1 (PD-1) (also called CD279) is a 288 amino acid protein receptor expressed on activated T-cells and B-cells, natural killer cells and monocytes. PD-1 is a member of the CD28/CTLA-4 (cytotoxic T lymphocyte antigen)/ ICOS (inducible co-stimulator) family of T-cell co-inhibitory receptors (Chen et al 2013, Nat. Rev. Immunol. 13: 227-242). The primary function of PD-1 is to attenuate the immune response (Riley 2009, Immunol. Rev. 229: 114-125). PD-1 has two ligands, PD-ligand1 (PD-L1) and PD-L2. PD-L1 (CD274, B7H1) is expressed widely on both lymphoid and non-lymphoid tissues such as CD4 and CD8 T-cells, macrophage lineage cells, peripheral tissues as well as on tumor cells, virally-infected cells and autoimmune tissue cells. PD-L2 (CD273, B7-DC) has a more restricted expression than PD-L1, being expressed on activated dendritic cells and macrophages (Dong et al 1999, Nature Med.). PD-L1 is expressed in most human cancers, including melanoma, glioma, non-small cell lung cancer, squamous cell carcinoma of head and neck, leukemia, pancreatic cancer, renal cell carcinoma, and hepatocellular carcinoma, and may be inducible in nearly all cancer types (Zou and Chen 2008, Nat. Rev. Immunol. 8: 467-77). PD-1 binding to its ligands results in decreased T-cell proliferation and cytokine secretion, compromising humoral and cellular immune responses in diseases such as cancer, viral infection and autoimmune disease. Blockade of PD-1 binding to reverse immunosuppression has been studied in autoimmune, viral and tumor immunotherapy (Ribas 2012, NEJM 366: 2517-2519; Watanabe et al 2012, Clin. Dev. Immunol. Volume 2012, Article ID: 269756; Wang et al 2013, J. Viral Hep. 20: 27-39).

[003] T-cell co-stimulatory and co-inhibitory molecules (collectively named co-signaling molecules) play a crucial role in regulating T-cell activation, subset differentiation, effector function and survival (Chen et al 2013, Nature Rev. Immunol. 13: 227-242). Following recognition of cognate peptide-MHC complexes on antigen-presenting cells by the T-cell receptor, co-signaling receptors co-localize with T-cell receptors at the immune synapse, where they synergize with TCR signaling to promote or inhibit T-cell activation and function (Flies et al 2011, Yale J. Biol. Med. 84: 409-421). The ultimate immune response is regulated by a balance between co-stimulatory and co-inhibitory signals ("immune checkpoints") (Pardoll 2012, Nature 12: 252-264). PD-1 functions as one such 'immune checkpoint' in mediating peripheral T-cell tolerance and in avoiding autoimmunity. PD-1 binds to PD-L1 or PD-L2 and inhibits T-cell activation. The ability of PD1 to inhibit T-cell activation is exploited by chronic viral infections and

tumors to evade immune response. In chronic viral infections, PD-1 is highly expressed on virus-specific T-cells and these T-cells become "exhausted" with loss of effector functions and proliferative capacity (Freeman 2008, PNAS 105: 10275-10276). PD-L1 is expressed on a wide variety of tumors and studies on animal models have shown that PD-L1 on tumors inhibits T-cell activation and lysis of tumor cells and may lead to increased death of tumor-specific T-cells. The PD-1: PD-L1 system also plays an important role in induced T-regulatory (Treg) cell development and in sustaining Treg function (Francisco et al 2010, Immunol. Rev. 236: 219-242).

[004] Since PD-1 plays an important role in autoimmunity, tumor immunity and infectious immunity, it is an ideal target for immunotherapy. Blocking PD-1 with antagonists, including monoclonal antibodies, has been studied in treatments of cancer and chronic viral infections (Sheridan 2012, Nature Biotechnology 30: 729-730).

[005] Monoclonal antibodies to PD-1 are known in the art and have been described, for example, in US Patent/Publication Nos. 8008449, 8168757, 20110008369, 20130017199, 20130022595, and in WO2006121168, WO20091154335, WO2012145493, WO2013014668, WO2009101611, EP2262837, and EP2504028.

BRIEF SUMMARY OF THE INVENTION

[006] The present invention provides antibodies and antigen-binding fragments thereof that bind PD-1. The antibodies of the present invention are useful, *inter alia*, for targeting T cells expressing PD-1, and for modulating PD-1 activity. In certain embodiments, the antibodies of the invention are useful for inhibiting or neutralizing PD-1 activity and/or for stimulating T cell activation, *e.g.*, under circumstances where T cell-mediated killing is beneficial or desirable. In alternate embodiments, the antibodies enhance PD-1 binding and/or activity and may be used to inhibit T-cell activation. The anti-PD-1 antibodies of the invention, or antigen-binding portions thereof, may be included as part of a multi-specific antigen-binding molecule, for example, to modulate the immune response and/or to target the antibodies to a specific cell type, such as a tumor cell, an autoimmune tissue cell or a virally infected cell. The antibodies are useful in treating a disease or disorder such as cancer, viral infection and autoimmune disease.

[007] The antibodies of the invention can be full-length (for example, an IgG1 or IgG4 antibody) or may comprise only an antigen-binding portion (for example, a Fab, $F(ab')_2$ or scFv fragment), and may be modified to affect functionality, e.g., to eliminate residual effector functions (Reddy et al., 2000, J. Immunol. 164:1925-1933). In certain embodiments, the antibodies may be bispecific.

[008] In a first aspect, the present invention provides isolated recombinant monoclonal antibodies or antigen-binding fragments thereof that bind specifically to PD-1. In certain embodiments, the antibodies are fully human. Exemplary anti-PD-1 antibodies of the present invention are listed in Tables 1 – 3 herein. Table 1 sets forth the amino acid sequence

identifiers of the heavy chain variable regions (HCVRs), light chain variable regions (LCVRs), heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3), and light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) of the exemplary anti-PD-1 antibodies. Table 2 sets forth the nucleic acid sequence identifiers of the HCVRs, LCVRs, HCDR1, HCDR2 HCDR3, LCDR1, LCDR2 and LCDR3 of the exemplary anti-PD-1 antibodies. Table 3 sets forth the amino acid sequence identifiers of heavy chain and light chain sequences of exemplary anti-PD-1 antibodies.

[009] The present invention provides antibodies, or antigen-binding fragments thereof, comprising an HCVR comprising an amino acid sequence selected from any of the HCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[010] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising an LCVR comprising an amino acid sequence selected from any of the LCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[011] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising an HCVR and an LCVR amino acid sequence pair (HCVR/LCVR) comprising any of the HCVR amino acid sequences listed in Table 1 paired with any of the LCVR amino acid sequences listed in Table 1. According to certain embodiments, the present invention provides antibodies, or antigen-binding fragments thereof, comprising an HCVR/LCVR amino acid sequence pair contained within any of the exemplary anti-PD-1 antibodies listed in Table 1. In certain embodiments, the HCVR/LCVR amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/202, 218/202, 226/202, 234/202, 242/202, 250/202, 258/202, 266/202, 274/202, 282/202, 290/202, 298/186, 306/186 and 314/186. In certain embodiments, the HCVR/LCVR amino acid sequence pair is selected from one of SEQ ID NOs: 130/138 (*e.g.*, H2M7795N), 162/170 (*e.g.*, H2M7798N), 234/202 (*e.g.*, H4xH9048P), or 314/186 (*e.g.*, H4xH9008P).

[012] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR1 (HCDR1) comprising an amino acid sequence selected from any of the HCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[013] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR2 (HCDR2) comprising an amino acid sequence selected from any of the HCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[014] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR3 (HCDR3) comprising an amino acid sequence selected from

any of the HCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[015] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR1 (LCDR1) comprising an amino acid sequence selected from any of the LCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[016] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR2 (LCDR2) comprising an amino acid sequence selected from any of the LCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[017] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR3 (LCDR3) comprising an amino acid sequence selected from any of the LCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[018] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising an HCDR3 and an LCDR3 amino acid sequence pair (HCDR3/LCDR3) comprising any of the HCDR3 amino acid sequences listed in Table 1 paired with any of the LCDR3 amino acid sequences listed in Table 1. According to certain embodiments, the present invention provides antibodies, or antigen-binding fragments thereof, comprising an HCDR3/LCDR3 amino acid sequence pair contained within any of the exemplary anti-PD-1 antibodies listed in Table 1. In certain embodiments, the HCDR3/LCDR3 amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 136/144 (*e.g.*, H2M7795N), 168/176 (*e.g.*, H2M7798N), 240/208 (*e.g.*, H4xH9048P), and 320/192 (*e.g.*, H4xH9008P).

[019] The present invention provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain comprising an amino acid sequence selected from any of the HC amino acid sequences listed in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[020] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a light chain comprising an amino acid sequence selected from any of the LC amino acid sequences listed in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[021] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a HC and a LC amino acid sequence pair (HC/LC) comprising any of the HC amino acid sequences listed in Table 3 paired with any of the LC amino acid sequences listed in Table 3. According to certain embodiments, the present invention provides antibodies, or antigen-binding fragments thereof, comprising an HC/LC amino acid sequence pair contained within any of the exemplary anti-PD-1 antibodies listed in Table 3. In certain embodiments, the HC/LC amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 330/331,

332/333, 334/335, and 336/337.

[022] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within any of the exemplary anti-PD-1 antibodies listed in Table 1. In certain embodiments, the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequence set is selected from the group consisting of SEQ ID NOs: 132-134-136-140-142-144 (*e.g.*, H2M7795N); 164-166-168-172-174-176 (*e.g.*, H2M7798N); 236-238-240-204-206-208 (*e.g.*, H4xH9048P); and 316-318-320-188-190-192 (*e.g.*, H4xH9008P).

[023] In a related embodiment, the present invention provides antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within an HCVR/LCVR amino acid sequence pair as defined by any of the exemplary anti-PD-1 antibodies listed in Table 1. For example, the present invention includes antibodies, or antigen-binding fragments thereof, comprising the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences set contained within an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 130/138 (*e.g.*, H2M7795N); 162/170 (*e.g.*, H2M7798N); 234/202 (*e.g.*, H4xH9048P); and 314/186 (*e.g.*, H4xH9008P). Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. *See*, *e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, *J. Mol. Biol. 273*:927-948 (1997); and Martin *et al.*, *Proc. Natl. Acad. Sci. USA 86*:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antibody.

[024] The present invention includes anti-PD-1 antibodies having a modified glycosylation pattern. In some embodiments, modification to remove undesirable glycosylation sites may be useful, or an antibody lacking a fucose moiety present on the oligosaccharide chain, for example, to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield et al. (2002) JBC 277:26733). In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[025] The present invention also provides for antibodies and antigen-binding fragments thereof that compete for specific binding to PD-1 with an antibody or antigen-binding fragment thereof comprising the CDRs of a HCVR and the CDRs of a LCVR, wherein the HCVR and LCVR each has an amino acid sequence selected from the HCVR and LCVR sequences listed in Table 1.

[026] The present invention also provides isolated antibodies and antigen-binding fragments

thereof that block PD-1 binding to PD-L1 or PD-L2. In some embodiments, the antibody or antigen-binding fragment thereof that blocks PD-1 binding to PD-L1 may bind to the same epitope on PD-1 as PD-L1 or may bind to a different epitope on PD-1 as PD-L1.

[027] In alternate embodiments, the present invention provides antibodies and antigen-binding fragments thereof that stimulate PD-1 binding to PD-L1. In certain embodiments, the present invention provides isolated antibodies or antigen-binding fragments thereof that bind PD-1, wherein the antibodies or antigen-binding fragments thereof enhance PD-1 binding to PD-L1. In some embodiments, the isolated antibodies or antigen-binding fragments thereof comprise the CDRs of a HCVR, wherein the HCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 98, and 250; and the CDRs of a LCVR, wherein the LCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 106, and 202. In some embodiments, the isolated antibodies or antigen-binding fragments thereof comprise an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10 (*e.g.*, H1M7789N), 98/106 (*e.g.*, H2M7791N), and 250/202 (*e.g.*, H4H9068P2).

[028] The present invention also provides antibodies and antigen-binding fragments thereof that bind specifically to PD-1 from human or other species. In certain embodiments, the antibodies may bind to human PD-1 and/or to cynomolgus PD-1.

[029] The present invention also provides antibodies and antigen-binding fragments thereof that cross-compete for binding to PD-1 with a reference antibody or antigen-binding fragment thereof comprising the CDRs of a HCVR and the CDRs of a LCVR, wherein the HCVR and LCVR each has an amino acid sequence selected from the HCVR and LCVR sequences listed in Table 1.

[030] In one embodiment, the invention provides an isolated antibody or antigen-binding fragment that has one or more of the following characteristics: (a) blocks the binding of PD-1 to PD-L1 or PD-L2; (b) binds specifically to human PD-1 and/or cynomolgus PD-1; (c) blocks PD-1-induced T-cell down regulation and rescues T-cell signaling; (d) suppresses tumor growth and increases survival in subjects with colon cancer; (e) inhibits T-cell proliferation in a mixed lymphocyte reaction (MLR) assay; and (f) increases IL-2 and/or interferon-gamma secretion in a MLR assay.

[031] In some embodiments, the antibody or antigen binding fragment thereof may bind specifically to PD-1 in an agonist manner, i.e., it may enhance or stimulate PD-1 binding and/or activity; in other embodiments, the antibody may bind specifically to PD-1 in an antagonist manner, i.e., it may block PD-1 from binding to its ligand.

[032] In certain embodiments, the antibodies or antigen-binding fragments of the present invention are bispecific comprising a first binding specificity to PD-1 and a second binding specificity for a second target epitope. The second target epitope may be another epitope on PD-1 or on a different protein. In certain embodiments, the target epitope may be on a different cell including a different T-cell, a B-cell, a tumor cell, an autoimmune tissue cell or a virally infected cell.

[033] In a second aspect, the present invention provides nucleic acid molecules encoding anti-PD-1 antibodies or portions thereof. For example, the present invention provides nucleic acid molecules encoding any of the HCVR amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[034] The present invention also provides nucleic acid molecules encoding any of the LCVR amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[035] The present invention also provides nucleic acid molecules encoding any of the HCDR1 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR1 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[036] The present invention also provides nucleic acid molecules encoding any of the HCDR2 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR2 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[037] The present invention also provides nucleic acid molecules encoding any of the HCDR3 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR3 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[038] The present invention also provides nucleic acid molecules encoding any of the LCDR1 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR1 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[039] The present invention also provides nucleic acid molecules encoding any of the LCDR2 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR2 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[040] The present invention also provides nucleic acid molecules encoding any of the LCDR3

amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR3 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[041] The present invention also provides nucleic acid molecules encoding an HCVR, wherein the HCVR comprises a set of three CDRs (*i.e.*, HCDR1-HCDR2-HCDR3), wherein the HCDR1-HCDR2-HCDR3 amino acid sequence set is as defined by any of the exemplary anti-PD-1 antibodies listed in Table 1.

[042] The present invention also provides nucleic acid molecules encoding an LCVR, wherein the LCVR comprises a set of three CDRs (*i.e.*, LCDR1-LCDR2-LCDR3), wherein the LCDR1-LCDR2-LCDR3 amino acid sequence set is as defined by any of the exemplary anti-PD-1 antibodies listed in Table 1.

[043] The present invention also provides nucleic acid molecules encoding both an HCVR and an LCVR, wherein the HCVR comprises an amino acid sequence of any of the HCVR amino acid sequences listed in Table 1, and wherein the LCVR comprises an amino acid sequence of any of the LCVR amino acid sequences listed in Table 1. In certain embodiments, the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto, and a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto. In certain embodiments according to this aspect of the invention, the nucleic acid molecule encodes an HCVR and LCVR, wherein the HCVR and LCVR are both derived from the same anti-PD-1 antibody listed in Table 1.

[044] The present invention provides nucleic acid molecules encoding any of the heavy chain amino acid sequences listed in Table 3. The present invention also provides nucleic acid molecules encoding any of the light chain amino acid sequences listed in Table 3.

[045] The present invention also provides nucleic acid molecules encoding both heavy chain (HC) and a light chain (LC), wherein the HC comprises an amino acid sequence of any of the HC amino acid sequences listed in Table 3, and wherein the LC comprises an amino acid sequence of any of the LC amino acid sequences listed in Table 3.

[046] In a related aspect, the present invention provides recombinant expression vectors capable of expressing a polypeptide comprising a heavy or light chain variable region of an anti-PD-1 antibody. For example, the present invention includes recombinant expression vectors comprising any of the nucleic acid molecules mentioned above, *i.e.*, nucleic acid molecules encoding any of the HCVR, LCVR, and/or CDR sequences as set forth in Table 1. The present invention also provides recombinant expression vectors capable of expressing a polypeptide comprising a heavy or light chain of an anti-PD-1 antibody. For example, the present invention

includes recombinant expression vectors comprising any of the nucleic acid molecules mentioned above, *i.e.*, nucleic acid molecules encoding any of the heavy chain or light chain sequences as set forth in Table 3. Also included within the scope of the present invention are host cells into which such vectors have been introduced, as well as methods of producing the antibodies or portions thereof by culturing the host cells under conditions permitting production of the antibodies or antibody fragments, and recovering the antibodies and antibody fragments so produced.

[047] In a third aspect, the present invention provides multi-specific antigen-binding molecules and antigen-binding fragments thereof comprising a first antigen-binding specificity that binds specifically to PD-1 and a second antigen-binding specificity that binds specifically to an antigen selected from the group consisting of a tumor cell-specific antigen, an autoimmune tissue-specific antigen, an infected-cell-specific antigen, a T-cell co-inhibitor, a T-cell receptor, a Fc receptor, PD-L1, and PD-1. In certain embodiments, the first antigen-binding specificity may comprise three CDRs derived from a HCVR with an amino acid sequence selected from the HCVR sequences in Table 1 and three CDRs derived from a LCVR with an amino acid sequence selected from the LCVR sequences in Table 1. In one embodiment, the first antigen-binding specificity may comprise the extracellular domain of PD-L1. The second antigen-binding specificity may target an antigen on the same cell as PD-1 or on a different cell of the same tissue type or of a different tissue type. For example, the multi-specific antigen-binding molecule may bind to a T-cell wherein the first antigen-binding specificity may bind specifically to PD-1 and the second antigen-binding specificity may bind to a T-cell receptor on the T-cell. Alternatively, in another embodiment, the first antigen-binding specificity may bind specifically to PD-1 on a T-cell and the second antigen-binding specificity may be targeted to an antigen/receptor on a B-cell or a macrophage or antigen-presenting cell. In certain embodiments, the second antigen-binding specificity may be directed to an antigen associated with an autoimmune tissue. In one embodiment, the first antigen-binding specificity may comprise an extracellular domain of PD-L1 and the second antigen-binding specificity may bind to another epitope on PD-1. In certain embodiments, the first antigen-binding specificity binds to PD-1 with a lower affinity, for example, with a K_D more than 10^{-7} M, more than 10^{-6} M, more than 10^{-5} M, or more than 10^{-4} M.

[048] In a fourth aspect, the invention provides a pharmaceutical composition comprising a recombinant human antibody or fragment thereof which specifically binds PD-1 and a pharmaceutically acceptable carrier. In a related aspect, the invention features a composition which is a combination of an anti-PD-1 antibody and a second therapeutic agent. In one embodiment, the second therapeutic agent is any agent that is advantageously combined with an anti-PD-1 antibody. Exemplary agents that may be advantageously combined with an anti-PD-1 antibody include, without limitation, other agents that bind and/or modulate PD-1 signaling (including other antibodies or antigen-binding fragments thereof, etc.) and/or agents which do

not directly bind PD-1 but nonetheless modulate immune cell activation. Additional combination therapies and co-formulations involving the anti-PD-1 antibodies of the present invention are disclosed elsewhere herein.

[049] In a fifth aspect, the invention provides methods to modulate the immune response in a subject, the method comprising administering a therapeutically effective amount of an anti-PD-1 antibody or antigen-binding fragment thereof of the invention to the subject in need thereof. In certain embodiments, the invention provides methods to enhance the immune response in a subject, the methods comprising administering to the subject an effective amount of an antibody or fragment thereof of the invention that binds PD-1 and blocks PD-1 binding to PD-L1. In one embodiment, the invention provides a method to stimulate or enhance T-cell stimulation in a subject. In one embodiment, the invention provides methods to inhibit a T-regulatory (Treg) cell in a subject, the methods comprising administering a therapeutically effective amount of a blocking antibody or antigen-binding fragment thereof of the invention to the subject in need thereof. In certain embodiments, the subject in need thereof may suffer from a disease or disorder such as cancer or viral infection. In alternate embodiments, the invention provides for methods to inhibit or suppress T-cell activation in a subject, the methods comprising administering a therapeutically effective amount of an activating antibody or fragment thereof of the invention to the subject in need thereof. In one embodiment, the subject may suffer from an autoimmune disease or disorder.

[050] In a sixth aspect, the invention provides therapeutic methods for treating a disease or disorder such as cancer, autoimmune disease or viral infection in a subject using an anti-PD-1 antibody or antigen-binding portion of an antibody of the invention, wherein the therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an antibody or fragment of an antibody of the invention to the subject in need thereof. The disorder treated is any disease or condition which is improved, ameliorated, inhibited or prevented by stimulation or inhibition of PD-1 activity or signaling. In certain embodiments, the antibody or antigen-binding fragment thereof the invention is administered in combination with a second therapeutic agent to the subject in need thereof. The second therapeutic agent may be selected from the group consisting of an antibody to another T-cell co-inhibitor, an antibody to a tumor cell antigen, an antibody to a T-cell receptor, an antibody to a Fc receptor, an antibody to an epitope on a virally infected cell, an antibody to an autoimmune tissue antigen, an antibody to PD-L1, a cytotoxic agent, an anti-cancer drug, an anti-viral drug, an anti-inflammatory drug (e.g., corticosteroids), chemotherapeutic agent, radiation therapy, an immunosuppressant and any other drug or therapy known in the art. In certain embodiments, the second therapeutic agent may be an agent that helps to counteract or reduce any possible side effect(s) associated with an antibody or antigen-binding fragment thereof of the invention, if such side effect(s) should occur.

[051] In certain embodiments, the present invention provides methods for suppressing tumor

growth. In certain embodiments, the present invention provides methods to enhance survival of cancer patients. Examples of cancer include, but are not limited to, primary and/or recurrent cancer, including brain cancer (*e.g.*, glioblastoma multiforme), lung cancer (*e.g.*, non-small cell lung cancer), squamous cell carcinoma of head and neck, renal cell carcinoma, melanoma, multiple myeloma, prostate cancer, and colon cancer. The methods comprise administering a pharmaceutical composition comprising a therapeutically effective amount of an anti-PD-1 antibody of the present invention in combination with a second therapeutic agent selected from the group consisting of a vascular endothelial growth factor (VEGF) antagonist (*e.g.*, aflibercept, bevacizumab), an angiopoietin-2 (Ang2) inhibitor (*e.g.*, an anti-Ang2 antibody such as nesvacumab), a lymphocyte activation gene 3 (LAG-3) inhibitor, a cytotoxic T-lymphocyte antigen 4 (CTLA-4) inhibitor (*e.g.*, ipilimumab), a chemotherapeutic agent, and radiation therapy. Additional examples of additional therapies/therapeutic agents that can be used in combination with an anti-PD-1 antibody of the invention for use in treating cancer are described elsewhere herein.

[052] The antibody or fragment thereof may be administered subcutaneously, intravenously, intradermally, intraperitoneally, orally, intramuscularly, or intracranially. The antibody or fragment thereof may be administered at a dose of about 0.1 mg/kg of body weight to about 100 mg/kg of body weight of the subject.

[053] The present invention also includes use of an anti-PD-1 antibody or antigen-binding fragment thereof of the invention in the manufacture of a medicament for the treatment of a disease or disorder that would benefit from the blockade or enhancement of PD-1 binding and/or signaling.

[054] Other embodiments will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[055] Figure 1 is a schematic of the luciferase-based PD-1 bioassay described in Example 8 herein. Panel A: Inactive Jurkat cells; Panel B: Jurkat cells are activated by T-cell receptor (TCR) clustering through the CD3xCD20 bispecific antibody; Panel C: PD-1 activation attenuates response in activated Jurkat cells; Panel D: Blocking PD-1 rescues the response in activated Jurkat cells.

[056] Figure 2 illustrates tumor growth and survival results for mice implanted with Colon-26 tumor cells at Day 0 and treated with the indicated combinations of molecules by injection at Days 3, 6, 10, 13 and 19 ("early-treatment tumor model"). The graph depicts tumor volume (in mm^3) for the different experimental groups at various time points after implantation. Upward arrows along the X-axis indicate the timing of treatment injections. "mIgG2a" is IgG2 isotype control; "Fc" is human Fc control; "VEGF Trap" is aflibercept; "anti-PD-1" is anti-mouse PD-1 clone RPMI-14; "anti-PD-L1" is an anti-PD-L1 monoclonal antibody as described elsewhere

herein.

[057] Figure 3 illustrates tumor growth and survival results for mice implanted with Colon-26 tumor cells at Day 0 and treated with the indicated combinations of molecules by injection at Days 3, 6, 10, 13 and 19 ("early-treatment tumor model"). The graph shows the tumor volume (in mm^3) of individual mice in each experimental group at Day 28 after implantation. "mIgG2a" is IgG2 isotype control; "Fc" is human Fc control; "VEGF Trap" is aflibercept; "anti-PD-1" is anti-mouse PD-1 clone RPMI-14; "anti-PD-L1" is an anti-PD-L1 monoclonal antibody as described elsewhere herein.

DETAILED DESCRIPTION

[058] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[059] 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 invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

[060] The term "PD-1" refers to the programmed death-1 protein, a T-cell co-inhibitor, also known as CD279. The amino acid sequence of full-length PD-1 is provided in GenBank as accession number NP_005009.2 and is also referred to herein as SEQ ID NO: 327. The term "PD-1" also includes protein variants of PD-1 having the amino acid sequence of SEQ ID NOs: 321, 322, 323, or 324. The term "PD-1" includes recombinant PD-1 or a fragment thereof. The term also encompasses PD-1 or a fragment thereof coupled to, for example, histidine tag, mouse or human Fc, or a signal sequence such as ROR1. For example, the term includes sequences exemplified by SEQ ID NOs: 323 or 324, comprising a mouse Fc (mIgG2a) or human Fc (hIgG1) at the C-terminal, coupled to amino acid residues 25 – 170 of full-length PD-1 with a C93S change. Protein variants as exemplified by SEQ ID NO: 321 comprise a histidine tag at the C-terminal, coupled to amino acid residues 25 – 170 of full length PD-1. Unless specified as being from a non-human species, the term "PD-1" means human PD-1.

[061] PD-1 is a member of the CD28/ CTLA-4/ICOS family of T-cell co-inhibitors. PD-1 is a 288-amino acid protein with an extracellular N-terminal domain which is IgV-like, a transmembrane domain and an intracellular domain containing an immunoreceptor tyrosine-based inhibitory (ITIM) motif and an immunoreceptor tyrosine-based switch (ITSM) motif (Chattopadhyay et al 2009, Immunol. Rev.). The PD-1 receptor has two ligands, PD-ligand-1 (PD-L1) and PD-L2.

[062] The term "PD-L1" refers to the ligand of the PD-1 receptor also known as CD274 and B7H1. The amino acid sequence of full-length PD-L1 is provided in GenBank as accession number NP_054862.1 and is also referred to herein as SEQ ID NO: 328. The term also encompasses PD-L1 or a fragment thereof coupled to, for example, histidine tag, mouse or human Fc, or a signal sequence such as ROR1. For example, the term includes sequences exemplified by SEQ ID NOs: 325 or 326, comprising a mouse Fc (mIgG2a) or human Fc (hIgG1) at the C-terminal, coupled to amino acid residues 19 – 239 of full-length PD-L1. PD-L1 is a 290 amino acid protein with an extracellular IgV-like domain, a transmembrane domain and a highly conserved intracellular domain of approximately 30 amino acids. PD-L1 is constitutively expressed on many cells such as antigen presenting cells (e.g., dendritic cells, macrophages, and B-cells) and on hematopoietic and non-hematopoietic cells (e.g., vascular endothelial cells, pancreatic islets, and sites of immune privilege). PD-L1 is also expressed on a wide variety of tumors, virally-infected cells and autoimmune tissue, and is a component of the immunosuppressive milieu (Ribas 2012, NEJM 366: 2517-2519).

[063] As used herein, the term "T-cell co-inhibitor" refers to a ligand and/or receptor which modulates the immune response via T-cell activation or suppression. The term "T-cell co-inhibitor", also known as T-cell co-signaling molecule, includes, but is not limited to, lymphocyte activation gene 3 protein (LAG-3, also known as CD223), cytotoxic T-lymphocyte antigen-4 (CTLA-4), B and T lymphocyte attenuator (BTLA), CD-28, 2B4, LY108, T-cell immunoglobulin and mucin 3(TIM3), T-cell immunoreceptor with immunoglobulin and ITIM (TIGIT; also known as VSIG9), leucocyte associated immunoglobulin-like receptor 1 (LAIR1; also known as CD305), inducible T-cell costimulator (ICOS; also known as CD278), V-domain Ig suppressor of T-cell activation (VISTA) and CD160.

[064] As used herein, the term "Fc receptor" refers to the surface receptor protein found on immune cells including B lymphocytes, natural killer cells, macrophages, basophils, neutrophils, and mast cells, which has a binding specificity for the Fc region of an antibody. The term "Fc receptor" includes, but is not limited to, a Fcγ receptor [e.g., FcγRI (CD64), FcγRIIA (CD32), FcγRIIB (CD32), FcγRIIIA (CD16a), and FcγRIIIB (CD16b)], Fcα receptor (e.g., FcαRI or CD89) and Fcε receptor [e.g., FcεRI, and FcεRII (CD23)].

[065] The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds (*i.e.*, "full antibody molecules"), as well as multimers thereof (*e.g.* IgM) or antigen-binding fragments thereof. Each heavy chain is comprised of a heavy chain variable region ("HCVR" or "V_H") and a heavy chain constant region (comprised of domains C_H1, C_H2 and C_H3). Each light chain is comprised of a light chain variable region ("LCVR or "V_L") and a light chain constant region (C_L). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed

of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In certain embodiments of the invention, the FRs of the antibody (or antigen binding fragment thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[066] Substitution of one or more CDR residues or omission of one or more CDRs is also possible. Antibodies have been described in the scientific literature in which one or two CDRs can be dispensed with for binding. Padlan *et al.* (1995 FASEB J. 9:133-139) analyzed the contact regions between antibodies and their antigens, based on published crystal structures, and concluded that only about one fifth to one third of CDR residues actually contact the antigen. Padlan also found many antibodies in which one or two CDRs had no amino acids in contact with an antigen (see also, Vajdos *et al.* 2002 J Mol Biol 320:415-428).

[067] CDR residues not contacting antigen can be identified based on previous studies (for example residues H60-H65 in CDRH2 are often not required), from regions of Kabat CDRs lying outside Chothia CDRs, by molecular modeling and/or empirically. If a CDR or residue(s) thereof is omitted, it is usually substituted with an amino acid occupying the corresponding position in another human antibody sequence or a consensus of such sequences. Positions for substitution within CDRs and amino acids to substitute can also be selected empirically. Empirical substitutions can be conservative or non-conservative substitutions.

[068] The fully human anti-PD-1 monoclonal antibodies disclosed herein may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present invention includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or

only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies of the present invention may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present invention.

[069] The present invention also includes fully human anti-PD-1 monoclonal antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present invention includes anti-PD-1 antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein.

[070] The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human mAbs 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*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include mAbs in which CDR sequences derived from the germline of another mammalian species (e.g., mouse), have been grafted onto human FR sequences. The term includes antibodies recombinantly produced in a non-human mammal, or in cells of a non-human mammal. The term is not intended to include antibodies isolated from or generated in a human subject.

[071] The term "recombinant", as used herein, refers to antibodies or antigen-binding fragments thereof of the invention created, expressed, isolated or obtained by technologies or methods known in the art as recombinant DNA technology which include, *e.g.*, DNA splicing and transgenic expression. The term refers to antibodies expressed in a non-human mammal (including transgenic non-human mammals, *e.g.*, transgenic mice), or a cell (*e.g.*, CHO cells) expression system or isolated from a recombinant combinatorial human antibody library.

[072] The term "multi-specific antigen-binding molecules", as used herein refers to bispecific, tri-specific or multi-specific antigen-binding molecules, and antigen-binding fragments thereof.

Multi-specific antigen-binding molecules may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for epitopes of more than one target polypeptide. A multi-specific antigen-binding molecule can be a single multifunctional polypeptide, or it can be a multimeric complex of two or more polypeptides that are covalently or non-covalently associated with one another. The term "multi-specific antigen-binding molecules" includes antibodies of the present invention that may be linked to or co-expressed with another functional molecule, *e.g.*, another peptide or protein. For example, an antibody or fragment thereof can be functionally linked (*e.g.*, by chemical coupling, genetic fusion, non-covalent association or otherwise) to one or more other molecular entities, such as a protein or fragment thereof to produce a bi-specific or a multi-specific antigen-binding molecule with a second binding specificity. According to the present invention, the term "multi-specific antigen-binding molecules" also includes bi-specific, tri-specific or multi-specific antibodies or antigen-binding fragments thereof. In certain embodiments, an antibody of the present invention is functionally linked to another antibody or antigen-binding fragment thereof to produce a bispecific antibody with a second binding specificity. Bispecific and multi-specific antibodies of the present invention are described elsewhere herein.

[073] The term "specifically binds," or "binds specifically to", or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiologic conditions. Specific binding can be characterized by an equilibrium dissociation constant of at least about $1x10^{-8}$ M or less (*e.g.*, a smaller K_D denotes a tighter binding). Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. As described herein, antibodies have been identified by surface plasmon resonance, *e.g.*, BIACORE™, which bind specifically to PD-1. Moreover, multi-specific antibodies that bind to one domain in PD-1 and one or more additional antigens or a bi-specific that binds to two different regions of PD-1 are nonetheless considered antibodies that "specifically bind", as used herein.

[074] The term "high affinity" antibody refers to those mAbs having a binding affinity to PD-1, expressed as K_D, of at least 10^{-7} M; preferably 10^{-8} M; more preferably 10^{-9}M, even more preferably 10^{-10} M, even more preferably 10^{-11} M, as measured by surface plasmon resonance, e.g., BIACORE™ or solution-affinity ELISA.

[075] By the term "slow off rate", "Koff" or "kd" is meant an antibody that dissociates from PD-1, with a rate constant of 1×10^{-3} s^{-1} or less, preferably 1×10^{-4} s^{-1} or less, as determined by surface plasmon resonance, *e.g.*, BIACORE™.

[076] The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen

to form a complex. The terms "antigen-binding fragment" of an antibody, or "antibody fragment", as used herein, refers to one or more fragments of an antibody that retain the ability to bind to PD-1.

[077] In specific embodiments, antibody or antibody fragments of the invention may be conjugated to a moiety such a ligand or a therapeutic moiety ("immunoconjugate"), such as an antibiotic, a second anti-PD-1 antibody, or an antibody to another antigen such a tumor-specific antigen, an autoimmune tissue antigen, a virally-infected cell antigen, a Fc receptor, a T-cell receptor, or a T-cell co-inhibitor, or an immunotoxin, or any other therapeutic moiety useful for treating a disease or condition including cancer, autoimmune disease or chronic viral infection.

[078] An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies (Abs) having different antigenic specificities (*e.g.,* an isolated antibody that specifically binds PD-1, or a fragment thereof, is substantially free of Abs that specifically bind antigens other than PD-1.

[079] A "blocking antibody" or a "neutralizing antibody", as used herein (or an "antibody that neutralizes PD-1 activity" or "antagonist antibody"), is intended to refer to an antibody whose binding to PD-1 results in inhibition of at least one biological activity of PD-1. For example, an antibody of the invention may prevent or block PD-1 binding to PD-L1.

[080] An "activating antibody" or an "enhancing antibody", as used herein (or an "agonist antibody"), is intended to refer to an antibody whose binding to PD-1 results in increasing or stimulating at least one biological activity of PD-1. For example, an antibody of the invention may increase PD-1 binding to PD-L1.

[081] The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biomolecular interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.).

[082] The term "K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[083] The term "epitope" refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. The term "epitope" also refers to a site on an antigen to which B and/or T cells respond. It also refers to a region of an antigen that is bound by an antibody. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may also be conformational, that is, composed of non-linear amino acids. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-

dimensional structural characteristics, and/or specific charge characteristics.

[084] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 90%, and more preferably at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or GAP, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[085] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 90% sequence identity, even more preferably at least 95%, 98% or 99% sequence identity. Preferably, residue positions, which are not identical, differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. See, *e.g.*, Pearson (1994) Methods Mol. Biol. 24: 307-331, which is herein incorporated by reference. Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartate and glutamate, and 7) sulfur-containing side chains: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443 45, herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[086] Sequence similarity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as GAP and

BESTFIT which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, *e.g.*, GCG Version 6.1. Polypeptide sequences also can be compared using FASTA with default or recommended parameters; a program in GCG Version 6.1. FASTA (*e.g.*, FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) *supra*). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. See, *e.g.*, Altschul *et al.* (1990) J. Mol. Biol. 215: 403-410 and (1997) Nucleic Acids Res. 25:3389-3402, each of which is herein incorporated by reference.

[087] By the phrase "therapeutically effective amount" is meant an amount that produces the desired effect for which it is administered. The exact amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

[088] As used herein, the term "subject" refers to an animal, preferably a mammal, in need of amelioration, prevention and/or treatment of a disease or disorder such as chronic viral infection, cancer or autoimmune disease.

[089] As used herein, "anti-cancer drug" means any agent useful to treat cancer including, but not limited to, cytotoxins and agents such as antimetabolites, alkylating agents, anthracyclines, antibiotics, antimitotic agents, procarbazine, hydroxyurea, asparaginase, corticosteroids, mytotane (O,P'-(DDD)), biologics (*e.g.*, antibodies and interferons) and radioactive agents. As used herein, "a cytotoxin or cytotoxic agent", also refers to a chemotherapeutic agent and means any agent that is detrimental to cells. Examples include Taxol® (paclitaxel), temozolamide, cytochalasin B, gramicidin D, ethidium bromide, emetine, cisplatin, mitomycin, etoposide, tenoposide, vincristine, vinbiastine, coichicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

[090] As used herein, the term "anti-viral drug" refers to any drug or therapy used to treat, prevent, or ameliorate a viral infection in a host subject. The term "anti-viral drug" includes, but is not limited to zidovudine, lamivudine, abacavir, ribavirin, lopinavir, efavirenz, cobicistat, tenofovir, rilpivirine, analgesics and corticosteroids. In the context of the present invention, the viral infections include long-term or chronic infections caused by viruses including, but not limited to, human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), human papilloma virus (HPV), lymphocytic choriomeningitis virus (LCMV), and simian immunodeficiency virus (SIV).

[091] The antibodies and antigen-binding fragments of the present invention specifically bind to

PD-1 and modulate the interaction of PD-1 with PD-L1. The anti-PD-1 antibodies may bind to PD-1 with high affinity or with low affinity. In certain embodiments, the antibodies of the present invention may be blocking antibodies wherein the antibodies may bind to PD-1 and block the interaction of PD-1 with PD-L1. In some embodiments, the blocking antibodies of the invention may block the binding of PD-1 to PD-L1 and/or stimulate or enhance T-cell activation. In some embodiments, the blocking antibodies may be useful for stimulating or enhancing the immune response and/or for treating a subject suffering from cancer, or a chronic viral infection. The antibodies when administered to a subject in need thereof may reduce the chronic infection by a virus such as HIV, LCMV or HBV in the subject. They may be used to inhibit the growth of tumor cells in a subject. They may be used alone or as adjunct therapy with other therapeutic moieties or modalities known in the art for treating cancer, or viral infection.

[092] In other embodiments, the antibodies of the present invention may be activating antibodies, wherein the antibodies may bind to PD-1 and enhance the interaction of PD-1 and PD-L1. In some embodiments, the activating antibodies may enhance binding of PD-1 to PD-L1 and/or inhibit or suppress T-cell activation. The activating antibodies of the present invention may be useful for inhibiting the immune response in a subject and/or for treating autoimmune disease.

[093] In certain embodiments, the anti-PD-1 antibodies may be multi-specific antigen-binding molecules, wherein they comprise a first binding specificity to PD-1 and a second binding specificity to an antigen selected from the group consisting of another T-cell co-inhibitor, an autoimmune tissue antigen, T-cell receptor, Fc receptor, T-cell receptor, PD-L1, and a different epitope of PD-1.

[094] In certain embodiments, the antibodies of the invention are obtained from mice immunized with a primary immunogen, such as a full length PD-1 [See GenBank accession number NP_005009.2 (SEQ ID NO: 327)] or with a recombinant form of PD-1 or modified human PD-1 fragments (SEQ ID NOs: 321, 323, or 324) or with modified cynomolgus PD-1 fragments (SEQ ID NO: 322), followed by immunization with a secondary immunogen, or with an immunogenically active fragment of PD-1.

[095] The immunogen may be a biologically active and/or immunogenic fragment of PD-1 or DNA encoding the active fragment thereof. The fragment may be derived from the N-terminal or C-terminal domain of PD-1. In certain embodiments of the invention, the immunogen is a fragment of PD-1 that ranges from amino acid residues 25 – 170 of SEQ ID NO: 327 with a C93S change.

[096] The peptides may be modified to include addition or substitution of certain residues for tagging or for purposes of conjugation to carrier molecules, such as, KLH. For example, a cysteine may be added at either the N terminal or C terminal end of a peptide, or a linker sequence may be added to prepare the peptide for conjugation to, for example, KLH for immunization.

[097] The full-length amino acid sequence of full length human PD-1 is shown as SEQ ID NO: 327.

[098] In certain embodiments, antibodies that bind specifically to PD-1 may be prepared using fragments of the above-noted regions, or peptides that extend beyond the designated regions by about 5 to about 20 amino acid residues from either, or both, the N or C terminal ends of the regions described herein. In certain embodiments, any combination of the above-noted regions or fragments thereof may be used in the preparation of PD-1 specific antibodies. In certain embodiments, any one or more of the above-noted regions of PD-1, or fragments thereof may be used for preparing monospecific, bispecific, or multispecific antibodies.

[099] Certain anti-PD-1 antibodies of the present invention are able to bind to and neutralize the activity of PD-1, as determined by *in vitro* or *in vivo* assays. The ability of the antibodies of the invention to bind to and neutralize the activity of PD-1 may be measured using any standard method known to those skilled in the art, including binding assays, or activity assays, as described herein.

[0100] Non-limiting, exemplary *in vitro* assays for measuring binding activity are illustrated in Examples herein. In Example 3, the binding affinities and kinetic constants of human anti-PD-1 antibodies for human PD-1 and cynomolgus PD-1 were determined by surface plasmon resonance and the measurements were conducted on a Biacore 4000 or T200 instrument. In Examples 4 and 5, blocking assays were used to determine the ability of the anti-PD-1 antibodies to block PD-L1-binding ability of PD-1 *in vitro*. In Example 6, blocking assays were used to determine cross-competition between anti-PD-1 antibodies. Example 7 describes the binding of the antibodies to cells overexpressing PD-1. In Example 8, a luciferase assay was used to determine the ability of anti-PD-1 antibodies to antagonize PD-1/PD-L1 signaling in T-cells.

[0101] In certain embodiments, the antibodies of the present invention are able to enhance or stimulate T-cell activation *in vitro* and in a subject with cancer or in a subject infected with a virus such as LCMV. In certain embodiments, the antibodies of the present invention are used in combination with a second therapeutic agent, such as an antibody to a second T-cell co-inhibitor, to enhance the immune response and inhibit tumor growth in a subject.

[0102] The antibodies specific for PD-1 may contain no additional labels or moieties, or they may contain an N-terminal or C-terminal label or moiety. In one embodiment, the label or moiety is biotin. In a binding assay, the location of a label (if any) may determine the orientation of the peptide relative to the surface upon which the peptide is bound. For example, if a surface is coated with avidin, a peptide containing an N-terminal biotin will be oriented such that the C-terminal portion of the peptide will be distal to the surface. In one embodiment, the label may be a radionuclide, a fluorescent dye or a MRI-detectable label. In certain embodiments, such labeled antibodies may be used in diagnostic assays including imaging assays.

Antigen-Binding Fragments of Antibodies

[0103] Unless specifically indicated otherwise, the term "antibody," as used herein, shall be understood to encompass antibody molecules comprising two immunoglobulin heavy chains and two immunoglobulin light chains (*i.e.*, "full antibody molecules") as well as antigen-binding fragments thereof. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. The terms "antigen-binding fragment" of an antibody, or "antibody fragment", as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to PD-1. An antibody fragment may include a Fab fragment, a $F(ab')_2$ fragment, a Fv fragment, a dAb fragment, a fragment containing a CDR, or an isolated CDR. In certain embodiments, the term "antigen-binding fragment" refers to a polypeptide fragment of a multi-specific antigen-binding molecule. In such embodiments, the term" antigen-binding fragment" includes, *e.g.*, an extracellular domain of PD-L1 which binds specifically to PD-1. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and (optionally) constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[0104] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[0105] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR, which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For

example, the variable region may be dimeric and contain V_H - V_H, V_H - V_L or V_L - V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[0106] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V_H -C_H1; (ii) V_H -C_H2; (iii) V_H -C_H3; (iv) V_H -C_H1-C_H2; (v) V_H -C_H1-C_H2-C_H3; (vi) V_H -C_H2-C_H3; (vii) V_H -C_L; (viii) V_L -C_H1; (ix) V_L -C_H2; (x) V_L -C_H3; (xi) V_L -C_H1-C_H2; (xii) V_L -C_H1-C_H2-C_H3; (xiii) V_L -C_H2-C_H3; and (xiv) V_L -C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids, which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[0107] As with full antibody molecules, antigen-binding fragments may be mono-specific or multi-specific (*e.g.*, bi-specific). A multi-specific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multi-specific antibody format, including the exemplary bi-specific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

Preparation of Human Antibodies

[0108] Methods for generating human antibodies in transgenic mice are known in the art. Any such known methods can be used in the context of the present invention to make human antibodies that specifically bind to PD-1.

[0109] An immunogen comprising any one of the following can be used to generate antibodies to PD-1. In certain embodiments, the antibodies of the invention are obtained from mice immunized with a full length, native PD-1 (See GenBank accession number NP_005009.2) (SEQ ID NO: 327), or with a recombinant PD-1 peptide. Alternatively, PD-1 or a fragment thereof may be produced using standard biochemical techniques and modified (SEQ ID NOS: 321 – 324) and used as immunogen.

[0110] In certain embodiments, the immunogen may be a peptide from the N terminal or C terminal end of PD-1. In one embodiment, the immunogen is the extracellular domain or the IgV-

like domain of PD-1. In certain embodiments of the invention, the immunogen is a fragment of PD-1 that ranges from about amino acid residues 25-170 of SEQ ID NO: 327 with a C93S change.

[0111] In some embodiments, the immunogen may be a recombinant PD-1 peptide expressed in *E. coli* or in any other eukaryotic or mammalian cells such as Chinese hamster ovary (CHO) cells.

[0112] In certain embodiments, antibodies that bind specifically to PD-1 may be prepared using fragments of the above-noted regions, or peptides that extend beyond the designated regions by about 5 to about 20 amino acid residues from either, or both, the N or C terminal ends of the regions described herein. In certain embodiments, any combination of the above-noted regions or fragments thereof may be used in the preparation of PD-1 specific antibodies.

[0113] Using VELOCIMMUNE® technology (see, for example, US 6,596,541, Regeneron Pharmaceuticals, VELOCIMMUNE®) or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to PD-1 are initially isolated having a human variable region and a mouse constant region. The VELOCIMMUNE® technology involves generation of a transgenic mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. The DNA encoding the variable regions of the heavy and light chains of the antibody are isolated and operably linked to DNA encoding the human heavy and light chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human antibody.

Bioequivalents

[0114] The anti-PD-1 antibodies and antibody fragments of the present invention encompass proteins having amino acid sequences that vary from those of the described antibodies, but that retain the ability to bind PD-1. Such variant antibodies and antibody fragments comprise one or more additions, deletions, or substitutions of amino acids when compared to parent sequence, but exhibit biological activity that is essentially equivalent to that of the described antibodies. Likewise, the antibody-encoding DNA sequences of the present invention encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to the disclosed sequence, but that encode an antibody or antibody fragment that is essentially bioequivalent to an antibody or antibody fragment of the invention.

[0115] Two antigen-binding proteins, or antibodies, are considered bioequivalent if, for example, they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose under similar experimental conditions, either single dose or multiple doses. Some antibodies will be considered equivalents or pharmaceutical alternatives if they are equivalent in

the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on, *e.g.*, chronic use, and are considered medically insignificant for the particular drug product studied.

[0116] In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, or potency.

[0117] In one embodiment, two antigen-binding proteins are bioequivalent if a patient can be switched one or more times between the reference product and the biological product without an expected increase in the risk of adverse effects, including a clinically significant change in immunogenicity, or diminished effectiveness, as compared to continued therapy without such switching.

[0118] In one embodiment, two antigen-binding proteins are bioequivalent if they both act by a common mechanism or mechanisms of action for the condition or conditions of use, to the extent that such mechanisms are known.

[0119] Bioequivalence may be demonstrated by *in vivo* and/or *in vitro* methods. Bioequivalence measures include, *e.g.*, (a) an *in vivo* test in humans or other mammals, in which the concentration of the antibody or its metabolites is measured in blood, plasma, serum, or other biological fluid as a function of time; (b) an *in vitro* test that has been correlated with and is reasonably predictive of human *in vivo* bioavailability data; (c) an *in vivo* test in humans or other mammals in which the appropriate acute pharmacological effect of the antibody (or its target) is measured as a function of time; and (d) in a well-controlled clinical trial that establishes safety, efficacy, or bioavailability or bioequivalence of an antibody.

[0120] Bioequivalent variants of the antibodies of the invention may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. In other contexts, bioequivalent antibodies may include antibody variants comprising amino acid changes, which modify the glycosylation characteristics of the antibodies, *e.g.*, mutations that eliminate or remove glycosylation.

Anti-PD-1 Antibodies Comprising Fc Variants

[0121] According to certain embodiments of the present invention, anti-PD-1 antibodies are provided comprising an Fc domain comprising one or more mutations which enhance or diminish antibody binding to the FcRn receptor, *e.g.*, at acidic pH as compared to neutral pH. For example, the present invention includes anti-PD-1 antibodies comprising a mutation in the C_H2 or a C_H3 region of the Fc domain, wherein the mutation(s) increases the affinity of the Fc

domain to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0). Such mutations may result in an increase in serum half-life of the antibody when administered to an animal. Non-limiting examples of such Fc modifications include, *e.g.*, a modification at position 250 (e.g., E or Q); 250 and 428 (e.g., L or F); 252 (e.g., L/Y/F/W or T), 254 (e.g., S or T), and 256 (e.g., S/R/Q/E/D or T); or a modification at position 428 and/or 433 (e.g., H/L/R/S/P/Q or K) and/or 434 (*e.g.,* A, W, H, F or Y [N434A, N434W, N434H, N434F or N434Y]); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.,* 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.,* T250Q and M428L); and a 307 and/or 308 modification (*e.g.,* 308F or 308P). In yet another embodiment, the modification comprises a 265A (*e.g.*, D265A) and/or a 297A (*e.g.*, N297A) modification.

[0122] For example, the present invention includes anti-PD-1 antibodies comprising an Fc domain comprising one or more pairs or groups of mutations selected from the group consisting of: 250Q and 248L (*e.g.*, T250Q and M248L); 252Y, 254T and 256E (*e.g.*, M252Y, S254T and T256E); 428L and 434S (*e.g.*, M428L and N434S); 257I and 311I (*e.g.*, P257I and Q311I); 257I and 434H (*e.g.*, P257I and N434H); 376V and 434H (*e.g.*, D376V and N434H); 307A, 380A and 434A (*e.g.*, T307A, E380A and N434A); and 433K and 434F (*e.g.*, H433K and N434F). In one embodiment, the present invention includes anti-PD-1 antibodies comprising an Fc domain comprising a S108P mutation in the hinge region of IgG4 to promote dimer stabilization. All possible combinations of the foregoing Fc domain mutations, and other mutations within the antibody variable domains disclosed herein, are contemplated within the scope of the present invention.

[0123] The present invention also includes anti-PD-1 antibodies comprising a chimeric heavy chain constant (C_H) region, wherein the chimeric C_H region comprises segments derived from the C_H regions of more than one immunoglobulin isotype. For example, the antibodies of the invention may comprise a chimeric C_H region comprising part or all of a C_H2 domain derived from a human IgG1, human IgG2 or human IgG4 molecule, combined with part or all of a C_H3 domain derived from a human IgG1, human IgG2 or human IgG4 molecule. According to certain embodiments, the antibodies of the invention comprise a chimeric C_H region having a chimeric hinge region. For example, a chimeric hinge may comprise an "upper hinge" amino acid sequence (amino acid residues from positions 216 to 227 according to EU numbering) derived from a human IgG1, a human IgG2 or a human IgG4 hinge region, combined with a "lower hinge" sequence (amino acid residues from positions 228 to 236 according to EU numbering) derived from a human IgG1, a human IgG2 or a human IgG4 hinge region. According to certain embodiments, the chimeric hinge region comprises amino acid residues derived from a human IgG1 or a human IgG4 upper hinge and amino acid residues derived from

a human IgG2 lower hinge. An antibody comprising a chimeric C_H region as described herein may, in certain embodiments, exhibit modified Fc effector functions without adversely affecting the therapeutic or pharmacokinetic properties of the antibody. (*See, e.g.*, USSN. 14/170,166, filed January 31, 2014, the disclosure of which is hereby incorporated by reference in its entirety).

Biological Characteristics of the Antibodies

[0124] In general, the antibodies of the present invention function by binding to PD-1. The present invention includes anti-PD-1 antibodies and antigen-binding fragments thereof that bind soluble monomeric or dimeric PD-1 molecules with high affinity. For example, the present invention includes antibodies and antigen-binding fragments of antibodies that bind monomeric PD-1 (*e.g.*, at 25°C or at 37°C) with a K_D of less than about 50nM as measured by surface plasmon resonance, e.g., using the assay format as defined in Example 3 herein. In certain embodiments, the antibodies or antigen-binding fragments thereof bind monomeric PD-1 with a K_D of less than about 40nM, less than about 30nM, less than about 20nM, less than about 10nM less than about 5nM, less than about 2nM or less than about 1nM, as measured by surface plasmon resonance, e.g., using the assay format as defined in Example 3 herein, or a substantially similar assay.

[0125] The present invention also includes antibodies and antigen-binding fragments thereof that bind dimeric PD-1 (*e.g.*, at 25°C or at 37°C) with a K_D of less than about 400 pM as measured by surface plasmon resonance, e.g., using the assay format as defined in Example 3 herein. In certain embodiments, the antibodies or antigen-binding fragments thereof bind dimeric PD-1 with a K_D of less than about 300 pM, less than about 250 pM, less than about 200 pM, less than about 100 pM, or less than about 50 pM, as measured by surface plasmon resonance, *e.g.*, using the assay format as defined in Example 3 herein, or a substantially similar assay.

[0126] The present invention also includes antibodies or antigen-binding fragments thereof that bind cynomolgus (*Macaca fascicularis*) PD-1 (*e.g.*, at 25°C or at 37°C) with a K_D of less than about 35 nM as measured by surface plasmon resonance, e.g., using the assay format as defined in Example 3 herein. In certain embodiments, the antibodies or antigen-binding fragments thereof bind cynomolgus PD-1 with a K_D of less than about 30 nM, less than about 20 nM, less than about 15 nM, less than about 10 nM, or less than about 5 nM, as measured by surface plasmon resonance, *e.g.*, using the assay format as defined in Example 3 herein, or a substantially similar assay.

[0127] The present invention also includes antibodies and antigen-binding fragments thereof that bind PD-1 with a dissociative half-life (t½) of greater than about 1.1 minutes as measured by surface plasmon resonance at 25°C or 37°C, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay. In certain embodiments, the antibodies or antigen-binding fragments of the present invention bind PD-1 with a t½ of greater than about 5

minutes, greater than about 10 minutes, greater than about 30 minutes, greater than about 50 minutes, greater than about 60 minutes, greater than about 70 minutes, greater than about 80 minutes, greater than about 90 minutes, greater than about 100 minutes, greater than about 200 minutes, greater than about 300 minutes, greater than about 400 minutes, greater than about 500 minutes, greater than about 600 minutes, greater than about 700 minutes, greater than about 800 minutes, greater than about 900 minutes, greater than about 1000 minutes, or greater than about 1200 minutes, as measured by surface plasmon resonance at 25°C or 37°C, *e.g.*, using an assay format as defined in Example 3 herein (*e.g.*, mAb-capture or antigen-capture format), or a substantially similar assay.

[0128] The present invention also includes antibodies or antigen-binding fragments thereof that block PD-1 binding to PD-L1 with an IC50 of less than about 3 nM as determined using a ELISA-based immunoassay assay, e.g., as shown in Example 4, or a substantially similar assay. The present invention also includes antibodies and antigen-binding fragments thereof that bind to PD-1 and enhance the binding of PD-1 to PD-L1.

[0129] In some embodiments, the antibodies of the present invention may bind to the extracellular domain of PD-1 or to a fragment of the domain. In some embodiments, the antibodies of the present invention may bind to more than one domain (cross-reactive antibodies). In certain embodiments, the antibodies of the present invention may bind to an epitope located in the extracellular domain comprising amino acid residues 21 – 171 of PD-1 (SEQ ID NO: 327). In one embodiment, the antibodies may bind to an epitope comprising one or more amino acids selected from the group consisting of amino acid residues 1 – 146 of SEQ ID NOs: 321 – 324.

[0130] In certain embodiments, the antibodies of the present invention may function by blocking or inhibiting the PD-L1-binding activity associated with PD-1 by binding to any other region or fragment of the full length protein, the amino acid sequence of which is shown in SEQ ID NO: 327. In certain embodiments, the antibodies may attenuate or modulate the interaction between PD-1 and PD-L1.

[0131] In certain embodiments, the antibodies of the present invention may be bi-specific antibodies. The bi-specific antibodies of the invention may bind one epitope in one domain and may also bind a second epitope in a different domain of PD-1. In certain embodiments, the bi-specific antibodies of the invention may bind two different epitopes in the same domain. In one embodiment, the multi-specific antigen-binding molecule comprises a first binding specificity wherein the first binding specificity comprises the extracellular domain or fragment thereof of PD-L1; and a second binding specificity to another epitope of PD-1.

[0132] In one embodiment, the invention provides an isolated fully human monoclonal antibody or antigen-binding fragment thereof that binds to PD-1, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (i) comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50,

66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 218, 226, 234, 242, 250, 258, 266, 274, 282, 290, 298, 306, and 314, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (ii) comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, and 202, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iii) comprises a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 224, 232, 240, 248, 256, 264, 272, 280, 288, 296, 304, 312, and 320, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, and 208, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iv) comprises a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 220, 228, 236, 244, 252, 260, 268, 276, 284, 292, 300, 308, and 316, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 222, 230, 238, 246, 254, 262, 270, 278, 286, 294, 302, 310, and 318, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, and 204, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, and 206, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (v) is a multi-specific antigen-binding molecule comprising a first binding specificity to PD-1 and a second binding specificity to an antigen selected from the group consisting of PD-1, a tumor specific antigen, an autoimmune tissue specific antigen, a virally infected cell antigen, a different T-cell co-inhibitor, T-cell receptor, and a Fc receptor; (vi) binds to human PD-1 with a K_D of about 28pM to about 1.5μM; (vii) binds to cynomolgus PD-1 with a K_D of about 3nM to about 7.5μM; (viii) blocks or enhances the binding of PD-1 to PD-L1 with an IC50 ≤ about 3.3nM; (ix) blocks PD-1-induced T-cell down regulation and/or rescues T-cell signaling in a T-cell/APC luciferase reporter assay; (x) stimulates T-cell proliferation and activity in a mixed lymphocyte reaction (MLR) assay; (xi) induces IL-2 and/or IFNγ production in a MLR assay; and (xii) suppresses tumor growth and increases survival in subjects with cancer.

[0133] In one embodiment, the invention provides an isolated fully human monoclonal

antibody or antigen-binding fragment thereof that blocks PD-1 binding to PD-L1, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (i) comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 130, 162, 234 and 314, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (ii) comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 138, 170, 186, and 202, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iii) comprises a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 136, 168, 240, and 320, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 144, 176, 192, and 208, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iv) comprises a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 132, 164, 236, and 316, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 134, 166, 238, and 318, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 140, 172, 188, and 204, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 142, 174, 190, and 206, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (v) is a multi-specific antigen-binding molecule comprising a first binding specificity to PD-1 and a second binding specificity to an antigen selected from the group consisting of a different epitope of PD-1, a tumor specific antigen, an autoimmune tissue specific antigen, a virally infected cell antigen, a different T-cell co-inhibitor, T-cell receptor, and a Fc receptor; (vi) binds to human PD-1 with a $K_D \leq 10^{-9}$M; (vii) binds to cynomolgus PD-1 with a $K_D \leq 10^{-8}$M; (viii) blocks the binding of PD-1 to PD-L1 with an IC50 $\leq 10^{-10}$M; (ix) blocks PD-1-induced T-cell down regulation and/or rescues T-cell signaling in a T-cell/APC luciferase reporter assay; (x) stimulates T-cell proliferation and activity in a mixed lymphocyte reaction (MLR) assay; (xi) induces IL-2 and/or IFNγ production in a MLR assay; and (xii) suppresses tumor growth and increases survival in subjects with cancer.

[0134] The antibodies of the present invention may possess one or more of the aforementioned biological characteristics, or any combinations thereof. Other biological characteristics of the antibodies of the present invention will be evident to a person of ordinary skill in the art from a review of the present disclosure including the working Examples herein.

Species Selectivity and Species Cross-Reactivity

[0135] According to certain embodiments of the invention, the anti-PD-1 antibodies bind to human PD-1 but not to PD-1 from other species. Alternatively, the anti-PD-1 antibodies of the invention, in certain embodiments, bind to human PD-1 and to PD-1 from one or more non-human species. For example, the anti-PD-1 antibodies of the invention may bind to human PD-1 and may bind or not bind, as the case may be, to one or more of mouse, rat, guinea pig, hamster, gerbil, pig, cat, dog, rabbit, goat, sheep, cow, horse, camel, cynomolgus, marmoset, rhesus or chimpanzee PD-1. In certain embodiments, the anti-PD-1 antibodies of the invention may bind to human and cynomolgus PD-1 with the same affinities or with different affinities, but do not bind to rat and mouse PD-1.

Epitope Mapping and Related Technologies

[0136] The present invention includes anti-PD-1 antibodies which interact with one or more amino acids found within one or more domains of the PD-1 molecule including, *e.g.*, extracellular (IgV-like) domain, a transmembrane domain, and an intracellular domain containing the immunoreceptor tyrosine-based inhibition motif (ITIM) and immunoreceptor tyrosine-based switch motif (ITSM). The epitope to which the antibodies bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acids located within any of the aforementioned domains of the PD-1 molecule (*e.g.* a linear epitope in a domain). Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) located within either or both of the aforementioned domains of the PD-1 molecule (*e.g.* a conformational epitope).

[0137] Various techniques known to persons of ordinary skill in the art can be used to determine whether an antibody "interacts with one or more amino acids" within a polypeptide or protein. Exemplary techniques include, for example, routine cross-blocking assays, such as that described in Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harbor, NY). Other methods include alanine scanning mutational analysis, peptide blot analysis (Reineke (2004) Methods Mol. Biol. 248: 443-63), peptide cleavage analysis crystallographic studies and NMR analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) Prot. Sci. 9: 487-496). Another method that can be used to identify the amino acids within a polypeptide with which an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water and exchangeable protons within amino acids that are protected by the antibody complex undergo deuterium-to-hydrogen back-exchange at a slower rate than exchangeable protons within amino acids that are not part of the interface. As a result, amino

acids that form part of the protein/antibody interface may retain deuterium and therefore exhibit relatively higher mass compared to amino acids not included in the interface. After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues which correspond to the specific amino acids with which the antibody interacts. *See, e.g.*, Ehring (1999) Analytical Biochemistry 267: 252-259; Engen and Smith (2001) Anal. Chem. 73: 256A-265A.

[0138] The term "epitope" refers to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

[0139] Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (see US 2004/0101920, herein specifically incorporated by reference in its entirety). Each category may reflect a unique epitope either distinctly different from or partially overlapping with epitope represented by another category. This technology allows rapid filtering of genetically identical antibodies, such that characterization can be focused on genetically distinct antibodies. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones that produce mAbs having the desired characteristics. MAP may be used to sort the antibodies of the invention into groups of antibodies binding different epitopes.

[0140] In certain embodiments, the anti-PD-1 antibodies or antigen-binding fragments thereof bind an epitope within any one or more of the regions exemplified in PD-1, either in natural form, as exemplified in SEQ ID NO: 327, or recombinantly produced, as exemplified in SEQ ID NOS: 321 – 324, or to a fragment thereof. In some embodiments, the antibodies of the invention bind to an extracellular region comprising one or more amino acids selected from the group consisting of amino acid residues 21 – 171 of PD-1. In some embodiments, the antibodies of the invention bind to an extracellular region comprising one or more amino acids selected from the group consisting of amino acid residues 1 – 146 of cynomolgus PD-1, as exemplified by SEQ ID NO: 322.

[0141] In certain embodiments, the antibodies of the invention, as shown in Table 1, interact with at least one amino acid sequence selected from the group consisting of amino acid residues ranging from about position 21 to about position 136 of SEQ ID NO: 327; or amino acid residues ranging from about position 136 to about position 171 of SEQ ID NO: 327. These regions are partially exemplified in SEQ ID NOs: 321 – 324.

[0142] The present invention includes anti-PD-1 antibodies that bind to the same epitope, or a

portion of the epitope, as any of the specific exemplary antibodies described herein in Table 1, or an antibody having the CDR sequences of any of the exemplary antibodies described in Table 1. Likewise, the present invention also includes anti-PD-1 antibodies that compete for binding to PD-1 or a PD-1 fragment with any of the specific exemplary antibodies described herein in Table 1, or an antibody having the CDR sequences of any of the exemplary antibodies described in Table 1. For example, the present invention includes anti-PD-1 antibodies that cross-compete for binding to PD-1 with one or more antibodies as defined in Example 6 herein (*e.g.*, H2aM7788N, H4xH8992P, H4xH8999P, H1M7799N, H2aM7780N, H1M7800N, H2aM7794N, H2aM7798N, H4xH9145P2, H4H9057P2, H4xH9120P2, H4xH9128P2, H4H9019P, H4xH9119P2, H4xH9135P2, H4xH9034P, H2aM7790N, H4xH9035P, H4xH9037P, H4xH9045P and H2aM7795N).

[0143] One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-PD-1 antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference anti-PD-1 antibody of the invention, the reference antibody is allowed to bind to a PD-1 protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the PD-1 molecule is assessed. If the test antibody is able to bind to PD-1 following saturation binding with the reference anti-PD-1 antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-PD-1 antibody. On the other hand, if the test antibody is not able to bind to the PD-1 protein following saturation binding with the reference anti-PD-1 antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-PD-1 antibody of the invention.

[0144] To determine if an antibody competes for binding with a reference anti-PD-1 antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to a PD-1 protein under saturating conditions followed by assessment of binding of the test antibody to the PD-1 molecule. In a second orientation, the test antibody is allowed to bind to a PD-1 molecule under saturating conditions followed by assessment of binding of the reference antibody to the PD-1 molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the PD-1 molecule, then it is concluded that the test antibody and the reference antibody compete for binding to PD-1. As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antibody may not necessarily bind to the identical epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

[0145] Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, *e.g.*, Junghans *et al.*, Cancer Res. 1990

50:1495-1502). Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0146] Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art.

Immunoconjugates

[0147] The invention encompasses a human anti-PD-1 monoclonal antibody conjugated to a therapeutic moiety ("immunoconjugate"), such as a cytotoxin or a chemotherapeutic agent to treat cancer. As used herein, the term "immunoconjugate" refers to an antibody which is chemically or biologically linked to a cytotoxin, a radioactive agent, a cytokine, an interferon, a target or reporter moiety, an enzyme, a toxin, a peptide or protein or a therapeutic agent. The antibody may be linked to the cytotoxin, radioactive agent, cytokine, interferon, target or reporter moiety, enzyme, toxin, peptide or therapeutic agent at any location along the molecule so long as it is able to bind its target. Examples of immunoconjugates include antibody drug conjugates and antibody-toxin fusion proteins. In one embodiment, the agent may be a second different antibody to PD-1. In certain embodiments, the antibody may be conjugated to an agent specific for a tumor cell or a virally infected cell. The type of therapeutic moiety that may be conjugated to the anti-PD-1 antibody and will take into account the condition to be treated and the desired therapeutic effect to be achieved. Examples of suitable agents for forming immunoconjugates are known in the art; see for example, WO 05/103081.

Multi-specific Antibodies

[0148] The antibodies of the present invention may be mono-specific, bi-specific, or multi-specific. Multi-specific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, *e.g.,* Tutt et al., 1991, J. Immunol. 147:60-69; Kufer et al., 2004, Trends Biotechnol. 22:238-244.

[0149] In one aspect, the present invention includes multi-specific antigen-binding molecules or antigen-binding fragments thereof wherein one specificity of an immunoglobulin is specific for the extracellular domain of PD-1, or a fragment thereof, and the other specificity of the immunoglobulin is specific for binding outside the extracellular domain of PD-1, or a second therapeutic target, or is conjugated to a therapeutic moiety. In certain embodiments, the first

antigen-binding specificity may comprise PD-L1 or PD-L2, or a fragment thereof. In certain embodiments of the invention, one specificity of an immunoglobulin is specific for an epitope comprising amino acid residues 21-171 of PD-1 (SEQ ID NO: 327) or a fragment thereof, and the other specificity of the immunoglobulin is specific for a second target antigen. The second target antigen may be on the same cell as PD-1 or on a different cell. In one embodiment, the second target cell is on an immune cell other than a T-cell such as a B-cell, antigen-presenting cell, monocyte, macrophage, or dendritic cell. In some embodiments, the second target antigen may be present on a tumor cell or an autoimmune tissue cell or on a virally infected cell.

[0150] In another aspect, the invention provides multi-specific antigen-binding molecules or antigen-binding fragments thereof comprising a first antigen-binding specificity that binds to PD-1 and a second antigen-binding specificity that binds to a T-cell receptor, a B-cell receptor or a Fc receptor. In a related aspect, the invention provides multi-specific antigen-binding molecules or antigen-binding fragments thereof comprising a first antigen-binding specificity that binds to PD-1 and a second antigen-binding specificity that binds to a different T-cell co-inhibitor such as LAG-3, CTLA-4, BTLA, CD-28, 2B4, LY108, TIGIT, TIM3, LAIR1, ICOS and CD160.

[0151] In another aspect, the invention provides multi-specific antigen-binding molecules or antigen-binding fragments thereof comprising a first antigen-binding specificity that binds to PD-1 and a second antigen-binding specificity that binds to an autoimmune tissue-specific antigen. In certain embodiments, the antibodies may be activating or agonist antibodies.

[0152] Any of the multi-specific antigen-binding molecules of the invention, or variants thereof, may be constructed using standard molecular biological techniques (e.g., recombinant DNA and protein expression technology), as will be known to a person of ordinary skill in the art.

[0153] In some embodiments, PD-1-specific antibodies are generated in a bi-specific format (a "bi-specific") in which variable regions binding to distinct domains of PD-1 are linked together to confer dual-domain specificity within a single binding molecule. Appropriately designed bi-specifics may enhance overall PD-1 inhibitory efficacy through increasing both specificity and binding avidity. Variable regions with specificity for individual domains, (*e.g.*, segments of the N-terminal domain), or that can bind to different regions within one domain, are paired on a structural scaffold that allows each region to bind simultaneously to the separate epitopes, or to different regions within one domain. In one example for a bi-specific, heavy chain variable regions (V_H) from a binder with specificity for one domain are recombined with light chain variable regions (V_L) from a series of binders with specificity for a second domain to identify non-cognate V_L partners that can be paired with an original V_H without disrupting the original specificity for that V_H. In this way, a single V_L segment (*e.g.*, V_L1) can be combined with two different V_H domains (*e.g.*, V_H1 and V_H2) to generate a bi-specific comprised of two binding "arms" (V_H1- V_L1 and V_H2- V_L1). Use of a single V_L segment reduces the complexity of the system and thereby simplifies and increases efficiency in cloning, expression, and purification

processes used to generate the bi-specific (See, for example, USSN13/022759 and US2010/0331527).

[0154] Alternatively, antibodies that bind more than one domains and a second target, such as, but not limited to, for example, a second different anti-PD-1 antibody, may be prepared in a bi-specific format using techniques described herein, or other techniques known to those skilled in the art. Antibody variable regions binding to distinct regions may be linked together with variable regions that bind to relevant sites on, for example, the extracellular domain of PD-1, to confer dual-antigen specificity within a single binding molecule. Appropriately designed bi-specifics of this nature serve a dual function. Variable regions with specificity for the extracellular domain are combined with a variable region with specificity for outside the extracellular domain and are paired on a structural scaffold that allows each variable region to bind to the separate antigens.

[0155] An exemplary bi-specific antibody format that can be used in the context of the present invention involves the use of a first immunoglobulin (Ig) C_H3 domain and a second Ig C_H3 domain, wherein the first and second Ig C_H3 domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bi-specific antibody to Protein A as compared to a bi-specific antibody lacking the amino acid difference. In one embodiment, the first Ig C_H3 domain binds Protein A and the second Ig C_H3 domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C_H3 may further comprise a Y96F modification (by IMGT; Y436F by EU). Further modifications that may be found within the second C_H3 include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 antibodies; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 antibodies; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 antibodies. Variations on the bi-specific antibody format described above are contemplated within the scope of the present invention.

[0156] Other exemplary bispecific formats that can be used in the context of the present invention include, without limitation, *e.g.*, scFv-based or diabody bispecific formats, IgG-scFv fusions, dual variable domain (DVD)-Ig, Quadroma, knobs-into-holes, common light chain (*e.g.*, common light chain with knobs-into-holes, etc.), CrossMab, CrossFab, (SEED)body, leucine zipper, Duobody, IgG1/IgG2, dual acting Fab (DAF)-IgG, and Mab^2 bispecific formats (*see*, *e.g.*, Klein *et al.* 2012, mAbs 4:6, 1-11, and references cited therein, for a review of the foregoing formats). Bispecific antibodies can also be constructed using peptide/nucleic acid conjugation, *e.g.*, wherein unnatural amino acids with orthogonal chemical reactivity are used to generate site-specific antibody-oligonucleotide conjugates which then self-assemble into multimeric complexes with defined composition, valency and geometry. (*See*, *e.g.*, Kazane *et al.*, *J. Am. Chem. Soc.* [*Epub*: *Dec. 4, 2012*]).

Therapeutic Administration and Formulations

[0157] The invention provides therapeutic compositions comprising the anti-PD-1 antibodies or antigen-binding fragments thereof of the present invention. Therapeutic compositions in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell *et al.* "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0158] The dose of antibody may vary depending upon the age and the size of a subject to be administered, target disease, conditions, route of administration, and the like. When an antibody of the present invention is used for treating a disease or disorder in an adult patient, or for preventing such a disease, it is advantageous to administer the antibody of the present invention normally at a single dose of about 0.1 to about 60 mg/kg body weight, more preferably about 5 to about 60, about 10 to about 50, or about 20 to about 50 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. In certain embodiments, the antibody or antigen-binding fragment thereof of the invention can be administered as an initial dose of at least about 0.1 mg to about 800 mg, about 1 to about 500 mg, about 5 to about 300 mg, or about 10 to about 200 mg, to about 100 mg, or to about 50 mg. In certain embodiments, the initial dose may be followed by administration of a second or a plurality of subsequent doses of the antibody or antigen-binding fragment thereof in an amount that can be approximately the same or less than that of the initial dose, wherein the subsequent doses are separated by at least 1 day to 3 days; at least one week, at least 2 weeks; at least 3 weeks; at least 4 weeks; at least 5 weeks; at least 6 weeks; at least 7 weeks; at least 8 weeks; at least 9 weeks; at least 10 weeks; at least 12 weeks; or at least 14 weeks.

[0159] Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, *e.g.,* encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, *e.g.*, Wu et al. (1987) J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, transdermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.,* oral mucosa, rectal and intestinal mucosa, etc.) and may be

administered together with other biologically active agents. Administration can be systemic or local. The pharmaceutical composition can be also delivered in a vesicle, in particular a liposome (see, for example, Langer (1990) Science 249:1527-1533).

[0160] The use of nanoparticles to deliver the antibodies of the present invention is also contemplated herein. Antibody-conjugated nanoparticles may be used both for therapeutic and diagnostic applications. Antibody-conjugated nanoparticles and methods of preparation and use are described in detail by Arruebo, M., et al. 2009 ("Antibody-conjugated nanoparticles for biomedical applications" in J. Nanomat. Volume 2009, Article ID 439389, 24 pages, doi: 10.1155/2009/439389), incorporated herein by reference. Nanoparticles may be developed and conjugated to antibodies contained in pharmaceutical compositions to target tumor cells or autoimmune tissue cells or virally infected cells. Nanoparticles for drug delivery have also been described in, for example, US 8257740, or US 8246995, each incorporated herein in its entirety.

[0161] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used. In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose.

[0162] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous, intracranial, intraperitoneal and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (*e.g.*, ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, *e.g.*, sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[0163] A pharmaceutical composition of the present invention can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable

cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0164] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but certainly are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Burghdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (Sanofi-Aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but certainly are not limited to the SOLOSTAR™ pen (Sanofi-Aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK ™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET ™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.) and the HUMIRA ™ Pen (Abbott Labs, Abbott Park, IL), to name only a few.

[0165] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the antibody contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the antibody is contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

Therapeutic Uses of the Antibodies

[0166] The antibodies of the invention are useful, *inter alia*, for the treatment, prevention and/or amelioration of any disease or disorder associated with or mediated by PD-1 expression, signaling, or activity, or treatable by blocking the interaction between PD-1 and a PD-1 ligand (*e.g.*, PD-L1, or PD-L2) or otherwise inhibiting PD-1 activity and/or signaling. For example, the present invention provides methods for treating cancer (tumor growth inhibition), chronic viral infections and/or autoimmune disease by administering an anti-PD-1 antibody (or pharmaceutical composition comprising an anti-PD-1 antibody) as described herein to a patient in need of such treatment. The antibodies of the present invention are useful for the treatment, prevention, and/or amelioration of disease or disorder or condition such as cancer, autoimmune disease or a viral infection and/or for ameliorating at least one symptom associated with such disease, disorder or condition. In the context of the methods of treatment described herein, the anti-PD-1 antibody may be administered as a monotherapy (*i.e.*, as the only therapeutic agent)

or in combination with one or more additional therapeutic agents (examples of which are described elsewhere herein).

[0167] In some embodiments of the invention, the antibodies described herein are useful for treating subjects suffering from primary or recurrent cancer, including, but not limited to, renal cell carcinoma, colorectal cancer, non-small-cell lung cancer, brain cancer (*e.g.*, glioblastoma multiforme), squamous cell carcinoma of head and neck, gastric cancer, prostate cancer, ovarian cancer, kidney cancer, breast cancer, multiple myeloma, and melanoma.

[0168] The antibodies may be used to treat early stage or late-stage symptoms of cancer. In one embodiment, an antibody or fragment thereof of the invention may be used to treat metastatic cancer. The antibodies are useful in reducing or inhibiting or shrinking tumor growth of both solid tumors and blood cancers. In certain embodiments, treatment with an antibody or antigen-binding fragment thereof of the invention leads to more than 50% regression, more than 60% regression, more than 70% regression, more than 80% regression or more than 90% regression of a tumor in a subject. In certain embodiments, the antibodies may be used to prevent relapse of a tumor. In certain embodiments, the antibodies are useful in extending overall survival in a subject with cancer. In some embodiments, the antibodies are useful in reducing toxicity due to chemotherapy or radiotherapy while maintaining long-term survival in a patient suffering from cancer.

[0169] In certain embodiments, the antibodies of the invention are useful to treat subjects suffering from a chronic viral infection. In some embodiments, the antibodies of the invention are useful in decreasing viral titers in the host and/or rescuing exhausted T-cells. In certain embodiments, an antibody or fragment thereof of the invention may be used to treat chronic viral infection by lymphocytic choriomeningitis virus (LCMV). In some embodiments, an antibody or antigen-binding fragment thereof the invention may be administered at a therapeutic dose to a patient with an infection by human immunodeficiency virus (HIV) or human papilloma virus (HPV) or hepatitis B/C virus (HBV/HCV). In a related embodiment, an antibody or antigen-binding fragment thereof of the invention may be used to treat an infection by simian immunodeficiency virus (SIV) in a simian subject such as cynomolgus.

[0170] In certain embodiments, a blocking antibody of the present invention may be administered in a therapeutically effective amount to a subject suffering from a cancer or a viral infection.

[0171] In certain embodiments, the antibodies of the invention are useful for treating an autoimmune disease, including but not limited to, alopecia areata, autoimmune hepatitis, celiac disease, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, hemolytic anemia, inflammatory bowel disease, inflammatory myopathies, multiple sclerosis, primary biliary cirrhosis, psoriasis, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic lupus erthyematosus, vitiligo, autoimmune pancreatitis, autoimmune urticaria, autoimmune thrombocytopenic purpura, Crohn's disease, diabetes type I, eosinophilic fasciitis, eosinophilic

enterogastritis, Goodpasture's syndrome, myasthenia gravis, psoriatic arthritis, rheumatic fever, ulcerative colitis, vasculitis and Wegener's granulomatosis. In certain embodiments, an activating antibody of the invention may be used to treat a subject suffering from autoimmune disease.

[0172] One or more antibodies of the present invention may be administered to relieve or prevent or decrease the severity of one or more of the symptoms or conditions of the disease or disorder.

[0173] It is also contemplated herein to use one or more antibodies of the present invention prophylactically to patients at risk for developing a disease or disorder such as cancer, autoimmune disease and chronic viral infection.

[0174] In a further embodiment of the invention the present antibodies are used for the preparation of a pharmaceutical composition for treating patients suffering from cancer, autoimmune disease or viral infection. In another embodiment of the invention, the present antibodies are used as adjunct therapy with any other agent or any other therapy known to those skilled in the art useful for treating cancer, autoimmune disease or viral infection.

Combination Therapies and Formulations

[0175] Combination therapies may include an anti-PD-1 antibody of the invention and any additional therapeutic agent that may be advantageously combined with an antibody of the invention, or with a biologically active fragment of an antibody of the invention.

[0176] The antibodies of the present invention may be combined synergistically with one or more anti-cancer drugs or therapy used to treat cancer, including, for example, renal cell carcinoma, colorectal cancer, glioblastoma multiforme, squamous cell carcinoma of head and neck, non-small-cell lung cancer, colon cancer, ovarian cancer, adenocarcinoma, prostate cancer, glioma, and melanoma. It is contemplated herein to use anti-PD-1 antibodies of the invention in combination with immunostimulatory and/or immunosupportive therapies to inhibit tumor growth, and/or enhance survival of cancer patients. The immunostimulatory therapies include direct immunostimulatory therapies to augment immune cell activity by either "releasing the brake" on suppressed immune cells or "stepping on the gas" to activate an immune response. Examples include targeting other checkpoint receptors, vaccination and adjuvants. The immunosupportive modalities may increase antigenicity of the tumor by promoting immunogenic cell death, inflammation or have other indirect effects that promote an anti-tumor immune response. Examples include radiation, chemotherapy, anti-angiogenic agents, and surgery.

[0177] In various embodiments, one or more antibodies of the present invention may be used in combination with an antibody to PD-L1, a second antibody to PD-1 (*e.g.*, nivolumab), a LAG-3 inhibitor, a CTLA-4 inhibitor (*e.g.*, ipilimumab), a TIM3 inhibitor, a BTLA inhibitor, a TIGIT inhibitor, a CD47 inhibitor, an antagonist of another T-cell co-inhibitor or ligand (e.g., an antibody

to CD-28, 2B4, LY108, LAIR1, ICOS, CD160 or VISTA), an indoleamine-2,3-dioxygenase (IDO) inhibitor, a vascular endothelial growth factor (VEGF) antagonist [*e.g.,* a "VEGF-Trap" such as aflibercept or other VEGF-inhibiting fusion protein as set forth in US 7,087,411, or an anti-VEGF antibody or antigen binding fragment thereof (*e.g.*, bevacizumab, or ranibizumab) or a small molecule kinase inhibitor of VEGF receptor (*e.g.*, sunitinib, sorafenib, or pazopanib)], an Ang2 inhibitor (*e.g.*, nesvacumab), a transforming growth factor beta (TGFβ) inhibitor, an epidermal growth factor receptor (EGFR) inhibitor (*e.g.*, erlotinib, cetuximab), an agonist to a co-stimulatory receptor (*e.g.*, an agonist to glucocorticoid-induced TNFR-related protein), an antibody to a tumor-specific antigen (*e.g.,* CA9, CA125, melanoma-associated antigen 3 (MAGE3), carcinoembryonic antigen (CEA), vimentin, tumor-M2-PK, prostate-specific antigen (PSA), mucin-1, MART-1, and CA19-9), a vaccine (*e.g.*, Bacillus Calmette-Guerin, a cancer vaccine), an adjuvant to increase antigen presentation (*e.g.*, granulocyte-macrophage colony-stimulating factor), a bispecific antibody (*e.g.*, CD3xCD20 bispecific antibody, PSMAxCD3 bispecific antibody), a cytotoxin, a chemotherapeutic agent (*e.g.*, dacarbazine, temozolomide, cyclophosphamide, docetaxel, doxorubicin, daunorubicin, cisplatin, carboplatin, gemcitabine, methotrexate, mitoxantrone, oxaliplatin, paclitaxel, and vincristine), cyclophosphamide, radiotherapy, an IL-6R inhibitor (*e.g.*, sarilumab), an IL-4R inhibitor (*e.g.*, dupilumab), an IL-10 inhibitor, a cytokine such as IL-2, IL-7, IL-21, and IL-15, an antibody-drug conjugate (ADC) (*e.g.*, anti-CD19-DM4 ADC, and anti-DS6-DM4 ADC), an anti-inflammatory drug (*e.g.*, corticosteroids, and non-steroidal anti-inflammatory drugs), a dietary supplement such as anti-oxidants or any palliative care to treat cancer. In certain embodiments, the anti-PD-1 antibodies of the present invention may be used in combination with cancer vaccines including dendritic cell vaccines, oncolytic viruses, tumor cell vaccines, etc. to augment the anti-tumor response. Examples of cancer vaccines that can be used in combination with anti-PD-1 antibodies of the present invention include MAGE3 vaccine for melanoma and bladder cancer, MUC1 vaccine for breast cancer, EGFRv3 (*e.g.*, Rindopepimut) for brain cancer (including glioblastoma multiforme), or ALVAC-CEA (for CEA+ cancers).

[0178] In certain embodiments, the anti-PD-1 antibodies of the invention may be administered in combination with radiation therapy in methods to generate long-term durable anti-tumor responses and/or enhance survival of patients with cancer. In some embodiments, the anti-PD-1 antibodies of the invention may be administered prior to, concomitantly or after administering radiation therapy to a cancer patient. For example, radiation therapy may be administered in one or more doses to tumor lesions followed by administration of one or more doses of anti-PD-1 antibodies of the invention. In some embodiments, radiation therapy may be administered locally to a tumor lesion to enhance the local immunogenicity of a patient's tumor (adjuvinating radiation) and/or to kill tumor cells (ablative radiation) followed by systemic administration of an anti-PD-1 antibody of the invention. For example, intracranial radiation may be administered to a patient with brain cancer (*e.g.*, glioblastoma multiforme) in combination with systemic

administration of an anti-PD-1 antibody of the invention. In certain embodiments, the anti-PD-1 antibodies of the invention may be administered in combination with radiation therapy and a chemotherapeutic agent (*e.g.*, temozolomide) or a VEGF antagonist (*e.g.*, aflibercept).

[0179] In certain embodiments, the anti-PD-1 antibodies of the invention may be administered in combination with one or more anti-viral drugs to treat chronic viral infection caused by LCMV, HIV, HPV, HBV or HCV. Examples of anti-viral drugs include, but are not limited to, zidovudine, lamivudine, abacavir, ribavirin, lopinavir, efavirenz, cobicistat, tenofovir, rilpivirine and corticosteroids. In some embodiments, the anti-PD-1 antibodies of the invention may be administered in combination with a LAG3 inhibitor, a CTLA-4 inhibitor or any antagonist of another T-cell co-inhibitor to treat chronic viral infection.

[0180] In certain embodiments, the anti-PD-1 antibodies of the invention may be combined with an antibody to a Fc receptor on immune cells for the treatment of an autoimmune disease. In one embodiment, an antibody or fragment thereof of the invention is administered in combination with an antibody or antigen-binding protein targeted to an antigen specific to autoimmune tissue. In certain embodiments, an antibody or antigen-binding fragment thereof of the invention is administered in combination with an antibody or antigen-binding protein targeted to a T-cell receptor or a B-cell receptor, including but not limited to, Fcα (e.g., CD89), Fcγ (e.g., CD64, CD32, CD16a, and CD16b), CD19, etc. The antibodies of fragments thereof of the invention may be used in combination with any drug or therapy known in the art (e.g., corticosteroids and other immunosuppressants) to treat an autoimmune disease or disorder including, but not limited to alopecia areata, autoimmune hepatitis, celiac disease, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, hemolytic anemia, inflammatory bowel disease, inflammatory myopathies, multiple sclerosis, primary biliary cirrhosis, psoriasis, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic lupus erthyematosus, vitiligo, autoimmune pancreatitis, autoimmune urticaria, autoimmune thrombocytopenic purpura, Crohn's disease, diabetes type I, eosinophilic fasciitis, eosinophilic enterogastritis, Goodpasture's syndrome, myasthenia gravis, psoriatic arthritis, rheumatic fever, ulcerative colitis, vasculitis and Wegener's granulomatosis.

[0181] The additional therapeutically active agent(s)/component(s) may be administered prior to, concurrent with, or after the administration of the anti-PD-1 antibody of the present invention. For purposes of the present disclosure, such administration regimens are considered the administration of an anti-PD-1 antibody "in combination with" a second therapeutically active component.

[0182] The additional therapeutically active component(s) may be administered to a subject prior to administration of an anti-PD-1 antibody of the present invention. For example, a first component may be deemed to be administered "prior to" a second component if the first component is administered 1 week before, 72 hours before, 60 hours before, 48 hours before, 36 hours before, 24 hours before, 12 hours before, 6 hours before, 5 hours before, 4 hours

before, 3 hours before, 2 hours before, 1 hour before, 30 minutes before, 15 minutes before, 10 minutes before, 5 minutes before, or less than 1 minute before administration of the second component. In other embodiments, the additional therapeutically active component(s) may be administered to a subject after administration of an anti-PD-1 antibody of the present invention. For example, a first component may be deemed to be administered "after" a second component if the first component is administered 1 minute after, 5 minutes after, 10 minutes after, 15 minutes after, 30 minutes after, 1 hour after, 2 hours after, 3 hours after, 4 hours after, 5 hours after, 6 hours after, 12 hours after, 24 hours after, 36 hours after, 48 hours after, 60 hours after, 72 hours after administration of the second component. In yet other embodiments, the additional therapeutically active component(s) may be administered to a subject concurrent with administration of an anti-PD-1 antibody of the present invention. "Concurrent" administration, for purposes of the present invention, includes, *e.g.*, administration of an anti-PD-1 antibody and an additional therapeutically active component to a subject in a single dosage form (*e.g.*, co-formulated), or in separate dosage forms administered to the subject within about 30 minutes or less of each other. If administered in separate dosage forms, each dosage form may be administered via the same route (*e.g.*, both the anti-PD-1 antibody and the additional therapeutically active component may be administered intravenously, subcutaneously, etc.); alternatively, each dosage form may be administered via a different route (*e.g.*, the anti-PD-1 antibody may be administered intravenously, and the additional therapeutically active component may be administered subcutaneously). In any event, administering the components in a single dosage from, in separate dosage forms by the same route, or in separate dosage forms by different routes are all considered "concurrent administration," for purposes of the present disclosure. For purposes of the present disclosure, administration of an anti-PD-1 antibody "prior to", "concurrent with," or "after" (as those terms are defined herein above) administration of an additional therapeutically active component is considered administration of an anti-PD-1 antibody "in combination with" an additional therapeutically active component).

[0183] The present invention includes pharmaceutical compositions in which an anti-PD-1 antibody of the present invention is co-formulated with one or more of the additional therapeutically active component(s) as described elsewhere herein using a variety of dosage combinations.

[0184] In exemplary embodiments in which an anti-PD-1 antibody of the invention is administered in combination with a VEGF antagonist (*e.g.*, a VEGF trap such as aflibercept), including administration of co-formulations comprising an anti-PD-1 antibody and a VEGF antagonist, the individual components may be administered to a subject and/or co-formulated using a variety of dosage combinations. For example, the anti-PD-1 antibody may be administered to a subject and/or contained in a co-formulation in an amount selected from the group consisting of 0.01 mg, 0.02 mg, 0.03 mg, 0.04 mg, 0.05 mg, 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1.0 mg, 1.5 mg, 2.0 mg, 2.5 mg, 3.0 mg, 3.5 mg,

4.0 mg, 4.5 mg, 5.0 mg, 6.0 mg, 7.0 mg, 8.0 mg, 9.0 mg, and 10.0 mg; and the VEGF antagonist (*e.g.*, a VEGF trap such as aflibercept) may be administered to the subject and/or contained in a co-formulation in an amount selected from the group consisting of 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1.0 mg, 1.1 mg, 1.2 mg, 1.3 mg, 1.4 mg, 1.5 mg, 1.6 mg, 1.7 mg, 1.8 mg, 1.9 mg, 2.0 mg, 2.1 mg, 2.2 mg, 2.3 mg, 2.4 mg, 2.5 mg, 2.6 mg, 2.7 mg, 2.8 mg, 2.9 mg and 3.0 mg. The combinations/co-formulations may be administered to a subject according to any of the administration regimens disclosed elsewhere herein, including, *e.g.*, twice a week, once every week, once every 2 weeks, once every 3 weeks, once every month, once every 2 months, once every 3 months, once every 4 months, once every 5 months, once every 6 months, etc.

Administrative Regimens

[0185] According to certain embodiments of the present invention, multiple doses of an anti-PD-1 antibody (or a pharmaceutical composition comprising a combination of an anti-PD-1 antibody and any of the additional therapeutically active agents mentioned herein) may be administered to a subject over a defined time course. The methods according to this aspect of the invention comprise sequentially administering to a subject multiple doses of an anti-PD-1 antibody of the invention. As used herein, "sequentially administering" means that each dose of anti-PD-1 antibody is administered to the subject at a different point in time, *e.g.*, on different days separated by a predetermined interval (*e.g.*, hours, days, weeks or months). The present invention includes methods which comprise sequentially administering to the patient a single initial dose of an anti-PD-1 antibody, followed by one or more secondary doses of the anti-PD-1 antibody, and optionally followed by one or more tertiary doses of the anti-PD-1 antibody. The anti-PD-1 antibody may be administered at a dose between 0.1 mg/kg to 100 mg/kg.

[0186] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the anti-PD-1 antibody of the invention. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of anti-PD-1 antibody, but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of anti-PD-1 antibody contained in the initial, secondary and/or tertiary doses varies from one another (*e.g.*, adjusted up or down as appropriate) during the course of treatment. In certain embodiments, two or more (*e.g.*, 2, 3, 4, or 5) doses are administered at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (*e.g.*, "maintenance doses").

[0187] In certain exemplary embodiments of the present invention, each secondary and/or

tertiary dose is administered 1 to 26 (*e.g.*, 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 5½, 6, 6½, 7, 7½, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12, 12½, 13, 13½, 14, 14½, 15, 15½, 16, 16½, 17, 17½, 18, 18½, 19, 19½, 20, 20½, 21, 21½, 22, 22½, 23, 23½, 24, 24½, 25, 25½, 26, 26½, or more) weeks after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of anti-PD-1 antibody which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0188] The methods according to this aspect of the invention may comprise administering to a patient any number of secondary and/or tertiary doses of an anti-PD-1 antibody. For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[0189] In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 1 to 2 weeks or 1 to 2 months after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 2 to 12 weeks after the immediately preceding dose. In certain embodiments of the invention, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

[0190] The present invention includes administration regimens in which 2 to 6 loading doses are administered to a patient at a first frequency (*e.g.*, once a week, once every two weeks, once every three weeks, once a month, once every two months, etc.), followed by administration of two or more maintenance doses to the patient on a less frequent basis. For example, according to this aspect of the invention, if the loading doses are administered at a frequency of, *e.g.*, once a month (e.g., two, three, four, or more loading doses administered once a month), then the maintenance doses may be administered to the patient once every five weeks, once every six weeks, once every seven weeks, once every eight weeks, once every ten weeks, once every twelve weeks, etc.).

Diagnostic Uses of the Antibodies

[0191] The anti-PD-1 antibodies of the present invention may be used to detect and/or measure PD-1 in a sample, *e.g.*, for diagnostic purposes. Some embodiments contemplate the use of one

or more antibodies of the present invention in assays to detect a disease or disorder such as cancer, autoimmune disease or chronic viral infection. Exemplary diagnostic assays for PD-1 may comprise, *e.g.*, contacting a sample, obtained from a patient, with an anti-PD-1 antibody of the invention, wherein the anti-PD-1 antibody is labeled with a detectable label or reporter molecule or used as a capture ligand to selectively isolate PD-1 from patient samples. Alternatively, an unlabeled anti-PD-1 antibody can be used in diagnostic applications in combination with a secondary antibody which is itself detectably labeled. The detectable label or reporter molecule can be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I; a fluorescent or chemiluminescent moiety such as fluorescein isothiocyanate, or rhodamine; or an enzyme such as alkaline phosphatase, β-galactosidase, horseradish peroxidase, or luciferase. Specific exemplary assays that can be used to detect or measure PD-1 in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).

[0192] Samples that can be used in PD-1 diagnostic assays according to the present invention include any tissue or fluid sample obtainable from a patient, which contains detectable quantities of either PD-1 protein, or fragments thereof, under normal or pathological conditions. Generally, levels of PD-1 in a particular sample obtained from a healthy patient (*e.g.*, a patient not afflicted with cancer or an autoimmune disease) will be measured to initially establish a baseline, or standard, level of PD-1. This baseline level of PD-1 can then be compared against the levels of PD-1 measured in samples obtained from individuals suspected of having a cancer-related condition, or symptoms associated with such condition.

[0193] The antibodies specific for PD-1 may contain no additional labels or moieties, or they may contain an N-terminal or C-terminal label or moiety. In one embodiment, the label or moiety is biotin. In a binding assay, the location of a label (if any) may determine the orientation of the peptide relative to the surface upon which the peptide is bound. For example, if a surface is coated with avidin, a peptide containing an N-terminal biotin will be oriented such that the C-terminal portion of the peptide will be distal to the surface.

[0194] Aspects of the invention relate to use of the disclosed antibodies as markers for predicting prognosis of cancer or an autoimmune disorder in patients. Antibodies of the present invention may be used in diagnostic assays to evaluate prognosis of cancer in a patient and to predict survival.

EXAMPLES

[0195] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be

accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, room temperature is about 25°C, and pressure is at or near atmospheric.

Example 1: Generation of Human Antibodies to PD-1

[0196] Human antibodies to PD-1 were generated using a fragment of PD-1 that ranges from about amino acids 25 – 170 of GenBank Accession NP_005009.2 (SEQ ID NO: 327) with a C93S change. The immunogen was administered directly, with an adjuvant to stimulate the immune response, to a VELOCIMMUNE® mouse comprising DNA encoding human Immunoglobulin heavy and kappa light chain variable regions. The antibody immune response was monitored by a PD-1-specific immunoassay. When a desired immune response was achieved splenocytes were harvested and fused with mouse myeloma cells to preserve their viability and form hybridoma cell lines. The hybridoma cell lines were screened and selected to identify cell lines that produce PD-1-specific antibodies. Using this technique, and the immunogen described above, several anti-PD-1 chimeric antibodies (*i.e.*, antibodies possessing human variable domains and mouse constant domains) were obtained; exemplary antibodies generated in this manner were designated as H1M7789N, H1M7799N, H1M7800N, H2M7780N, H2M7788N, H2M7790N, H2M7791N, H2M7794N, H2M7795N, H2M7796N, and H2M7798N.

[0197] Anti-PD-1 antibodies were also isolated directly from antigen-positive B cells without fusion to myeloma cells, as described in U.S. 2007/0280945A1, herein specifically incorporated by reference in its entirety. Using this method, several fully human anti-PD-1 antibodies (*i.e.*, antibodies possessing human variable domains and human constant domains) were obtained; exemplary antibodies generated in this manner were designated as follows: H4H9019P, H4xH9034P2, H4xH9035P2, H4xH9037P2, H4xH9045P2, H4xH9048P2, H4H9057P2, H4H9068P2, H4xH9119P2, H4xH9120P2, H4xH9128P2, H4xH9135P2, H4xH9145P2, H4xH8992P, H4xH8999P, and H4xH9008P.

[0198] The biological properties of the exemplary antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

Example 2: Heavy and Light Chain Variable Region Amino Acid and Nucleotide Sequences

[0199] Table 1 sets forth the amino acid sequence identifiers of the heavy and light chain variable regions and CDRs of selected anti-PD-1 antibodies of the invention. The corresponding nucleic acid sequence identifiers are set forth in Table 2.

Table 1: Amino Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**

Designation								
H1M7789N	2	4	6	8	10	12	14	16
H1M7799N	18	20	22	24	26	28	30	32
H1M7800N	34	36	38	40	42	44	46	48
H2M7780N	50	52	54	56	58	60	62	64
H2M7788N	66	68	70	72	74	76	78	80
H2M7790N	82	84	86	88	90	92	94	96
H2M7791N	98	100	102	104	106	108	110	112
H2M7794N	114	116	118	120	122	124	126	128
H2M7795N	130	132	134	136	138	140	142	144
H2M7796N	146	148	150	152	154	156	158	160
H2M7798N	162	164	166	168	170	172	174	176
H4H9019P	178	180	182	184	186	188	190	192
H4xH9034P2	194	196	198	200	202	204	206	208
H4xH9035P2	210	212	214	216	202	204	206	208
H4xH9037P2	218	220	222	224	202	204	206	208
H4xH9045P2	226	228	230	232	202	204	206	208
H4xH9048P2	234	236	238	240	202	204	206	208
H4H9057P2	242	244	246	248	202	204	206	208
H4H9068P2	250	252	254	256	202	204	206	208
H4xH9119P2	258	260	262	264	202	204	206	208
H4xH9120P2	266	268	270	272	202	204	206	208
H4xH9128P2	274	276	278	280	202	204	206	208
H4xH9135P2	282	284	286	288	202	204	206	208
H4xH9145P2	290	292	294	296	202	204	206	208
H4xH8992P	298	300	302	304	186	188	190	192
H4xH8999P	306	308	310	312	186	188	190	192
H4xH9008P	314	316	318	320	186	188	190	192

Table 2: Nucleic Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	HCVR	HCDR1	HCDR2	HCDR3	LCVR	LCDR1	LCDR2	LCDR3
H1M7789N	1	3	5	7	9	11	13	15
H1M7799N	17	19	21	23	25	27	29	31
H1M7800N	33	35	37	39	41	43	45	47

H2M7780N	49	51	53	55	57	59	61	63
H2M7788N	65	67	69	71	73	75	77	79
H2M7790N	81	83	85	87	89	91	93	95
H2M7791N	97	99	101	103	105	107	109	111
H2M7794N	113	115	117	119	121	123	125	127
H2M7795N	129	131	133	135	137	139	141	143
H2M7796N	145	147	149	151	153	155	157	159
H2M7798N	161	163	165	167	169	171	173	175
H4H9019P	177	179	181	183	185	187	189	191
H4xH9034P2	193	195	197	199	201	203	205	207
H4xH9035P2	209	211	213	215	201	203	205	207
H4xH9037P2	217	219	221	223	201	203	205	207
H4xH9045P2	225	227	229	231	201	203	205	207
H4xH9048P2	233	235	237	239	201	203	205	207
H4H9057P2	241	243	245	247	201	203	205	207
H4H9068P2	249	251	253	255	201	203	205	207
H4xH9119P2	257	259	261	263	201	203	205	207
H4xH9120P2	265	267	269	271	201	203	205	207
H4xH9128P2	273	275	277	279	201	203	205	207
H4xH9135P2	281	283	285	287	201	203	205	207
H4xH9145P2	289	291	293	295	201	203	205	207
H4xH8992P	297	299	301	303	185	187	189	191
H4xH8999P	305	307	309	311	185	187	189	191
H4xH9008P	313	315	317	319	185	187	189	191

[0200] Antibodies are typically referred to herein according to the following nomenclature: Fc prefix (*e.g.* "H4xH," "H1M," "H2M," etc.), followed by a numerical identifier (*e.g.* "7789," "7799," etc., as shown in Table 1), followed by a "P," "P2," "N," or "B" suffix. Thus, according to this nomenclature, an antibody may be referred to herein as, *e.g.*, "H1H7789N," "H1M7799N," "H2M7780N," etc. The H4xH, H1M, H2M and H2aM prefixes on the antibody designations used herein indicate the particular Fc region isotype of the antibody. For example, an "H4xH" antibody has a human IgG4 Fc with 2 or more amino acid changes as disclosed in US20100331527, an "H1M" antibody has a mouse IgG1 Fc, and an "H2M" antibody has a mouse IgG2 Fc (a or b isotype) (all variable regions are fully human as denoted by the first 'H' in the antibody designation). As will be appreciated by a person of ordinary skill in the art, an antibody having a particular Fc isotype can be converted to an antibody with a different Fc isotype (*e.g.*, an antibody with a mouse IgG1 Fc can be converted to an antibody with a human

IgG4, etc.), but in any event, the variable domains (including the CDRs) – which are indicated by the numerical identifiers shown in Table 1 – will remain the same, and the binding properties to antigen are expected to be identical or substantially similar regardless of the nature of the Fc domain.

[0201] In certain embodiments, selected antibodies with a mouse IgG1 Fc were converted to antibodies with human IgG4 Fc. In one embodiment, the IgG4 Fc domain comprises a serine to proline mutation in the hinge region (S108P) to promote dimer stabilization. Table 3 sets forth the amino acid sequence identifiers of heavy chain and light chain sequences of selected anti-PD-1 antibodies with human IgG4 Fc.

Table 3

	SEQ ID NOs:	
Antibody Designation	**Heavy Chain**	**Light Chain**
H4H7798N	**330**	**331**
H4H7795N2	**332**	**333**
H4H9008P	**334**	**335**
H4H9048P2	**336**	**337**

[0202] Each heavy chain sequence in Table 3 comprised a variable region (V_H or HCVR; comprising HCDR1, HCDR2 and HCDR3) and a constant region (comprising C_H1, C_H2 and C_H3 domains). Each light chain sequence in Table 3 comprised a variable region (V_L or LCVR; comprising LCDR1, LCDR2 and LCDR3) and a constant region (C_L). SEQ ID NO: 330 comprised a HCVR comprising amino acids 1 – 117 and a constant region comprising amino acids 118 – 444. SEQ ID NO: 331 comprised a LCVR comprising amino acids 1 – 107 and a constant region comprising amino acids 108 – 214. SEQ ID NO: 332 comprised a HCVR comprising amino acids 1 – 122 and a constant region comprising amino acids 123 – 449. SEQ ID NO: 333 comprised a LCVR comprising amino acids 1 – 107 and a constant region comprising amino acids 108 – 214. SEQ ID NO: 334 comprised a HCVR comprising amino acids 1 – 119 and a constant region comprising amino acids 120 – 446. SEQ ID NO: 335 comprised a LCVR comprising amino acids 1 – 108 and a constant region comprising amino acids 109 – 215. SEQ ID NO: 336 comprised a HCVR comprising amino acids 1 – 121 and a constant region comprising amino acids 122 – 448. SEQ ID NO: 337 comprised a LCVR comprising amino acids 1 – 108 and a constant region comprising amino acids 109 – 215.

Example 3: Antibody binding to PD-1 as determined by Surface Plasmon Resonance

[0203] Binding association and dissociation rate constants (k_a and k_d, respectively), equilibrium dissociation constants and dissociation half-lives (K_D and $t_{½}$, respectively) for antigen

binding to purified anti-PD1 antibodies were determined using a real-time surface plasmon resonance biosensor assay on a Biacore 4000 or Biacore T200 instrument. The Biacore sensor surface was derivatized with either a polyclonal rabbit anti-mouse antibody (GE, # BR-1008-38) or with a monoclonal mouse anti-human Fc antibody (GE, # BR-1008-39) to capture approximately 100-900 RUs of anti-PD-1 monoclonal antibodies, expressed with either a mouse Fc or a human Fc, respectively. The PD-1 reagents tested for binding to the anti-PD-1 antibodies included recombinant human PD-1 expressed with a C-terminal myc-myc-hexahistidine tag (hPD-1-MMH; SEQ ID NO: 321), recombinant cynomolgus monkey PD-1 expressed with a C-terminal myc-myc-hexahistidine tag (MfPD-1-MMH; SEQ ID NO: 322), recombinant human PD-1 dimer expressed with either a C-terminal mouse IgG2a Fc tag (hPD-1-mFc; SEQ ID NO: 323) or with a C-terminal human IgG1 Fc (hPD1-hFc; SEQ ID NO: 324), and monkey PD-1 with mFc (SEQ ID NO: 329). Different concentrations of PD-1 reagents ranging from 200nM to 3.7nM were injected over the anti-PD-1 monoclonal antibody captured surface at a flow rate of 30μL/min on Biacore 4000 or at 50μL/min on Biacore T200. The binding of the PD-1 reagents to captured monoclonal antibodies was monitored for 3 to 5 minutes while their dissociation from the antibodies was monitored for 7 to 10 minutes in HBST running buffer (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.05% v/v Surfactant P20). Experiments were performed at 25°C and 37°C. Kinetic association (k_a) and dissociation (k_d) rate constants were determined by processing and fitting the data to a 1:1 binding model using Scrubber 2.0c curve fitting software. Binding dissociation equilibrium constants (K_D) and dissociative half-lives ($t_{1/2}$) were then calculated from the kinetic rate constants as: K_D (M) = k_d / k_a and $t_{½}$ (min) = [ln2/(60*k_d)]. Binding kinetics parameters for different anti-PD-1 monoclonal antibodies binding to different PD-1 reagents at 25°C and 37°C are tabulated in Tables 4 – 11.

Table 4: Binding Kinetics parameters of anti-PD-1 monoclonal antibodies binding to human PD-1-MMH at 25°C.

Antibody	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H2aM7780N	9.32E+03	3.59E-04	3.85E-08	32
H2aM7788N	1.97E+04	3.88E-04	1.96E-08	30
H1M7789N	2.53E+04	5.31E-05	2.10E-09	218
H2aM7790N	4.63E+04	8.23E-04	1.78E-08	14
H2aM7791N	3.01E+04	7.06E-04	2.34E-08	16
H2aM7794N	5.50E+04	2.12E-03	3.80E-08	5.4
H2aM7795N	4.91E+04	1.15E-03	2.35E-08	10
H2aM7796N	6.73E+03	1.93E-03	2.86E-07	6.0
H2aM7798N	1.32E+05	3.06E-04	2.31E-09	38
H1M7799N	5.04E+04	1.23E-02	2.44E-07	0.9
H1M7800N	5.88E+04	9.47E-03	1.61E-07	1.2
H4H9019P	2.05E+04	8.08E-04	3.94E-08	14

H4xH9034P	1.02E+05	1.49E-03	1.45E-08	7.8
H4xH9035P	1.03E+05	4.75E-04	4.62E-09	24
H4xH9037P	7.32E+04	7.95E-04	1.09E-08	15
H4xH9045P	5.40E+04	4.03E-03	7.46E-08	2.9
H4xH9048P2	1.37E+05	1.23E-03	8.95E-09	9.4
H4H9057P2	4.60E+04	1.34E-02	2.91E-07	0.9
H4H9068P2	NB*	NB*	NB*	NB*
H4xH9119P2	7.84E+04	1.22E-03	1.56E-08	9.5
H4xH9120P2	3.32E+04	9.98E-04	3.01E-08	12
H4xH9128P2	4.95E+04	7.19E-04	1.45E-08	16
H4xH9135P2	1.17E+05	1.20E-03	1.02E-08	10
H4xH9145P2	3.47E+04	1.34E-03	3.85E-08	8.6
H4xH8992P	1.50E+05	2.13E-02	1.41E-07	0.5
H4xH8999P	2.83E+05	1.23E-03	4.33E-09	9.4
H4xH9008P	4.29E+04	1.33E-03	3.10E-08	8.7
H4H7795N2	6.35E+04	1.48E-03	2.33E-08	8
H4H7798N	1.47E+05	4.43E-04	3.01E-09	26

*NB indicates that under the experimental conditions, PD-1 reagent did not bind to the captured anti-PD-1 monoclonal antibody

Table 5: Binding Kinetics parameters of anti-PD-1 monoclonal antibodies binding to human PD-1-MMH at 37°C.

Antibody	k_a **(1/Ms)**	k_d **(1/s)**	K_D **(M)**	**t½ (min)**
H2aM7780N	2.72E+04	1.52E-03	5.58E-08	7.6
H2aM7788N	2.88E+04	1.49E-03	5.19E-08	7.7
H1M7789N	4.53E+04	2.95E-04	6.52E-09	39
H2aM7790N	6.13E+04	5.20E-03	8.49E-08	2.2
H2aM7791N	4.18E+04	2.24E-03	5.35E-08	5.2
H2aM7794N	1.20E+05	7.92E-03	6.61E-08	1.5
H2aM7795N	6.75E+04	4.58E-03	6.78E-08	2.5
H2aM7796N	1.09E+04	1.65E-02	1.51E-06	0.7
H2aM7798N	1.73E+05	6.56E-04	3.79E-09	18
H1M7799N	7.94E+04	4.25E-02	5.36E-07	0.3
H1M7800N	7.83E+04	3.99E-02	5.10E-07	0.3
H4H9019P	1.20E+04	5.44E-03	4.53E-07	2.1
H4xH9034P	2.79E+05	1.12E-02	4.02E-08	1.0
H4xH9035P	2.98E+05	4.26E-03	1.43E-08	2.7
H4xH9037P	2.26E+05	6.68E-03	2.95E-08	1.7
H4xH9045P	8.04E+04	5.32E-02	6.62E-07	0.2
H4xH9048P2	3.70E+05	8.60E-03	2.32E-08	1.3
H4H9057P2	NB*	NB*	NB*	NB*
H4H9068P2	NB*	NB*	NB*	NB*

H4xH9119P2	2.40E+05	1.04E-02	4.35E-08	1.1
H4xH9120P2	6.88E+04	7.01E-03	1.02E-07	1.6
H4xH9128P2	1.04E+05	4.36E-03	4.20E-08	2.6
H4xH9135P2	4.18E+05	1.11E-02	2.66E-08	1.0
H4xH9145P2	1.31E+05	1.23E-02	9.40E-08	0.9
H4xH8992P	IC*	IC*	IC*	IC*
H4xH8999P	5.99E+05	9.42E-03	1.57E-08	1.2
H4xH9008P	1.29E+05	8.09E-03	6.26E-08	1.4
H4H7795N2	6.41E+04	6.64E-03	1.04E-07	1.7
H4H7798N	2.27E+05	1.70E-03	7.48E-09	7

*NB indicates that under the experimental conditions, PD-1 reagent did not bind to the captured anti-PD-1 monoclonal antibody. IC indicates that under the experimental conditions, PD-1 binding is inconclusive.

Table 6: Binding Kinetics parameters of anti-PD-1 monoclonal antibodies binding to human PD-1 dimer (human PD-1-mFc or human PD-1-hFc) at 25°C.

Antibody	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H2aM7780N	4.21E+04	9.94E-06	2.36E-10	1162
H2aM7788N	8.94E+04	2.82E-05	3.15E-10	410
H1M7789N	3.91E+04	4.31E-05	1.10E-09	268
H2aM7790N	1.86E+05	3.02E-05	1.62E-10	383
H2aM7791N	4.05E+04	1.01E-04	2.49E-09	114
H2aM7794N	1.79E+05	1.06E-04	5.93E-10	109
H2aM7795N	1.38E+05	3.14E-05	2.27E-10	368
H2aM7796N	2.61E+04	8.67E-05	3.32E-09	133
H2aM7798N	3.50E+05	2.29E-05	6.55E-11	505
H1M7799N	2.38E+05	8.55E-05	3.60E-10	135
H1M7800N	1.52E+05	7.72E-05	5.09E-10	150
H4H9019P	4.38E+04	8.61E-05	1.97E-09	134
H4xH9034P	2.15E+05	1.51E-04	7.01E-10	77
H4xH9035P	2.01E+05	1.03E-04	5.13E-10	112
H4xH9037P	1.50E+05	1.29E-04	8.62E-10	89
H4xH9045P	9.13E+04	1.60E-04	1.75E-09	72
H4xH9048P2	2.36E+05	1.88E-04	7.98E-10	61
H4H9057P2	1.01E+05	1.77E-04	1.75E-09	65
H4H9068P2	4.72E+04	2.80E-03	5.94E-08	4
H4xH9119P2	1.63E+05	1.62E-04	9.92E-10	71
H4xH9120P2	6.52E+04	1.19E-04	1.82E-09	97
H4xH9128P2	8.37E+04	1.33E-04	1.59E-09	87
H4xH9135P2	2.12E+05	1.38E-04	6.51E-10	84
H4xH9145P2	6.58E+04	1.58E-04	2.40E-09	73
H4xH8992P	2.35E+05	1.60E-04	6.80E-10	72

H4xH8999P	5.55E+05	1.20E-04	2.17E-10	96
H4xH9008P	3.52E+04	2.80E-05	7.96E-10	412
H4H7795N2	1.50E+05	9.25E-05	6.15E-10	125
H4H7798N	4.41E+05	5.40E-05	1.22E-10	214

Table 7: Binding Kinetics parameters of anti-PD-1 monoclonal antibodies binding to human PD-1 dimer (human PD-1-mFc or human PD-1-hFc) at 37°C.

Antibody	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H2aM7780N	9.94E+04	2.29E-05	2.30E-10	505
H2aM7788N	1.31E+05	2.13E-05	1.63E-10	542
H1M7789N	1.09E+05	≤ 1.0E-05	≤ 9.17E-11	≥ 1155
H2aM7790N	2.01E+05	8.49E-05	4.22E-10	136
H2aM7791N	4.98E+04	1.79E-04	3.59E-09	65
H2aM7794N	4.68E+05	2.11E-04	4.52E-10	55
H2aM7795N	1.65E+05	6.13E-05	3.71E-10	188
H2aM7796N	2.21E+04	4.34E-04	1.96E-08	27
H2aM7798N	4.90E+05	1.40E-05	2.80E-11	825
H1M7799N	4.41E+05	1.81E-04	4.11E-10	64
H1M7800N	4.00E+05	1.81E-04	4.50E-10	64
H4H9019P	7.17E+04	1.95E-04	2.71E-09	59
H4xH9034P	3.02E+05	6.30E-04	2.09E-09	18
H4xH9035P	3.16E+05	5.54E-04	1.75E-09	21
H4xH9037P	2.63E+05	9.21E-04	3.50E-09	13
H4xH9045P	2.14E+05	1.10E-03	5.13E-09	11
H4xH9048P2	3.61E+05	1.10E-03	3.05E-09	10
H4H9057P2	2.33E+05	2.11E-03	9.07E-09	5
H4H9068P2	9.69E+04	1.20E-02	1.24E-07	1
H4xH9119P2	2.40E+05	9.09E-04	3.80E-09	13
H4xH9120P2	8.08E+04	4.82E-04	5.96E-09	24
H4xH9128P2	1.86E+05	6.86E-04	3.68E-09	17
H4xH9135P2	3.10E+05	7.02E-04	2.27E-09	16
H4xH9145P2	1.60E+05	5.71E-04	3.58E-09	20
H4xH8992P	3.49E+05	1.02E-03	2.91E-09	11
H4xH8999P	7.57E+05	4.51E-04	5.96E-10	26
H4xH9008P	5.52E+04	≤ 1.0E-05	≤ 1.81E-10	≥ 1155
H4H7795N2	1.60E+05	2.64E-04	1.65E-09	44
H4H7798N	6.60E+05	1.15E-04	1.75E-10	100

Table 8: Binding Kinetics parameters of anti-PD-1 monoclonal antibodies binding to MfPD-1-MMH at 25°C.

Antibody	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H2aM7780N	1.00E+04	3.15E-04	3.15E-08	37
H2aM7788N	8.63E+03	6.62E-04	7.66E-08	17
H1M7789N	1.55E+04	1.23E-04	7.89E-09	94
H2aM7790N	3.11E+04	9.37E-04	3.01E-08	12
H2aM7791N	1.61E+04	5.53E-04	3.44E-08	21
H2aM7794N	3.60E+04	5.99E-03	1.66E-07	1.9
H2aM7795N	4.44E+04	8.89E-04	2.01E-08	13
H2aM7796N	NB*	NB*	NB*	NB*
H2aM7798N	8.72E+04	3.93E-04	4.50E-09	29
H1M7799N	5.78E+04	1.30E-02	2.24E-07	0.9
H1M7800N	5.89E+04	1.04E-02	1.76E-07	1.1
H4H9019P	1.94E+04	8.33E-04	4.29E-08	14
H4xH9034P	9.61E+04	2.69E-03	2.80E-08	4.3
H4xH9035P	9.36E+04	4.34E-04	4.64E-09	27
H4xH9037P	6.99E+04	9.15E-04	1.31E-08	13
H4xH9045P	6.25E+04	7.05E-03	1.13E-07	1.6
H4xH9048P2	1.28E+05	8.97E-04	7.00E-09	13
H4H9057P2	3.46E+04	1.91E-02	5.51E-07	0.6
H4H9068P2	NB*	NB*	NB*	NB*
H4xH9119P2	7.50E+04	1.66E-03	2.22E-08	6.9
H4xH9120P2	3.17E+04	1.08E-03	3.41E-08	11
H4xH9128P2	3.68E+04	6.49E-04	1.77E-08	18
H4xH9135P2	1.24E+05	1.31E-03	1.06E-08	8.8
H4xH9145P2	2.86E+04	1.24E-03	4.31E-08	9.3
H4xH8992P	1.88E+05	3.76E-02	2.00E-07	0.3
H4xH8999P	4.29E+05	1.33E-03	3.09E-09	8.7
H4xH9008P	1.05E+05	2.49E-03	2.38E-08	4.6
H4H7795N2	6.59E+04	1.48E-03	2.24E-08	8
H4H7798N	1.43E+05	5.51E-04	3.86E-09	21

*NB indicates that under the experimental conditions, PD-1 reagent did not bind to the captured anti-PD-1 monoclonal antibody

Table 9: Binding Kinetics parameters of anti-PD-1 monoclonal antibodies binding to MfPD-1-MMH at 37°C.

Antibody	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H2aM7780N	2.29E+04	1.38E-03	6.05E-08	8.3
H2aM7788N	1.88E+04	3.28E-03	1.74E-07	3.5
H1M7789N	4.79E+04	4.08E-04	8.50E-09	28
H2aM7790N	2.55E+04	6.93E-03	2.71E-07	1.7
H2aM7791N	3.79E+04	1.91E-03	5.05E-08	6.0
H2aM7794N	6.66E+04	2.01E-02	3.02E-07	0.6

H2aM7795N	6.47E+04	3.89E-03	6.02E-08	3.0
H2aM7796N	NB*	NB*	NB*	NB*
H2aM7798N	1.42E+05	9.93E-04	7.00E-09	12
H1M7799N	8.80E+04	4.67E-02	5.30E-07	0.2
H1M7800N	8.40E+04	4.43E-02	5.27E-07	0.3
H4H9019P	2.14E+04	7.63E-03	3.56E-07	1.5
H4xH9034P	2.83E+05	2.47E-02	8.73E-08	0.5
H4xH9035P	3.06E+05	4.29E-03	1.40E-08	2.7
H4xH9037P	2.22E+05	8.80E-03	3.97E-08	1.3
H4xH9045P	1.40E+04	1.05E-01	7.54E-06	0.1
H4xH9048P2	4.15E+05	6.97E-03	1.68E-08	1.7
H4H9057P2	NB*	NB*	NB*	NB*
H4H9068P2	NB*	NB*	NB*	NB*
H4xH9119P2	2.40E+05	1.23E-02	5.14E-08	0.9
H4xH9120P2	6.98E+04	7.48E-03	1.07E-07	1.5
H4xH9128P2	9.06E+04	4.18E-03	4.61E-08	2.8
H4xH9135P2	4.62E+05	1.34E-02	2.89E-08	0.9
H4xH9145P2	1.71E+05	1.43E-02	8.37E-08	0.8
H4xH8992P	IC*	IC*	IC*	IC*
H4xH8999P	9.83E+05	9.26E-03	9.41E-09	1.2
H4xH9008P	5.86E+05	1.38E-02	2.35E-08	0.8
H4H7795N2	7.80E+04	6.89E-03	8.83E-08	1.7
H4H7798N	2.13E+05	2.23E-3	1.05E-08	5

*NB indicates that under the experimental conditions, PD-1 reagent did not bind to the captured anti-PD-1 monoclonal antibody. IC indicates that under the experimental conditions, PD-1 binding is inconclusive.

Table 10: Binding Kinetics parameters of anti-PD-1 monoclonal antibodies binding to monkey PD-1 dimer (monkey PD-1-mFc) at 25°C

Antibody	Amount of mAb Captured (RU)	100nM Monkey PD-1-mFc Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H4H9019P	116	31	4.55E+04	8.96E-05	1.97E-09	129
H4xH9034P	215	95	2.03E+05	1.66E-04	8.18E-10	70
H4xH9035P	153	78	2.16E+05	9.96E-05	4.60E-10	116
H4xH9037P	137	58	1.50E+05	1.37E-04	9.12E-10	84
H4xH9045P	202	78	9.78E+04	1.68E-04	1.72E-09	69
H4xH9048P2	227	115	2.43E+05	1.84E-04	7.54E-10	63
H4H9057P2	196	75	1.02E+05	3.03E-04	2.98E-09	38
H4H9068P2	178	17	5.70E+04	3.09E-03	5.42E-08	4
H4xH9119P2	209	83	1.63E+05	1.72E-04	1.05E-09	67

H4xH9120P2	195	52	5.84E+04	1.12E-04	1.91E-09	104
H4xH9128P2	175	64	7.87E+04	1.24E-04	1.57E-09	94
H4xH9135P2	150	74	2.38E+05	1.43E-04	6.02E-10	81
H4xH9145P2	304	84	7.24E+04	1.50E-04	2.08E-09	77
H4xH8992P	260	122	2.03E+05	2.51E-04	1.24E-09	46
H4xH8999P	217	126	5.50E+05	1.15E-04	2.10E-10	100
H4xH9008P	248	93	1.20E+05	5.77E-05	4.80E-10	200
H4H7795N2	204	60	1.60E+05	9.92E-05	6.21E-10	116
H4H7798N	223	93	4.49E+05	6.14E-05	1.37E-10	188

Table 11: Binding Kinetics parameters of anti-PD-1 monoclonal antibodies binding to monkey PD-1 dimer (monkey PD-1-mFc) at 37°C

Antibody	Amount of mAb Captured (RU)	100nM Monkey PD-1-mFc Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H4H9019P	89	36	8.16E+04	2.59E-04	3.17E-09	45
H4xH9034P	184	81	3.07E+05	7.49E-04	2.44E-09	15
H4xH9035P	88	40	3.67E+05	6.23E-04	1.70E-09	19
H4xH9037P	55	24	2.80E+05	8.97E-04	3.21E-09	13
H4xH9045P	161	65	2.41E+05	1.36E-03	5.66E-09	8
H4xH9048P2	184	84	4.94E+05	1.13E-03	2.29E-09	10
H4H9057P2	105	28	1.61E+05	4.77E-03	2.96E-08	2.4
H4H9068P2	90	6	1.21E+05	1.05E-02	8.63E-08	1.1
H4xH9119P2	98	40	2.79E+05	8.85E-04	3.17E-09	13
H4xH9120P2	141	46	8.29E+04	5.02E-04	6.06E-09	23
H4xH9128P2	148	60	1.87E+05	8.16E-04	4.36E-09	14
H4xH9135P2	106	52	3.42E+05	7.94E-04	2.32E-09	15
H4xH9145P2	284	94	1.51E+05	6.09E-04	4.04E-09	19
H4xH8992P	206	86	3.50E+05	1.53E-03	4.38E-09	8
H4xH8999P	160	83	7.30E+05	5.10E-04	7.00E-10	23
H4xH9008P	216	98	2.04E+05	1.00E-05*	4.90E-11*	1155*
H4H7795N2	164	47	1.70E+05	2.90E-04	1.71E-09	40
H4H7798N	203	88	6.30E+05	1.27E-04	2.02E-10	91

* indicates that under the current experimental conditions, no dissociation of PD-1 reagent was observed and the value of kd was manually fixed at 1.00E-05

[0204] As shown in Table 4, at 25°C, 28 of the 29 anti-PD-1 antibodies of the invention bound to hPD-1-MMH with K_D values ranging from 2.1nM to 291nM. One antibody, H4H9068P2, did not demonstrate any measurable binding to hPD-1-MMH at 25°C. As shown in Table 5, at 37°C,

26 of the 29 anti-PD-1 antibodies of the invention bound to hPD-1-MMH with K_D values ranging from 3.79nM to 1.51μM. Three antibodies of the invention did not demonstrate any conclusive binding to hPD-1-MMH at 37°C. As shown in Table 6, at 25°C, all 29 anti-PD-1 antibodies of the invention bound to hPD-1 dimer proteins with K_D values ranging from 65.5pM to 59.4nM. As shown in Table 7, at 37°C, all 27 anti-PD-1 antibodies of the invention bound to hPD-1 dimer proteins with K_D values ranging from 3.09pM to 551nM. As shown in Table 8, at 25°C, 27 of the 29 anti-PD-1 antibodies of the invention bound to MfPD-1-MMH with K_D values ranging from 3.09nM to 551nM. Two antibodies of the invention did not demonstrate any conclusive binding to MfPD-1-MMH at 25°C. As shown in Table 9, at 37°C, 25 of the 29 anti-PD-1 antibodies of the invention bound to MfPD-1-MMH with K_D values ranging from 7.00nM to 7.54μM. Four antibodies of the invention did not demonstrate any conclusive binding to MfPD-1-MMH at 37°C. As shown in Table 10, at 25°C, all 18 of the tested anti-PD-1 antibodies of the invention bound to MfPD-1 dimer with K_D values ranging from 137pM to 54.2nM. As shown in Table 11, at 37°C, all 18 of the tested anti-PD-1 antibodies of the invention bound to MfPD-1 dimer with K_D values ranging from less than 49pM to 86.3nM.

Example 4: Blocking of PD-1 binding to PD-L1 as determined by ELISA

[0205] The ability of anti-PD-1 antibodies to block human PD-1 binding to its ligand, the PD-L1 receptor, was measured using three competition sandwich ELISA formats. Dimeric human PD-L1 proteins, comprised of a portion of the human PD-L1 extracellular domain expressed with either a C-terminal human Fc tag (hPD-L1-hFc; SEQ ID: 325) or a C-terminal mouse Fc tag (hPD-L1-mFc; SEQ ID: 326), or dimeric human PD-L2, comprised of the human PD-L2 extracellular region produced with a C-terminal human Fc tag (hPD-L2-hFc; R&D Systems, #1224-PL) were separately coated at a concentration of 2 μg/mL in PBS on a 96-well microtiter plate overnight at 4°C. Nonspecific binding sites were subsequently blocked using a 0.5% (w/v) solution of BSA in PBS. In a first competition format, a constant concentration of 1.5nM of a dimeric human PD-1 protein, comprised of the human PD-1 extracellular domain expressed with a C-terminal mouse Fc tag (hPD-1-mFc; SEQ ID: 323) was added to serial dilutions of anti-PD-1 antibodies or isotype control antibodies so that the final concentrations of antibodies ranged from 0 to 200nM. In a second competition format, a constant concentration of 200 pM of dimeric biotinylated human PD-1 protein, comprised of the human PD-1 extracellular domain that was expressed with a C-terminal human Fc tag (biot-hPD-1-hFc; SEQ ID: 323), was similarly added to serial dilutions of anti-PD-1 antibodies or an isotype control at final antibody concentrations ranging from 0 to 50nM. In a third competition format, a constant concentration of 100 pM of dimeric hPD-1-mFc protein was similarly added to serial dilutions of anti-PD-1 antibodies or an isotype control at final antibody concentrations ranging from 0 to 100nM. These antibody-protein complexes were then incubated for 1 hour at room temperature (RT). Antibody-protein

complexes with 1.5 nM constant hPD-1-mFc were transferred to microtiter plates coated with hPD-L1-hFc, antibody-protein complexes with 200 pM constant biot-hPD-1-hFc were transferred to hPD-L1-mFc coated plates, and antibody-protein complexes with 100 pM constant hPD-1-mFc were transferred to microtiter plates coated with hPD-L2-hFc. After incubating for 1 hour at RT, the wells were washed, and plate-bound hPD-1-mFc was detected with an anti-mFc polyclonal antibody conjugated with horseradish peroxidase (HRP) (Jackson ImmunoResearch Inc., #115-035-164), and plate-bound biot-hPD-1-hFc was detected with streptavidin conjugated with HRP (Thermo Scientific, #N200). Samples were developed with a TMB solution (BD Biosciences, #51-2606KC and #51-2607KC) to produce a colorimetric reaction and then color development was stabilized by addition of 1M sulfuric acid before measuring absorbance at 450nm on a Victor X5 plate reader. Data analysis was performed using a sigmoidal dose-response model within Prism™ software (GraphPad). The calculated IC_{50} value, defined as the concentration of antibody required to reduce 50% of human PD-1 binding to human PD-L1 or PD-L2, was used as an indicator of blocking potency. Percent maximum blockade was calculated as a measure of the ability of the antibodies to completely block binding of human PD-1 to human PD-L1 or PD-L2 on the plate as determined from the dose curve. This percent maximum blockade was calculated by subtracting from 100% the ratio of the reduction in signal observed in the presence of the highest tested concentration for each antibody relative to the difference between the signal observed for a sample of human PD-1 containing no anti-PD-1 antibody (0% blocking) and the background signal from HRP-conjugated secondary antibody alone (100% blocking).

[0206] Percent maximum blockade and the calculated IC_{50} values for antibodies blocking greater than 35% of the hPD-1 binding signal are shown in Tables 12 – 14. Antibodies that showed a decrease in the hPD-1 binding signal of 35% or less were defined as non-blockers. Antibodies that showed an increase of 35% or more in the binding signal of human PD-1 were characterized as non-blocker/enhancers. The theoretical assay bottom, defined as the minimum antibody concentration theoretically needed to occupy 50% binding sites of human PD-1 in the assay, is 0.75nM for the format using 1.5nM constant hPD-1-mFc, 100pM for the format using 200pM constant biot-hPD-1-hFc, and 50pM for the format using 100pM constant hPD-1-mFc, indicating that lower calculated IC_{50} values may not represent quantitative protein-antibody site binding. For this reason, antibodies with calculated IC_{50} values less than 0.75nM in the assay with hPD-1-mFc constant and hPD-L1 coat, less than 100pM in the assay with biot-hPD-1-hFc constant and hPD-L1 coat, and less than 50pM in the assay with hPD-1-mFc constant and hPD-L2 coat are reported in Tables 12 – 14 as <7.5E-10M, <1.0E-10M and <5.0E-11M, respectively.

Table 12: ELISA blocking of human PD-1 binding to human PD-L1 by anti-PD-1 antibodies

Antibody	Blocking 1.5nM of hPD-1-mFc binding to hPD-L1-hFc, **IC_{50} (M)**	200nM Antibody blocking 1.5nM hPD-1-mFc binding to hPD-L1-hFc, **% blocking**
H4H9019P	1.3E-09	98
H4xH9034P	5.1E-10*	98
H4xH9045P	2.8E-10*	98
H4xH9048P2	3.3E-09	67
H4xH9120P2	1.0E-09	98
H4xH9128P2	6.4E-10*	98
H4xH9035P	6.2E-10*	99
H4xH9135P2	1.1E-09	97
H4xH9145P2	9.3E-10	90
H4xH9119P2	2.0E-10*	78
H4H9057P2	1.9E-10*	98
H4H9068P2	NBI/ Enchancer	-142
H4xH9037P	8.9E-10	100
H2aM7780N	6.9E-10*	94
H2aM7788N	2.2E-10*	74
H1M7789N	NBI/ Enchancer	-170
H2aM7790N	1.5E-09	74
H2aM7791N	NBI/ Enchancer	-154
H2aM7794N	1.1E-09	95
H2aM7795N2	8.6E-10	93
H2aM7796N	NBI	-20
H2aM7798N	6.8E-10*	93
H1M7799N	2.2E-10*	82
H1M7800N	6.0E-10*	83
H4xH8992P	1.3E-09	93
H4xH8999P	1.3E-09	88
H4xH9008P	2.4E-09	88
Isotype control 1	NBI	-3
Isotype control 2	NBI	-34
Isotype control 2	NBI	-7
Isotype control 2	NBI	-16

Assay theoretical bottom: for blocking ELISA with hPD-1-mFc constant and hPD-L1 coat is 7.5E-10 M
(*) - Below theoretical bottom of the assay;
NT- not tested; NBI - non-blocker;
NBI/Enhancer – non-blocker/enhancer; IC – inconclusive

Table 13: ELISA blocking of biotinylated human PD-1 binding to human PD-L1 by anti-PD-1 antibodies

Antibody	Blocking 200pM biot-hPD-1-hFc binding to hPD-L1-mFc, **IC_{50} (M)**	50nM Antibody blocking 200pM biot-hPD-1-hFc binding to hPD-L1-mFc, **% blocking**
H4H9019P	6.4E-10	97
H4xH9034P	6.6E-11*	96

H4xH9045P	1.3E-10	95
H4xH9048P2	IC	76
H4xH9120P2	3.9E-10	96
H4xH9128P2	1.9E-10	97
H4xH9035P	8.0E-11*	95
H4xH9135P2	1.5E-10	96
H4xH9145P2	3.5E-10	97
H4xH9119P2	8.2E-11*	96
H4H9057P2	NBI / Enhancer	-57
H4H9068P2	NBI / Enhancer	-43
H4xH9037P	7.8E-11*	95
H2aM7780N	9.1E-11*	100
H2aM7788N	6.5E-11*	100
H1M7789N	NBI	9
H2aM7790N	1.9E-10	99
H2aM7791N	NBI / Enhancer	-45
H2aM7794N	2.3E-10	99
H2aM7795N2	6.9E-11*	99
H2aM7796N	1.3E-09	60
H2aM7798N	7.3E-11*	100
H1M7799N	5.9E-11*	100
H1M7800N	6.5E-11*	99
H4xH8992P	1.6E-10	97
H4xH8999P	1.8E-10	92
H4xH9008P	1.3E-09	93
Isotype control 1	NBI	19
Isotype control 2	NBI	35
Isotype control 2	NBI	-18
Isotype control 2	NBI	-11

Assay theoretical bottom: for blocking ELISA with biot-hPD-1-mFc constant and hPD-L1 coat is 1.0E-10 M
(*) - Below theoretical bottom of the assay;
NT- not tested; NBI - non-blocker;
NBI/Enhancer – non-blocker/enhancer; IC – inconclusive

Table 14: ELISA blocking of human PD-1 binding to human PD-L2 by anti-PD-1 antibodies

Antibody	Blocking 100pM of hPD-1-mFc binding to hPD-L2-hFc, **IC_{50} (M)**	100nM Antibody blocking 100pM hPD-1-mFc binding to hPD-L2-hFc, **% blocking**
H4xH9048P2	1.4E-10	98
H2aM7795N2	2.6E-10	100
H2aM7798N	1.3E-10	100
H4xH9008P	1.3E-09	94
Isotype control 2	NBI	-27

Assay theoretical bottom: blocking ELISA with hPD-1-mFc constant and hPD-L2 coat is 5.0E-11 M
NBI - non-blocker

[0207] As indicated in Table 12, in the first assay format, 23 of the 27 anti-PD-1 antibodies blocked 1.5nM of hPD-1-mFc from binding to hPD-L1-hFc with IC_{50} values ranging from 190pM

to 3.3nM with the percent maximum blockage ranging from 67% to 100%. One antibody, H2aM7796N, was identified as a non-blocker. Three anti-PD-1 antibodies (H4H9068P2, H1M7789N, and H2aM7791N) were identified as non-blockers/ enhancers.

[0208] As shown in Table 13, in the second assay format, 23 of the 27 anti-PD-1 antibodies blocked 200pM of biot-hPD-1-hFc from binding to hPD-L1-mFc with IC_{50} values ranging from 59pM to 1.3nM with maximum percent blockade ranging from 60% to 101%. One antibody, H1M7789N, was identified as a non-blocker. Three anti-PD-1 antibodies (H4H9057P2, H4H9068P2, and H2aM7791N) were identified as non-blockers/ enhancers.

[0209] In the third assay format as shown in Table 14, four anti-PD-1 antibodies of the invention, and an Isotype control were tested. All 4 anti-PD-1 antibodies of the invention blocked 100pM (fixed concentration) of hPD-1-mFc from binding to plate-coated hPD-L2-hFc with IC_{50} values ranging from 0.13nM to 1.3nM and with maximum percent blockade ranging from 94% to 100%.

Example 5: Blocking of PD-1 binding to PD-L1 as determined by biosensor assay and by surface plasmon resonance

[0210] Inhibition of human PD-1 from binding to human PD-L1 by different anti-PD-1 monoclonal antibodies was studied either using real time bio-layer interferometry assay on an Octet Red96 biosensor instrument (Fortebio Inc.) or using a real-time surface plasmon resonance biosensor assay on a Biacore 3000 instrument.

[0211] Inhibition studies for anti-PD-1 monoclonal antibodies expressed with a mouse Fc were performed on an Octet Red 96 instrument. First, 100nM of a recombinant human PD-1 expressed with a C-terminal mouse IgG2a Fc tag (hPD-1-mFc; SEQ ID NO: 323) was incubated with 500nM of each anti-PD-1 monoclonal antibody for at least 1 hour before running the inhibition assay. Around 0.8nm to 1.2nm of recombinant human PD-L1 expressed with a C-terminal human IgG1 Fc tag (hPD-L1-hFc; SEQ ID NO: 325) was captured using anti-human IgG Fc capture Octet biosensor. The Octet biosensors coated with hPD-L1-hFc were then dipped into wells containing the mixture of hPD-1-mFc and different anti-PD-1 monoclonal antibodies. The entire experiment was performed at 25°C in Octet HBST buffer (0.01 M HEPES pH7.4, 0.15M NaCl, 3 mM EDTA, 0.05% v/v Surfactant P20, 0.1mg/mL BSA) with the plate shaking at a speed of 1000rpm. The biosensors were washed in Octet HBST buffer in between each step of the experiment. The real-time binding responses were monitored during the entire course of the experiment and the binding response at the end of every step was recorded. Binding of hPD-1-mFc to the captured hPD-L1-hFc was compared in the presence and absence of different anti-PD-1 monoclonal antibodies and was used to determine the blocking behavior of the tested antibodies as shown in Table 15.

Table 15: Inhibition of human PD-L1 binding to PD-1 by anti-PD-1 monoclonal antibodies expressed with mouse Fc as measured on an Octet Red 96 instrument

Anti-PD-1 antibody	Amount of hPD-L1-hFc Captured (nm)	Binding of the mixture of 100nM hPD-1-mFc and 500nM anti-PD-1 monoclonal antibody (nm)	% Blocking
No Antibody	0.77	0.07	0
H2aM7780N	1.07	-0.01	114
H2aM7788N	0.74	0.00	100
H1M7789N	0.80	0.05	29
H2aM7790N	0.90	-0.01	114
H2aM7791N	1.17	0.23	-229
H2aM7794N	0.87	-0.01	114
H2aM7795N	0.28	-0.01	114
H2aM7796N	0.82	-0.02	129
H2aM7798N	0.85	0.01	86
H1M7799N	0.79	0.00	100
H1M7800N	0.96	0.00	100

[0212] As shown in Table 15, 9 of the 11 anti-PD-1 antibodies tested on the Octet Red 96 instrument demonstrated strong blocking of hPD-1-mFc from binding to hPD-L1-hFc ranging from 86% to complete blockade of binding. One anti-PD-1 antibody (H1M7789N) tested showed weaker blocking of hPD-1-mFc binding to hPD-L1-hFc with 29% blockade. One antibody (H2aM7791N) tested demonstrated the ability to enhance the binding of hPD-1-mFc to hPD-L1-hFc.

[0213] Next, inhibition studies for anti-PD-1 monoclonal antibodies expressed with human Fc were performed on a Biacore 3000 instrument. First, 100nM of a recombinant human PD-1 expressed with a C-terminal human IgG1 Fc tag (hPD-1-hFc; SEQ ID: 324) was incubated with 500nM of each anti-PD-1 monoclonal antibody for at least 2 hours before running the inhibition assay. A CM5 Biacore sensor surface was first derivatized with polyclonal rabbit anti-mouse antibody (GE Catalog# BR-1008-38) using standard EDC-NHS chemistry. Around 730 RUs of recombinant human PD-L1 expressed with a C-terminal mouse IgG2a Fc tag (hPD-L1.mFc; SEQ ID: 326) was then captured followed by the injection of 100nM of hPD-1.hFc in the presence and absence of different anti-PD-1 monoclonal antibodies at a flow rate of 25µL/min for 3 minutes. The entire experiment was performed at 25°C in running buffer comprised of 0.01 M HEPES pH7.4, 0.15M NaCl, 3mM EDTA, 0.05% v/v Surfactant Tween-20 (HBS-ET running buffer).The real-time binding responses were monitored during the entire course of the experiment and the binding response at the end of every step was recorded. Binding of hPD-1-hFc to the captured hPD-L1-mFc was compared in the presence and absence of different anti-

PD-1 monoclonal antibodies and was used to determine the blocking behavior of the tested antibodies as shown in Table 16.

Table 16: Inhibition of human PD-L1 binding to PD-1 by anti-PD-1 monoclonal antibodies expressed with human Fc as measured on a Biacore 3000 instrument

Anti-PD-1 monoclonal antibody	500nM of anti-PD-1 monoclonal antibody (RU)	Binding of the mixture of 100nM hPD-1.hFc and 500nM anti-PD-1 monoclonal antibody (RU)	% Blocking
No Antibody	N/A	100 ± 1.78	N/A
H4H9019P	-2	-1	101
H4xH9034P	-4	-5	105
H4xH9035P	-3	-4	104
H4xH9037P	-4	-4	104
H4xH9045P	-4	-5	105
H4H9048P2	-7	9	91
H4H9057P2	58	57	43
H4H9068P2	-2	365	-265
H4xH9119P2	-5	-5	105
H4xH9120P2	1	0	100
H4xH9128P2	-5	-5	105
H4xH9135P2	-3	-3	102
H4xH9145P2	-8	-6	106
H4xH8992P	3	2	98
H4xH8999P	1	0	100
H4xH9008P	0	1	99
H4H7795N2	-5	-6	106
H4H7798N	-6	-6	106
H4H9008P	-7	-7	107
H4H9048P2	-4	6	94

[0214] As shown in Table 16, 18 out of 20 anti-PD-1 antibodies of the invention tested on the Biacore 3000 instrument demonstrated strong blocking of hPD-1-hFc from binding to hPD-L1-mFc with the blockade ranging from 96% to 100%. One antibody demonstrated the ability to enhance the binding of hPD-1-hFc binding to hPD-L1-mFc. In this study, one of the tested antibodies of the invention (H4H9057P2) demonstrated non-specific background binding to the anti-mouse Fc capture surface.

Example 6: Octet cross-competition between anti-PD-1 antibodies

[0215] Binding competition between anti-PD-1 monoclonal antibodies was determined using a real time, label-free bio-layer interferometry assay on an Octet RED384 biosensor (Pall ForteBio Corp.). The entire experiment was performed at 25°C in 0.01 M HEPES pH7.4, 0.15M NaCl, 3 mM EDTA, 0.05% v/v Surfactant Tween-20, 0.1mg/mL BSA (Octet HBS-ET buffer) with the plate shaking at the speed of 1000rpm. To assess whether 2 antibodies were able to compete with one another for binding to their respective epitopes on a recombinantly expressed human PD-1 with a C-terminal myc-myc-hexahistidine tag (hPD-1-MMH; SEQ ID: 321), around 0.1nM of hPD-1-MMH was first captured onto anti-Penta-His antibody coated Octet biosensor tips (Pall ForteBio Corp., # 18-5079) by submerging the tips for 5 minutes into wells containing a 50µg/mL solution of hPD-1-MMH. The antigen captured biosensor tips were then saturated with the first anti-PD-1 monoclonal antibody (subsequently referred to as mAb-1) by dipping into wells containing 50µg/mL solution of mAb-1 for 5 minutes. The biosensor tips were then subsequently dipped into wells containing a 50µg/mL solution of a second anti-PD-1 monoclonal antibody (subsequently referred to as mAb-2). The biosensor tips were washed in Octet HBS-ET buffer in between every step of the experiment. The real-time binding response was monitored during the course of the experiment and the binding response at the end of every step was recorded. The response of mAb-2 binding to hPD-1-MMH pre-complexed with mAb-1 was compared and competitive/non-competitive behavior of different anti-PD-1 monoclonal antibodies was determined. Results are summarized in Table 17 (*Self-competing mAb2s are not listed).

Table 17: Cross-competition between pairs of selected anti-PD-1 antibodies

First antibody applied ("mAb1")	mAb2 Antibodies shown to compete with mAb1*
H4xH8992P	H4xH8999P, H1M7799N, H2aM7780N, H1M7800N, H2aM7788N, H2aM7794N, H2aM7798N, H4xH9145P2, H4H9057P2, H4xH9120P2, H4xH9128P2, H4H9019P, H4xH9119P2, H4xH9135P2, H4xH9034P, H2aM7790N, H4xH9035P, H4xH9037P, H4xH9045P, H2aM7795N
H4xH8999P	H4xH8992P, H1M7799N, H2aM7780N, H1M7800N, H2aM7788N, H2aM7794N, H2aM7798N, H4xH9145P2, H4H9057P2, H4xH9120P2, H4xH9128P2, H4H9019P, H4xH9119P2, H4xH9135P2, H4xH9034P, H2aM7790N, H4xH9035P, H4xH9037P, H4xH9045P, H2aM7795N, H4xH9008P
H1M7799N	H4xH8992P, H4xH8999P, H2aM7780N, H1M7800N, H2aM7788N, H2aM7794N, H2aM7798N, H4xH9145P2, H4H9057P2, H4xH9120P2, H4xH9128P2, H4H9019P, H4xH9119P2, H4xH9135P2, H4xH9034P, H2aM7790N, H4xH9035P, H4xH9037P, H4xH9045P, H2aM7795N
H2aM7780N	H4xH8992P, H4xH8999P, H1M7799N, H1M7800N, H2aM7788N, H2aM7794N, H2aM7798N, H4xH9145P2, H4H9057P2, H4xH9120P2, H4xH9128P2, H4H9019P, H4xH9119P2, H4xH9135P2, H4xH9034P, H2aM7790N, H4xH9035P, H4xH9037P, H4xH9045P, H2aM7795N, H4xH9008P
H1M7800N	H4xH8992P, H4xH8999P, H1M7799N, H2aM7780N, H2aM7788N, H2aM7794N, H2aM7798N, H4xH9145P2, H4H9057P2, H4xH9120P2, H4xH9128P2, H4H9019P, H4xH9119P2, H4xH9135P2, H4xH9034P, H2aM7790N, H4xH9035P, H4xH9037P, H4xH9045P, H2aM7795N, H4xH9008P
H2aM7788N	H4xH8992P, H4xH8999P, H1M7799N, H2aM7780N, H1M7800N, H2aM7794N, H2aM7798N, H4xH9145P2, H4H9057P2, H4xH9120P2, H4xH9128P2, H4H9019P, H4xH9119P2, H4xH9135P2, H4xH9034P,

	H2aM7790N, H4xH9035P, H4xH9037P, H4xH9045P, H2aM7795N, H2aM7791N
H2aM7794N	H4xH8992P, H4xH8999P, H1M7799N, H2aM7780N, H1M7800N, H2aM7788N, H2aM7798N, H4xH9145P2, H4H9057P2, H4xH9120P2, H4xH9128P2, H4H9019P, H4xH9119P2, H4xH9135P2, H4xH9034P, H2aM7790N, H4xH9035P, H4xH9037P, H4xH9045P, H2aM7795N, H4xH9008P
H2aM7798N	H4xH8992P, H4xH8999P, H1M7799N, H2aM7780N, H1M7800N, H2aM7788N, H2aM7794N, H4xH9145P2, H4H9057P2, H4xH9120P2, H4xH9128P2, H4H9019P, H4xH9119P2, H4xH9135P2, H4xH9034P, H2aM7790N, H4xH9035P, H4xH9037P, H4xH9045P, H2aM7795N, H4xH9008P
H4xH9145P2	H4xH8992P, H4xH8999P, H1M7799N, H2aM7780N, H1M7800N, H2aM7788N, H2aM7794N, H2aM7798N, H4H9057P2, H4xH9120P2, H4xH9128P2, H4H9019P, H4xH9119P2, H4xH9135P2, H4xH9034P, H2aM7790N, H4xH9035P, H4xH9037P, H4xH9045P, H4xH9008P
H4H9057P2	H4xH8992P, H4xH8999P, H1M7799N, H2aM7780N, H1M7800N, H2aM7788N, H2aM7794N, H2aM7798N, H4xH9145P2, H4xH9120P2, H4xH9128P2, H4H9019P, H4xH9119P2, H4xH9135P2, H4xH9034P, H2aM7790N, H4xH9035P, H4xH9037P, H4xH9045P, H2aM7795N, H2aM7791N, H4xH9048P2
H4xH9120P2	H4xH8992P, H4xH8999P, H1M7799N, H2aM7780N, H1M7800N, H2aM7788N, H2aM7794N, H2aM7798N, H4xH9145P2, H4H9057P2, H4xH9128P2, H4H9019P, H4xH9119P2, H4xH9135P2, H4xH9034P, H2aM7790N, H4xH9035P, H4xH9037P, H4xH9045P, H4xH9048P2
H4xH9128P2	H4xH8992P, H4xH8999P, H1M7799N, H2aM7780N, H1M7800N, H2aM7788N, H2aM7794N, H2aM7798N, H4xH9145P2, H4H9057P2, H4xH9120P2, H4H9019P, H4xH9119P2, H4xH9135P2, H4xH9034P, H2aM7790N, H4xH9035P, H4xH9037P, H4xH9045P, H4xH9008P, H4H9066P2, H4xH9048P2
H4H9019P	H4xH8992P, H4xH8999P, H1M7799N, H2aM7780N, H1M7800N, H2aM7794N, H2aM7798N, H4xH9145P2, H4H9057P2, H4xH9120P2, H4xH9128P2, H2aM7788N, H4xH9119P2, H4xH9135P2, H4xH9034P, H4xH9035P, H4xH9037P, H4xH9045P, H2aM7795N, H2aM7791N
H4xH9119P2	H4xH8992P, H4xH8999P, H1M7799N, H2aM7780N, H1M7800N, H2aM7794N, H2aM7798N, H4xH9145P2, H4H9057P2, H4xH9120P2, H4xH9128P2, H2aM7788N, H4H9019P, H4xH9135P2, H4xH9034P, H4xH9035P, H4xH9037P, H4xH9045P, H2aM7795N, H2aM7791N
H4xH9135P2	H4xH8992P, H4xH8999P, H1M7799N, H2aM7780N, H1M7800N, H2aM7794N, H2aM7798N, H4xH9145P2, H4H9057P2, H4xH9120P2, H4xH9128P2, H2aM7788N, H4H9019P, H4xH9119P2, H4xH9034P, H4xH9035P, H4xH9037P, H4xH9045P, H2aM7795N, H2aM7791N
H4xH9034P	H4xH8992P, H4xH8999P, H1M7799N, H2aM7780N, H1M7800N, H2aM7794N, H2aM7798N, H4xH9145P2, H4H9057P2, H4xH9120P2, H4xH9128P2, H4H9019P, H4xH9119P2, H4xH9135P2, H2aM7788N, H2aM7790N, H4xH9035P, H4xH9037P, H4xH9045P, H2aM7795N, H2aM7791N
H2aM7790N	H4xH8992P, H1M7799N, H2aM7780N, H1M7800N, H2aM7788N, H2aM7794N, H2aM7798N, H4xH9145P2, H4H9057P2, H4xH9120P2, H4xH9128P2, H4xH9034P, H4xH8999P, H4xH9008P
H4xH9035P	H4xH8992P, H4xH8999P, H1M7799N, H2aM7780N, H1M7800N, H2aM7794N, H2aM7798N, H4xH9145P2, H4H9057P2, H4xH9120P2, H4xH9128P2, H2aM7788N, H4H9019P, H4XH9119P2, H4xH9034P, H4xH9135P2, H4xH9037P, H4xH9045P, H2aM7795N, H2aM7791N
H4xH9037P	H4xH8992P, H4xH8999P, H1M7799N, H2aM7780N, H1M7800N, H2aM7794N, H2aM7798N, H4xH9145P2, H4H9057P2, H4xH9120P2, H4xH9128P2, H2aM7788N, H4H9019P, H4xH9119P2, H4xH9034P, H4xH9135P2, H4xH9035P, H4xH9045P, H2aM7795N, H2aM7791N
H4xH9045P	H4xH8992P, H4xH8999P, H1M7799N, H2aM7780N, H1M7800N, H2aM7794N, H2aM7798N, H4xH9145P2, H4H9057P2, H4xH9120P2,

	H4xH9128P2, H2aM7788N, H4H9019P, H4xH9119P2, H4xH9034P, H4xH9135P2, H4xH9035P, H4xH9037P, H2aM7795N, H2aM7791N
H2aM7795N	H4xH8992P, H4xH8999P, H1M7799N, H2aM7780N, H1M7800N, H2aM7794N, H2aM7798N, H4H9057P2, H2aM7788N, H4H9019P, H4xH9119P2, H4xH9034P, H4xH9135P2, H4xH9035P, H4xH9037P, H4xH9045P, H2aM7791N
H4xH9008P	H4xH8999P, H2aM7780N, H2aM7794N, H2aM7798N, H4xH9145P2, H4xH9128P2, H2aM7790N, H4H9068P2, H1M7799N, H4xH9048P2
H2aM7791N	H2aM7788N, H4H9057P2, H4H9019P, H4xH9119P2, H4xH9135P2, H4xH9034P, H4xH9035P, H4xH9037P, H4xH9045P, H2aM7795N
H4H9068P2	H4xH9128P2, H4xH9008P, H1M7789N, H4xH9048P2
H1M7789N	H4xH9008P, H4H9068P2, H4xH9048P2
H4xH9048P2	H4H9057P2, H4xH9120P2, H4xH9128P2, H4H9019P, H4xH9008P, H4H9068P2, H1M7799N

[0216] A second binding competition between a panel of selected anti-PD-1 monoclonal antibodies was determined using a real time, label-free bio-layer interferometry assay on an Octet HTX biosensor (Pall ForteBio Corp.). The entire experiment was performed at 25°C in 0.01 M HEPES pH7.4, 0.15M NaCl, 3 mM EDTA, 0.05% v/v Surfactant Tween-20, 0.1mg/mL BSA (Octet HBS-ET buffer) with the plate shaking at the speed of 1000rpm. To assess whether 2 antibodies were able to compete with one another for binding to their respective epitopes on the hPD-1-MMH, around 0.25nm of hPD-1-MMH was first captured onto anti-Penta-His antibody coated Octet biosensor tips (Fortebio Inc, # 18-5079) by submerging the tips for 150 seconds into wells containing a 10µg/mL solution of hPD-1-MMH. The antigen-captured biosensor tips were then saturated with a first anti-PD-1 monoclonal antibody (subsequently referred to as mAb-1) by dipping into wells containing 100µg/mL solution of mAb-1 for 5 minutes. The biosensor tips were then subsequently dipped into wells containing a 100µg/mL solution of second anti-PD-1 monoclonal antibody (subsequently referred to as mAb-2) for 4 minutes. All the biosensors were washed in Octet HBS-ET buffer in between every step of the experiment. The real-time binding response was monitored during the course of the experiment and the binding response at the end of every step was recorded as shown in Figure 2. The response of mAb-2 binding to hPD-1-MMH pre-complexed with mAb-1 was compared and competitive/non-competitive behavior of different anti-PD-1 monoclonal antibodies was determined. Results are summarized in Table 18 (*Self-competing mAb2s are not listed).

Table 18: Cross-competition between pairs of selected anti-PD-1 antibodies

First Antibody applied ("mAb1")	**mAb2 Antibodies Shown to Compete with mAb1***
H4H7795N2	H4H7798N
H4H7798N	H4H7795N2; H4H9008P
H4H9008P	H4H7798N; H4H9068P2
H4H9068P2	H4H9008P; H4H9048P2
H4H9048P2	H4H9068P2

[0217] Under the experimental conditions disclosed in this Example, H4H7795N2 cross-

competed with H4H7798N; H4H7798N cross-competed with H4H7795N2 and H4H9008P; H4H9008P cross-competed with H4H7798N and H4H9068P2; H4H9068P2 cross-competed with H4H9008P and H4H9048P2.

Example 7: Antibody binding to cells overexpressing PD-1

[0218] The binding of anti-PD-1 antibodies to a human embryonic kidney cell line (HEK293; ATCC, #CRL-1573) stably transfected with full length human PD-1 (amino acids 1 to 289 of accession number NP_005009.2) (HEK293/hPD-1) was determined by FACS.

[0219] For the assay, adherent cells were detached using trypsin or enzyme-free dissociation buffer and blocked with complete medium. Cells were centrifuged and resuspended at a concentration of 2.5-6x10^6 cells/mL in cold PBS containing 2% FBS. HEK293 parental and HEK293/hPD-1 cells were then incubated for 15-30min on ice with 100nM of each anti-PD-1 antibody. Unbound antibodies were removed by washing with D-PBS containing 2% FBS, and cells were subsequently incubated with an allophycocyanin-conjugated secondary F(ab')2 recognizing either human Fc (Jackson ImmunoResearch, # 109-136-170) or mouse Fc (Jackson ImmunoResearch, #115-136-146) for 15-30 minutes on ice. Cells were washed with D-PBS containing 2% FBS to remove unbound secondary F(ab')2 and fluorescence measurements were acquired using either a HyperCyte (IntelliCyt, Inc.) flow cytometer or an Accuri flow cytometer (BD Biosciences). Data was analyzed using FlowJo software (Tree Star).

Table 19: FACS binding of anti-PD-1 antibodies to HEK293/hPD-1 cells and parental HEK293 cells

Antibody	FACS on HEK293 parental cells [MFI]	FACS on HEK293/hPD-1 cells [MFI]	Ratio of HEK293/hPD-1 to HEK293 parental cells
H1M7789N	262	24166	92.3
H1M7799N	255	6855	26.9
H1M7800N	275	6812	24.7
H2aM7780N	320	23656	73.8
H2aM7788N	305	23112	75.7
H2aM7790N	270	47310	175.5
H2aM7791N	274	4948	18.0
H2aM7794N	270	19127	71.0
H2aM7795N	288	817	2.8
H2aM7796N	297	49755	167.8
H2aM7798N	300	23443	78.1
H4H9019P	111	8610	77.2
H4H9057P2	141	6501	46.1
H4H9068P2	285	1940	6.8
H4xH8992P	358	17502	48.9
H4xH8999P	809	28875	35.7

H4xH9008P	509	26233	51.5
H4xH9034P	147	10115	69.0
H4xH9035P	108	9915	91.7
H4xH9037P	108	8787	81.4
H4xH9045P	95	8884	93.7
H4xH9048P2	102	7196	70.8
H4xH9119P2	109	9142	84.0
H4xH9120P2	109	9975	91.9
H4xH9128P2	135	9081	67.5
H4xH9135P2	114	9380	82.2
H4xH9145P2	226	11552	51.2

[0220] As shown in Table 19, 25 of the 27 anti-PD-1 antibodies of the invention showed strong binding to the HEK293/ hPD-1 cells compared to binding on the parental HEK293 line. Two antibodies of the invention (H2aM7795N and H4H9068P2) bound weaker to human PD-1 expressing cells compared to the other antibodies tested.

[0221] To further characterize anti-PD1 antibodies of the invention, dose-dependent binding to a human embryonic kidney cell line (HEK293; ATCC, #CRL-1573) stably transfected with full length human PD-1 (amino acids 1 to 289 of accession number NP_005009.2) (HEK293/hPD-1) was determined by FACS.

[0222] For the assay, adherent cells were detached using trypsin and blocked with complete medium. Cells were centrifuged and resuspended at a concentration of 6x10^6 cells/mL in staining buffer (1% FBS in PBS). To determine the EC_{50} and E_{max} of the anti-PD1 antibodies, 90uL of cell suspension was incubated for 30 minutes on ice with a serial dilution of anti-PD-1 antibodies and controls diluted to a final concentration ranging from 5 pM to 100 nM (no mAb sample was included as negative control) in staining buffer. Cells were then centrifuged and pellets were washed once with staining buffer to remove unbound antibodies. Cells were subsequently incubated for 30 minutes on ice either with an allophycocyanin-conjugated secondary F(ab')2 recognizing human Fc (Jackson ImmunoResearch, # 109-136-170) or mouse Fc (Jackson ImmunoResearch, #115-136-071). Cells were centrifuged and pellets were washed once with staining buffer to remove unbound secondary F(ab')2 and then fixed overnight with a 1:1 dilution of Cytofix (BD Biosciences, # 554655) and staining buffer. The following day, cells were centrifuged and pellets were washed once with staining buffer, resuspended and filtered. Fluorescence measurements were acquired on Hypercyt® cytometer and analyzed in ForeCyt™ (IntelliCyt; Albuquerque, NM) to determine the mean fluorescence intensities (MFI). The EC_{50} values were calculated from a four-parameter logistic equation over an 11-point response curve using GraphPad Prism. E_{max} for each antibody was defined as the binding at the highest antibody dose (100nM) tested.

Table 20: Dose dependent FACS binding of anti-PD-1 antibodies to HEK293/hPD-1 cells

Antibody	EC_{50} [M]	Max Geom. Mean [MFI] @ 100nM
H2aM7779N	2.59E-09	16832
H2aM7780N	1.69E-09	18415
H2aM7781N	5.67E-10	13740
H2aM7782N	1.26E-09	17302
H2aM7787N	2.40E-09	15744
H2aM7788N	3.21E-10	14827
H2aM7790N	1.71E-10	19196
H2aM7791N	No EC_{50} determined	1397
H2aM7794N	1.37E-09	16406
H2aM7795N	No EC_{50} determined	624
H2aM7798N	6.985E-11	20900
H1M7799N	3.318E-11	24405
H1M7800N	4.80E-11	20763
H4xH8992P	5.45E-11	11368
H4xH8999P	5.27E-11	28341
H4H9019P	1.40E-09	29201
H4xH9034P	2.09E-10	32388
H4xH9035P	1.15E-10	28708
H4xH9037P	6.74E-10	36441
H4xH9045P	9.17E-11	24662
H4xH9048P2	6.68E-10	33687
H4H9057P2	2.363E-10	19953
H4H9068P2	No EC_{50} determined	639
H4xH9119P2	3.476E-10	37789
H4xH9120P2	4.797E-10	34057
H4xH9128P2	1.551E-09	37167
H4xH9135P2	1.048E-10	32793
H4xH9145P2	2.321E-10	30613
mIgG1 isotype	N/A	200
mIgG2a isotype	N/A	239
hIgG4 isotype	N/A	459

Table 21: Dose dependent FACS binding of anti-PD-1 antibodies to HEK293/hPD-1 cells

Antibody	EC_{50} [M]	Max Geom. Mean [MFI] @ 100nM
H4H7795N2	Inconclusive	15188
H4H7798N	5.09E-10	20305
H4H9008P	Inconclusive	32230
H4H9048P2	1.60E-09	39774
H1M7789N	Inconclusive	35574
H2aM7796N	4.81E-09	14111
mIgG1 isotype	N/A	858
mIgG2a isotype	N/A	352

hIgG4 isotype	N/A	809

[0223] As shown in Table 20, 25 of 28 anti-PD1 antibodies of the invention showed dose dependent binding to HEK293/hPD-1 cells with EC_{50} values ranging from 33.18pM to 2.59nM and E_{max} values ranging from 37,789 to 11,368 MFI. Three anti-PD1 antibodies of the invention did not demonstrate strong binding to HEK293/hPD-1 cells and therefore an EC_{50} value could not be determined. None of the isotype controls demonstrated any measurable binding in this assay.

[0224] As shown in Table 21, 3 of 6 anti-PD1 antibodies of the invention showed dose dependent binding to HEK293/hPD-1 cells with EC_{50} values ranging from 509pM to 4.81nM and E_{max} values ranging from 39,774 to 14,111 MFI. Three antibodies of the invention tested bound to HEK293/hPD-1 cells, but did not reach a plateau. Therefore their precise EC_{50} values could not be determined and their EC_{50} values are referred to as inconclusive. None of the isotype controls demonstrated any measurable binding in this assay.

Example 8: Blocking of PD-1-induced T-cell down-regulation in a T-cell/APC luciferase reporter assay

[0225] T-cell activation is achieved by stimulating T-cell receptors (TcR) that recognize specific peptides presented by major histocompatibility complex class I or II proteins on antigen-presenting cells (APC). Activated TcRs in turn initiate a cascade of signaling events that can be monitored by reporter genes driven by transcription factors such as activator-protein 1 (AP-1), Nuclear Factor of Activated T-cells (NFAT) or Nuclear factor kappa-light-chain-enhancer of activated B cells (NFκb). T-cell response is modulated via engagement of co-receptors expressed either constitutively or inducibly on T-cells. One such receptor is PD-1, a negative regulator of T-cell activity. PD-1 interacts with its ligand, PD-L1, which is expressed on target cells including APCs or cancer cells, and acts to deliver inhibitory signals by recruiting phosphatases to the TcR signalosome, resulting in the suppression of positive signaling.

[0226] The ability of anti-PD-1 antibodies to antagonize PD-1/PD-L1-mediated signaling through the PD-1 receptor in human T cell lines was assessed using an in vitro cell based assay shown in Figure 1. The bioassay was developed to measure T cell signaling induced by interaction between APC and T cells by utilizing a mixed culture derived from two mammalian cell lines: Jurkat cells (an immortalized T cell line) and Raji cells (a B cell line). For the first component of the bioassay, Jurkat Clone E6-1 cells (ATCC, #TIB-152) were transduced with the Cignal Lenti AP-1 Luc Reporter (Qiagen – Sabiosciences, #CLS-011L) as per the manufacturer's instructions. The lentivirus encodes the firefly luciferase gene under the control of a minimal CMV promoter, tandem repeats of the TPA-inducible transcriptional response element (TRE) and a puromycin resistance gene. The engineered Jurkat cell line was subsequently transduced with a PD-1 chimera comprising the extracellular domain of human

PD-1 (amino acids from 1 to 170 of human PD1; accession number NP_005009.2) and the trans-membrane and cytoplasmic domains of human CD300a (amino acids from 181 to 299 of human CD300a; accession number NP_009192.2). The resulting stable cell line (Jurkat/AP1-Luc/ hPD1-hCD300a) was selected and maintained in RPMI/10% FBS/ penicillin/streptomycin/glutamine supplemented with 500ug/mL G418+1ug/mL puromycin.

[0227] For the second component of the bioassay, Raji cells (ATCC, #CCL-86) were transduced with human PD-L1 gene (amino acids 1-290 of accession number NP_054862.1) that had been cloned into a lentiviral (pLEX) vector system (Thermo Scientific Biosystems, #OHS4735). Raji cells, positive for PD-L1 (Raji/ hPD-L1) were isolated by FACS using a PD-L1 antibody and maintained in Iscove/10% FBS/penicillin/streptomycin/glutamine supplemented with 1ug/mL puromycin.

[0228] To simulate the APC/T cell interaction, a bispecific antibody composed of one Fab arm that binds to CD3 on T cells and the other one Fab arm binding that binds to CD20 on Raji cells (CD3xCD20 bispecific antibody; *e.g.*, as disclosed in US20140088295) was utilized. The presence of the bispecific molecule in the assay results in the activation of the T cell and APC by bridging the CD3 subunits on T-cells to CD20 endogenously expressed on Raji cells. Ligation of CD3 with anti-CD3 antibodies has been demonstrated to lead to activation of T cells. In this bioassay, antibodies blocking the PD1/PD-L1 interaction rescue T-cell activity by disabling the inhibitory signaling and subsequently leading to increased AP1-Luc activation.

[0229] In the luciferase-based bioassay, RPMI1640 supplemented with 10% FBS and penicillin/streptomycin/glutamine was used as assay medium to prepare cell suspensions and antibody dilutions to carry out the screening of anti-PD1 monoclonal antibodies (mAbs). On the day of the screening, EC50 values of anti-PD1 mAbs, in the presence of a fixed concentration of CD3xCD20 bispecific antibody (30 pM), as well as the EC50 of the bispecific antibody alone, were determined. In the following order, cells and reagents were added to 96 well white, flat-bottom plates. For the anti-PD1 mAb EC50 determinations, first a fixed concentration of CD3xCD20 bispecific antibody (final 30 pM) was prepared and added to the microtiter plate wells. Then 12-point serial dilutions of anti-PD1 mAbs and controls were added (final concentrations ranging from 1.7 pM to 100 nM; plus wells with assay medium alone). For the bispecific antibody (alone) EC_{50} determination, the bispecific antibody, at final concentrations ranging from 0.17 pM to 10 nM (plus wells with assay medium alone), was added to the microtiter plate wells. Subsequently, a 2.5x10^6/mL Raji/hPD-L1 cell suspension was prepared and 20 uL per well was added (final cell number/well 5x10^4 cells). Plates were left at room temperature (15-20 minutes), while a suspension of 2.5x10^6/mL of Jurkat/AP1-Luc/hPD1(ecto)-hCD300a(TM-Cyto) was prepared. 20 uL of the Jurkat suspension (final cell number/well 5x10^4 cells) was added per well. Plates containing the co-culture were incubated for 5 to 6 hours at 37°C/5% CO_2. Samples were tested in duplicates and luciferase activity was then detected after the addition of ONE-Glo™ (Promega, # E6051) reagent and relative light

units (RLUs) were measured on a Victor luminometer.

[0230] RLU values for each screened antibody were normalized by setting the assay condition with fixed (30 pM) concentration of the CD3/CD20 bispecific antibody, but without anti-PD-1 antibody to 100%. This condition corresponds to the maximal AP1-Luc response elicited by the bispecific molecule in the presence of the PD-1/PD-L1 inhibitory signal. Upon addition of the anti-PD-1 antibody, the inhibitory signal is suppressed, and the increased stimulation is shown here as E_{max}, the percentage increase in the signal in the presence of the highest antibody dose tested (100 nM). To compare potency of the anti-PD1 antibodies tested, the concentration of antibody at which the normalized RLU value reached 150% activation was determined from a four-parameter logistic equation over a 12-point response curve using GraphPad Prism. The results are summarized in Table 22 and Table 23, respectively.

Table 22: Anti-PD1 antibody blocking PD-1/PD-L1 dependent inhibition of AP1-Luc signaling in Experiment 1

Antibody	Antagonistic assay Concentration (M) of Antibody at 150% activation Experiment 1	Antagonistic assay E_{max} mean [%] @ 100nM Experiment 1
H1M7789N	N/A	135
H1M7799N	2.97E-08	183
H1M7800N	1.65E-08	182
H2aM7779N	8.92E-09	214
H2aM7780N	6.52E-09	228
H2aM7781N	6.70E-09	230
H2aM7782N	9.96E-09	215
H2aM7787N	1.38E-08	215
H2aM7788N	4.72E-09	189
H2aM7790N	5.24E-09	234
H2aM7791N	N/A	103
H2aM7794N	4.09E-08	170
H2aM7795N	N/A	109
H2aM7796N	N/A	121
H2aM7798N	7.99E-10	239
H4H9019P	1.79E-08	180
H4xH9034P	2.62E-09	202
H4xH9035P	1.20E-09	227
H4xH9037P	2.82E-09	195
H4xH9045P	2.23E-08	176
H4xH9048P2	N/A	138
H4H9057P2	2.68E-08	212
H4H9068P2	N/A	102
H4xH9119P2	1.11E-08	163
H4xH9120P2	1.10E-08	166
H4xH9128P2	3.99E-09	187
H4xH9135P2	1.55E-09	193

H4xH9145P2	2.40E-09	185
H4xH8992P	5.32E-09	178
H4xH8999P	8.63E-10	217
H4H7798N	1.54E-09	202
mIgG1 isotype control	N/A	92
mIgG2a isotype control	N/A	91
hIgG4 isotype control	N/A	94

N/A= not applicable because at the concentrations tested these antibodies did not activate 150%

Table 23: Anti-PD1 antibody blocking PD-1/PD-L1 dependent inhibition of AP1-Luc signaling in Experiment 2

Antibody	**Antagonistic assay Concentration (M) of Antibody at 150% activation Experiment 2**	**Antagonistic assay E_{max} mean [%] @ 100nM Experiment 2**
H4H7795N2	N/A	110
H4H7798N	1.59E-10	343
H4H9008P	9.84E-08	150
H4H9048P	N/A	134
hIgG4 isotype control	N/A	98

N/A= not applicable because at the concentrations tested these antibodies did not activate 150%

[0231] As shown in Table 22, 25 out of the 31 anti-PD-1 antibodies of the invention tested blocked PD-1/PD-L1 inhibition with E_{max} values ranging from 239 to 163. Six out of the 31 anti-PD-1 antibodies of the invention did not demonstrate substantial blockade of PD1/PD-L1 interaction when tested in this assay.

[0232] As shown in Table 23, 2 out of the 4 anti-PD-1 antibodies of the invention tested blocked PD-1/PD-L1 inhibition with E_{max} values of 150 and 343%, respectively. 2 out of the 4 anti-PD-1 antibodies of the invention did not demonstrate substantial blockade of PD1/PD-L1 interaction when tested in this assay.

Example 9: In vivo efficacy of anti-PD-1 antibodies

[0233] To determine the effect of a select number of anti-PD-1 antibodies of the invention in a relevant *in vivo* model, three MC38.ova tumor growth studies, involving subcutaneous injection of tumor cells and started on different days, were conducted in mice that were homozygous for the expression of the extracellular domain of human PD-1 in place of extracellular domain of mouse PD-1 (PD-1 HumIn mice) on a 75% C57/Bl6 / 25% 129 strain background.

[0234] For the studies, mice were divided evenly according to body weight into 5 treatment or control groups for Study 1 (5 mice per group), 8 treatment or control groups for Study 2 (5 mice per group), and 5 treatment or control groups for Study 3 (7 mice per group). At day 0, mice were anesthetized by isoflurane inhalation and then injected subcutaneously into the right flank with $5x10^5$ MC38.ova cells in suspension of 100uL of DMEM for Study 1 or $1x10^6$ MC38.ova

cells in suspension of 100 uL of DMEM for Study 2 and Study 3. For Study 1, treatment groups were intraperitoneally injected with 200ug of either one of three anti-PD-1 antibodies of the invention, or an isotype control antibody with irrelevant specificity on days 3, 7, 10, 14, and 17 of the experiment, while one group of mice was left untreated. For Study 2, treatment groups were intraperitoneally injected with either one of three anti-PD-1 antibodies of the invention at 10mg/kg or 5mg/kg per/dose, one antibody of the invention (H4H7795N2) at 10mg/kg per dose, or an isotype control antibody with irrelevant specificity at 10mg/kg on days 3, 7, 10, 14, and 17 of the experiment. For Study 3, treatment groups were intraperitoneally injected with either one of two anti-PD-1 antibodies of the invention at 5mg/kg or 2.5mg/kg per/dose, or an isotype control antibody with irrelevant specificity at 5mg/kg on days 3, 7, 10, 14, and 17 of the experiment. Experimental dosing and treatment protocol for groups of mice are shown in Table 24.

Table 24: Experimental dosing and treatment protocol for groups of mice

Study #	Samples Tested	Dosage amount at each dosage time point	Dosing interval
1	Isotype Control	200ug	Days 3, 7, 10, 14, 17
	No treatment	N/A	N/A
	H4H7798N	200ug	Days 3, 7, 10, 14, 17
	H4H7795N2	200ug	Days 3, 7, 10, 14, 17
	H4H9008P	200ug	Days 3, 7, 10, 14, 17
2	Isotype Control	10mg/kg	Days 3, 7, 10, 14, 17
	H4H7795N2	10mg/kg	Days 3, 7, 10, 14, 17
	H4H7798N	10mg/kg	Days 3, 7, 10, 14, 17
	H4H7798N	5mg/kg	Days 3, 7, 10, 14, 17
	H4H9048P2	10mg/kg	Days 3, 7, 10, 14, 17
	H4H9048P2	5mg/kg	Days 3, 7, 10, 14, 17
	H4H9008P	10mg/kg	Days 3, 7, 10, 14, 17
	H4H9008P	5mg/kg	Days 3, 7, 10, 14, 17
3	Isotype Control	5mg/kg	Days 3, 7, 10, 14, 17
	H4H7798N	5mg/kg	Days 3, 7, 10, 14, 17
	H4H7798N	2.5mg/kg	Days 3, 7, 10, 14, 17
	H4H9008P	5mg/kg	Days 3, 7, 10, 14, 17
	H4H9008P	2.5mg/kg	Days 3, 7, 10, 14, 17

[0235] For the studies, average tumor volumes determined by caliper measurements and percent survival at Day 14 or 17 and Day 23 or 24 of each experiment for each treatment group were recorded. In addition, the number of tumor-free mice were also assessed at the end of the study (Day 42 for Study 1 and Day 31 for Study 2 and Study 3). Results, expressed as mean tumor volume (mm^3)(±SD), percent survival, and number of tumor-free mice are shown in Table 23 for Study 1, Table 3 for Study 2, and Table 4 for Study 3.

Table 25: Mean tumor volume, percent survival and numbers of tumor free mice in each treatment group from in vivo tumor Study 1

Treatment group (n=5)	Tumor Volume, mm^3 mean (±SD)		Survival, %		Tumor-Free Mice
	Day 17	Day 23	Day 17	Day 23	Day 42
	200 ug/mouse	200 ug/mouse	200 ug/mouse	200 ug/mouse	200 ug/mouse
No treatment	189 (±110)	554 (±317)	100%	100%	1/5 (20%)
Isotype control	86 (±114)	515 (±859)	100%	60%	2/5 (40%)
H4H7798N	0 (0)	0 (0)	100%	100%	5/5 (100%)
H4H9008P	14 (±19)	205 (±312)	100%	100%	3/5 (60%)
H4H7795N2	89 (±176)	445 (±889)	100%	80%	3/5 (60%)

[0236] As shown in Table 25 for Study 1, mice treated with one antibody of the invention, H4H7798N did not develop any detectable tumors during the course of the study. Mice treated with H4H9008P exhibited a sustained reduced tumor volume as compared to controls at days 17 and 24 of the study with 3 out of 5 mice or 4 out of 5 mice being tumor free by the end of the experiment, respectively. In contrast, treatment with one of the anti-PD1 antibodies, H4H7795N2, did not demonstrate significant efficacy in reducing tumor volume in this study as compared to controls. By day 23 of the study, 1 out of 5 mice died in the H4H7795N2 group, and 2 out of 5 mice died in the isotype control treatment group. In non-treatment group and isotype control group some mice exhibited spontaneous regression of tumors (1 out of 5 mice and 2 out of 5 mice, respectively).

Table 26: Mean tumor volume, percent survival and numbers of tumor free mice in each treatment group from in vivo tumor Study 2

Treatment group (n=5)	Tumor Volume, mm^3 mean (±SD)				Survival, %				Tumor-Free Mice	
	Days 17		Day 24		Day 17		Day 24		Day 31	
	5 mg/kg	10 mg/kg	5 mg/kg	10 mg/kg	5 mg/kg	10 mg/kg	5 mg/kg	10 mg/kg	5 mg/kg	10 mg/kg
Isotype control	N/A	449 (±434)	N/A	824(±858)	N/A	100%	N/A	60%	N/A	1/5 (20%)
H4H7798N	17 (±38)	0 (0)	104 (±233)	0 (0)	100%	100%	100%	100%	4/5 (80%)	5/5/ (100%)
H4H9008P	91 (±204)	12 (±28)	228 (±509)	96 (±215)	100%	100%	80%	100%	4/5 (80%)	4/5 (80%)
H4H9048P2	94 (±160)	10 (±21)	328 (±559)	67 (±150)	100%	100%	80%	100%	3/5 (60%)	4/5 (80%)
H4H7795N2	N/A	124 (±209)	N/A	359 (±657)	N/A	100%	N/A	80%	N/A	2/5 (40%)

[0237] As shown in Table 26 for Study 2, mice treated with one antibody of the invention, H4H7798N at 10mg/kg did not develop detectable tumors during the course of the study. Groups of mice treated with 10 mg/kg of either H4H9008P or H4H9048P2 exhibited substantially reduced tumor volume as compared to controls at days 17 and 24 of the study. Four out of 5 mice in each group treated with 10mg/kg of either H4H9008P or H4H9048P2 were

tumor free at Day 31, whereas in the isotype control treatment group only 1 out of 5 animals was tumor free as a result of spontaneous tumor regression. One antibody tested at 10mg/kg, H4H7795N2, demonstrated substantially reduced tumor volume as compared to controls at days 17 and 24 of the study, but this antibody was the least efficacious anti-PD1 antibody with only 2 out of 5 mice surviving at the end of the experiment.

[0238] A dose-dependent response in tumor suppression at the tested doses (5 mg/kg and 10 mg/kg) was observed in groups treated with H4H7798N, H4H9008P, and H4H9048P2. H4H7798N or H4H9008P therapy at 5 mg/kg was less efficacious, with 4 out of 5 tumor-free mice at the end of experiment on day 21, whereas 5 out of 5 mice remained tumor-free in both 10 mg/kg dose groups of H4H7798N, and H4H9008P.

[0239] Dunett's test in 2 way ANOVA multiple comparisons revealed that the differences in tumor growth between the group treated with isotype control antibody at 10 mg/kg as reference and the groups treated at 10 mg/kg with either H4H7798N, H4H9008P, or H4H9048P2 were statistically significant with p value<0.005. The differences in tumor growth between the group treated with isotype control antibody at 10 mg/kg as reference and the groups treated at 5 mg/kg with either H4H7798N , H4H9008P, or H4H9048P2 were also statistically significant with a p value<0.05.

Table 27: Mean tumor volume, percent survival and numbers of tumor free mice in each treatment group from in vivo tumor Study 3

Treatment group (n=7)	Tumor Volume, mm^3 mean (±SD)				Survival, %				Tumor-Free Mice	
	Days 14		Day 21		Day 14		Day 21		Day 31	
	2.5 mg/kg	5 mg/kg	2.5 mg/kg	5 mg/kg	2.5 mg/kg	5 mg/kg	2.5 mg/kg	5 mg/kg	2.5 mg/kg	5 mg/kg
Isotype control	N/A	94(±44)	N/A	405 (±326)	N/A	100%	N/A	86%	N/A	0/7 (0%)
H4H7798N	0 (0)	0 (0)	19 (±51)	13 (±35)	100%	100%	100%	100%	6/7 (86%)	6/7 (86%)
H4H9008P	41 (±68)	7 (±20)	87 (±123)	16 (±42)	100%	100%	100%	100%	4/7 (57%)	6/7 (86%)

[0240] As shown in Table 27 for Study 3, 6 out or 7 mice treated with one antibody of the invention, H4H7798N, or another antibody of the invention, H4H9008P, at 5mg/kg were tumor free at the end of the experiment, whereas there were no tumor free animals in the isotype control group. One tumor-bearing mouse in the IgG4 control group died on post-implantation day 17. Only 4 out of 7 mice treated with H4H9008P at 2.5mg/kg dose remained tumor free at the end of the experiment. The difference in tumor volumes at day 21 between anti-PD-1 antibodies tested and an isotype control group was statistically significant as determined by one-way ANOVA with Dunnett's multiple comparison post-test with p<0.01. All four anti-PD-1 antibodies were equally more efficacious at the 5 mg/kg dose than at the 2.5 mg/kg dose.

Example 10: Anti-tumor effects of a combination of an anti-PD-1 antibody and a VEGF

antagonist in a mouse early-treatment tumor model

[0241] An early-treatment tumor model was developed to test the efficacy of a combination of an anti-PD-1 antibody and a VEGF antagonist. In this model, the combination therapy is administered shortly after tumor implantation. The experiment also used an anti-PD-L1 antibody alone and in combination with the VEGF antagonist. The anti-PD-1 antibody used in this experiment was anti-mouse PD-1 clone "RPMI-14" with rat IgG2b (Bio X Cell, West Lebanon, NH). The VEGF antagonist used in this experiment was aflibercept (a VEGF receptor-based chimeric molecule, also known as "VEGF-trap" or "VEGFR1R2-FcΔC1(a)," a full description of which is provided elsewhere herein). The anti-PD-L1 antibody used in this experiment was an anti-PD-L1 monoclonal antibody with V_H/V_L sequences of antibody "YW243.55S70" according to US20100203056A1 (Genentech, Inc.), with mouse IgG2a and which was cross-reactive with mouse PD-L1.

[0242] For this experimental model, $1.0x10^6$ Colon-26 tumor cells were implanted sub-cutaneously into BALB/c mice at Day 0. Starting on Day 3, prior to the establishment of measurable tumors, mice were treated with one of the mono- or combination therapies, or control combination, as set forth in Table 28.

Table 28: Experimental dosing and treatment groups

Treatment Group	First Agent	Second Agent
Control Combination	IgG2a isotype control (250 µg, IP)	hFc control (250 µg, SC)
VEGF Trap only	IgG2a isotype control (250 µg, IP)	Aflibercept (10 mg/kg, SC)
anti-PD-1 only	anti-PD-1 mAb RPMI-14 (250 µg, IP)	hFc control (250 µg, SC)
anti-PD-L1 only	anti-PD-L1 mAb (250 µg, IP)	hFc control (250 µg, SC)
VEGF Trap + anti-PD-1	anti-PD-1 mAb RPMI-14 (250 µg, IP)	Aflibercept (10 mg/kg, SC)
VEGF Trap + anti-PD-L1	anti-PD-L1 mAb (250 µg, IP)	Aflibercept (10 mg/kg, SC)

[0243] The various therapies were administered at five different time points over a two week period (i.e., injections at Day 3, Day 6, Day 10, Day 13 and Day 19).

[0244] Animals in each therapy group were evaluated in terms of tumor incidence, tumor volume, median survival time, and number of tumor-free animals at Day 50. The extent of tumor growth is summarized in Figure 2 (tumor growth curves) and Figure 3 (tumor volume at Day 28). Results are also summarized in Table 29.

Table 29: Tumor-free mice in treatment groups

Treatment Group	No. of Tumor-Free Animals by Day 50
Control Combination	0/10
VEGF Trap only	3/10
anti-PD-1 only	4/10
anti-PD-L1 only	5/10

VEGF Trap + anti-PD-1	7/10
VEGF Trap + anti-PD-L1	9/10

[0245] Tumor growth was substantially reduced in animals treated with the combination of VEGF Trap + anti-PD-1 antibody as compared with treatment regimens involving either therapeutic agent alone (*see* Figures 2 and 3). Furthermore, survival was substantially increased in the VEGF Trap + anti-PD-1 antibody group, with 70% of animals surviving to at least day 50 after tumor implantation. By contrast, for the anti-PD-1 and VEGF Trap monotherapy groups, survival to Day 50 was only 40% and 30% respectively (*see* Figure 3 and Table 29).

Example 11: Clinical trial study of repeat dosing with anti-PD-1 antibody as single therapy and in combination with other anti-cancer therapies in patients with advanced malignancies

[0246] This is a dose-escalation study of anti-PD-1 antibody, alone or in combination with radiation therapy, cyclophosphamide, or both in patients with advanced malignancies. The exemplary anti-PD-1 antibody ("mAb") used in this Example comprises HCVR of SEQ ID NO: 162 and LCVR of SEQ ID NO: 170.

Study Objectives

[0247] The primary objective of the study is to characterize the safety, tolerability, DLTs of mAb administered IV as monotherapy, or in combination with targeted radiation (with the intent to have this serve as an immuno-stimulatory, rather than primarily tumor-ablative therapy), low-dose cyclophosphamide (a therapy shown to inhibit regulatory T-cell responses), or both in patients with advanced malignancies.

[0248] The secondary objectives of the study are: (1) to determine a recommended phase 2 dose (RP2D) of mAb as monotherapy and in combination with other anti-cancer therapies (targeted radiation, low-dose cyclophosphamide, or both); (2) to describe preliminary antitumor activity of mAb, alone and with each combination partner (s); (3) to characterize the PK of mAb as monotherapy and in combination with other anti-cancer therapies (targeted radiation, low-dose cyclophosphamide, or both); and (4) to assess immunogenicity of mAb.

Study Design

[0249] Safety will be assessed in separate, standard 3 + 3 dose escalation cohorts (in monotherapy, combination with radiation therapy, combination with cyclophosphamide, and combination with radiation therapy plus cyclophosphamide). The choice of combination therapy with radiation, cyclophosphamide, or both will be based on investigator assessment of the best choice of therapy for an individual patient in consultation with the sponsor. To be enrolled in a radiotherapy cohort, a patient must have a lesion that can be safely irradiated and for which

radiation at the limited, palliative doses contemplated would be considered medically appropriate, and at least one other lesion suitable for response evaluation. A patient will be allowed to enroll only if a slot is available in the cohort for the chosen treatment.

[0250] Patients will undergo screening procedures to determine eligibility within 28 days prior to the initial administration of mAb. Following enrollment of patients into a mAb monotherapy cohort, enrollment of subsequent cohorts will be determined by occurrence of DLTs in prior cohorts (*i.e.*, no DLT in a cohort of 3 patients, or no more than 1 DLT in an expanded cohort of 6 patients), and the availability of patient slots. The planned monotherapy dose levels are 1, 3, or 10 mg/kg administered IV every 14 days (2 weeks).

[0251] Once one or both of the 1 mg/kg or 3 mg/kg mAb monotherapy cohort DLT observation periods are completed without a DLT in a cohort of 3 patients or with no more than 1 DLT in an expanded cohort of 6 patients, patients can be enrolled into a cohort combining cyclophosphamide or radiotherapy with mAb at that monotherapy dose level. Patients can be enrolled into a combination mAb + cyclophosphamide/radiotherapy cohort once the DLT observation periods for both the cohort for that mAb dose level + cyclophosphamide and the cohort for that mAb dose level + the same radiotherapy regimen are completed with no DLT in a cohort of 3 patients, or no more than 1 DLT in an expanded cohort of 6 patients.

[0252] Once the 3 mg/kg mAb monotherapy cohort DLT observation period is completed with no DLT in a cohort of 3 patients, or no more than 1 DLT in an expanded cohort of 6 patients, a 10 mg/kg mAb monotherapy cohort may also enroll.

[0253] mAb 3 mg/kg and 10 mg/kg monotherapy cohorts will enroll only after the requisite number of patients in the prior monotherapy dose cohort (ie, 1 mg/kg and 3 mg/kg, respectively) have cleared the 28 day DLT observation period without a maximum tolerated dose (MTD) being demonstrated for that dose level. A mAb 1 mg/kg combination treatment cohort will enroll only after completion of the DLT observation period for the 1 mg/kg monotherapy cohort. Combination cohorts receiving 3 mg/kg mAb will enroll only when the requisite number of patients in the respective 1 mg/kg mAb combination cohorts has cleared the DLT observation period without demonstrating a MTD. Triple combination cohorts combining mAb with cyclophosphamide and a radiation regimen will enroll only when the requisite number of patients in both corresponding double combination cohorts at that dosage level have cleared the DLT observation period without a MTD being demonstrated.

[0254] Table 30 summarizes the dose-escalation cohorts in which patients will be enrolled.

Table 30: Possible Dose-escalation Cohorts

n	Possible Assigned Treatment Cohort
3–6	0.3 mg/kg mAb monotherapy ***(to be enrolled only if MTD < 1 mg/kg mAb)***
3–6	1 mg/kg mAb monotherapy
3–6	3 mg/kg mAb monotherapy[a)]
3–6	10 mg/kg mAb monotherapy[b)]
3–6	1 mg/kg[a)] mAb + radiotherapy (6 Gy × 5)
3–6	1 mg/kg[a)] mAb + radiotherapy (9 Gy × 3)
3–6	3 mg/kg[b)] (or MTD) mAb + cyclophosphamide
3–6	3 mg/kg[b)] (or MTD) mAb + radiotherapy (6 Gy × 5)
3–6	3 mg/kg[b)] (or MTD) mAb + radiotherapy (9 Gy × 3)
3–6	3 mg/kg[b)] (or MTD) mAb + radiotherapy (6 Gy × 5) + cyclophosphamide
3–6	3 mg/kg[b)] (or MTD) mAb + radiotherapy (9 Gy × 3) + cyclophosphamide

[0255] A DLT is defined as any of the following: a non-hematologic toxicity (*e.g.*, uveitis, or any other irAE), or a hematologic toxicity (*e.g.*, neutropenia, thrombocytopenia, febrile neutropenia).

[0256] The maximum tolerated dose (MTD) is defined as the highest dose at which fewer than a third of an expanded cohort of 6 patients experience a DLT during the first cycle of treatment. Thus, the MTD is defined as the dose level immediately below the level at which dosing is stopped due to the occurrence of 2 or more DLTs in an expanded cohort of 6 patients. If dose escalation is not stopped due to the occurrence of DLTs, it will be considered that the MTD has not been determined. It is possible that an MTD may not be defined in this study, either for a monotherapy group or for individual combination groups. Additionally, it is possible that mAb MTDs may differ between monotherapy and each combination treatment regimen.

Study Duration

[0257] Patients will receive up to 48 weeks of treatment, after which there will be a 24 week follow-up period. A patient will receive treatment until the 48 week treatment period is complete, or until disease progression, unacceptable toxicity, withdrawal of consent, or meeting of another study withdrawal criterion. After a minimum of 24 weeks of treatment, patients with confirmed complete responses (CR) may elect to discontinue treatment and continue with all relevant study assessments (eg, efficacy assessments). After a minimum of 24 weeks of treatment, patients with tumor burden assessments of stable disease (SD) or partial response (PR) that have been unchanged for 3 successive tumor evaluations may also elect to discontinue treatment and continue with all relevant study assessments (*e.g.*, efficacy assessments).

Study Population

[0258] The target population for this study comprises patients with advanced malignancies who are not candidates for standard therapy, unwilling to undergo standard therapy, or for whom no available therapy is expected to convey clinical benefit; and patients with malignancies that are incurable and have failed to respond to or showed tumor progression despite standard therapy.

[0259] Inclusion criteria: A patient must meet with the following criteria to be eligible for

inclusion in the study: (1) demonstrated progression of a solid tumor with no alternative standard-of-care therapeutic option available; (2) at least 1 lesion for response assessment. Patients assigned to radiotherapy require at least one additional lesion that can be safely irradiated while sparing the index lesions and for which radiation at the limited, palliative doses contemplated would be considered medically appropriate; (3) Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1; (4) more than 18 years old; (5) hepatic function: a. total bilirubin ≤ 1.5x upper limit of normal (ULN; if liver metastases ≤ 3x ULN), b. transaminases ≤ 3x ULN (or ≤5.0x ULN, if liver metastases), c. alkaline phosphatase (ALP) ≤2.5x ULN (or 5.0x ULN, if liver metastases); (6) renal function: serum creatinine ≤ 1.5x ULN; (7) neutrophil count (ANC) ≥ 1.5 x 10^9/L, c. platelet count ≥ 75 x 10^9/L; (8) ability to provide signed informed consent; and (9) ability and willingness to comply with scheduled visits, treatment plans, laboratory tests, and other study-related procedures.

[0260] Exclusion criteria: A patient who meets any of the following criteria will be excluded from the study: (1) Ongoing or recent (within 5 years) evidence of significant autoimmune disease that required treatment with systemic immunosuppressive treatments, which may suggest risk for irAEs; (2) Prior treatment with an agent that blocks the PD-1/PD-L1 pathway; (3) Prior treatment with other immune modulating agents within fewer than 4 weeks or 4 half-lives, whichever is greater, prior to the first dose of mAb; (4) Examples of immune modulating agents include blockers of CTLA-4, 4-1BB (CD137), OX-40, therapeutic vaccines, or cytokine treatments; (5) Untreated brain metastasis (es) that may be considered active. Patients with previously treated brain metastases may participate provided they are stable (ie, without evidence of progression by imaging for at least 4 weeks prior to the first dose of study treatment, and any neurologic symptoms have returned to baseline), and there is no evidence of new or enlarging brain metastases; (6) Immunosuppressive corticosteroid doses (>10 mg prednisone daily or equivalent) within 4 weeks prior to the first dose of mAb; (7) Deep vein thrombosis, pulmonary embolism (including asymptomatic pulmonary embolism identified on imaging), or other thromboembolic event within the 6 months preceding the first dose of mAb; (8) Active infection requiring therapy, including known infection with human immunodeficiency virus, or active infection with hepatitis B or hepatitis C virus; (9) History of pneumonitis within the last 5 years; (10) Any investigational or antitumor treatment within 30 days prior to the initial administration of mAb; (11) History of documented allergic reactions or acute hypersensitivity reaction attributed to treatment with antibody therapies in general, or to agents specifically used in the study; (12) Known allergy to doxycycline or tetracycline (precaution due to presence of trace components in mAb); (13) Breast-feeding; (14) Positive serum pregnancy test; (15) History within the last 5 years of an invasive malignancy other than the one treated in this study, with the exception of resected/ablated basal or squamous-cell carcinoma of the skin or carcinoma in situ of the cervix, or other local tumors considered cured by local treatment; (16) Acute or chronic psychiatric problems that, under the evaluation of the investigator, make the patient

ineligible for participation; and (17) Continued sexual activity in men or women of childbearing potential who are unwilling to practice adequate contraception during the study.

Study Treatments

[0261] mAb will be supplied as a liquid in sterile, single-use vials. Each vial will contain a volume sufficient to withdraw 10 mL of mAb at a concentration of 25 mg/mL. Instructions on dose preparation are provided in the study reference manuals. mAb will be administered in an outpatient setting as a 30 minute IV infusion. Each patient's dose will depend on individual body weight. The dose of mAb must be adjusted each cycle for changes in body weight of ≥10%. mAb will be administered alone and in combination with radiation and or cyclophosphamide.

Monotherapy

[0262] mAb will be administered in an outpatient setting by IV infusion over 30 minutes every 14 days for 48 weeks (ie, Days 1, 15±3, 29±3, and 43±3 of a 56 day cycle). Planned monotherapy regimens to be assigned may include: (i) 1 mg/kg IV infusion over 30 minutes every 14 days for 48 weeks; (ii) 3 mg/kg infusion over 30 minutes every 14 days for 48 weeks; (iii) 10 mg/kg infusion over 30 minutes every 14 days for 48 weeks; and (iv) 0.3 mg/kg infusion over 30 minutes every 14 days for 48 weeks (if MTD is determined to be below 1 mg/kg).

Combination Therapy

[0263] Concomitant radiation therapy and cyclophosphamide will be supplied through a prescription and their usage, dose, dose modifications, reductions, or delays, as well as any potential AEs resulting from their use, will be tracked along with that of mAb.

[0264] **Co-administration of mAb and radiation**: mAb will be administered by IV infusion over 30 minutes every 14 days for 48 weeks in combination with radiation treatment from day 8 to day 12. Planned combination mAb and radiation therapy regimens may include:

- 1 mg/kg mAb infusion over 30 minutes every 14 days for 48 weeks plus 30 Gy radiotherapy (6 Gy × 5 times/week; given 1 week after the first dose of mAb, preferably on consecutive days)
- 1 mg/kg mAb infusion over 30 minutes every 14 days for 48 weeks plus 27 Gy radiotherapy (9 Gy × 3 times/week; given 1 week after the first dose of mAb, preferably not on consecutive days)
- 3 mg/kg mAb infusion over 30 minutes every 14 days for 48 weeks plus 30 Gy radiotherapy (6 Gy × 5 times/week; given 1 week after the first dose of mAb, preferably on consecutive days)
- 3 mg/kg mAb infusion over 30 minutes every 14 days for 48 weeks plus 27 Gy radiotherapy (9 Gy × 3 times/week; given 1 week after the first dose of mAb, preferably not on consecutive days)

[0265] Patients will receive either 30 Gy given as 5 fractions of 6 Gy administered daily starting 1 week after the first dose of mAb, or 27 Gy given as 3 fractions of 9 Gy administered

every other day starting 1 week after the first dose of mAb. The lesion selected for radiation should be a lesion that can be safely irradiated with focal irradiation while sparing the index lesion(s), and for which radiation at the limited, palliative doses contemplated would be considered medically appropriate. The target dose for a patient will be based on cohort assignment and should conform to the normal tissue requirements, in accord with standard radiation oncology practice. Treatment at the protocol-specified dosing regimen is permitted only if the normal tissue criteria are met. If the normal tissue criteria cannot be met at either of the radiation therapy regiments specified in the protocol, the patient is not eligible for enrollment in a combination radiation treatment cohort in this study.

[0266] **Co-administration of mAb and cyclophosphamide**: mAb will be administered by IV infusion over 30 minutes every 14 days (2 weeks) for 48 weeks in combination with cyclophosphamide 200 mg/m^2 every 14 days for 4 doses. Each of the 4 cyclophosphamide doses will be administered 1 day before each of the first 4 mAb doses (days –1, 14, 28, and 42 of the first 56 day cycle).

[0267] Though cyclophosphamide has been used successfully concurrently with other drugs, the rate of metabolism and the leukopenic activity of cyclophosphamide reportedly are increased by chronic administration of high doses of phenobarbital. Cyclophosphamide treatment causes a marked and persistent inhibition of cholinesterase activity, thus potentiating the effect of succinylcholine chloride. The planned combination mAb and cyclophosphamide regimen to be assigned is:

- Cyclophosphamide 200 mg/m^2 every 14 days (days –1, 14, 28, and 42 of the first 56 day cycle) for a total of 4 doses plus
- 3 mg/kg mAb infusion over 30 minutes every 14 days for 48 weeks (provided monotherapy dose of 3 mg/kg < MTD; if 3 mg/kg > MTD, dose will be 1 mg/kg).

[0268] **Co-administration of mAb, radiation and cyclophosphamide**: The planned combination mAb, radiation, and cyclophosphamide regimen includes:

- Cyclophosphamide 200 mg/m^2 every 14 days (days –1, 14, 28, and 42 of the first 56 day cycle) for a total of 4 doses
  plus
- 27 Gy radiotherapy (9 Gy × 3 times/week; given 1 week after the first dose of mAb, preferably not on consecutive days) OR
  30 Gy radiotherapy (6 Gy × 5 times/week; given 1 week after the first dose of mAb, preferably on consecutive days)
  plus
- 3 mg/kg mAb infusion over 30 minutes every 14 days for 48 weeks (provided monotherapy dose of 3 mg/kg < MTD; if 3 mg/kg > MTD, dose will be 1 mg/kg)

Study Variables

[0269] Primary Variables: Primary safety variables include incidence of DLTs, incidence and severity of treatment-emergent adverse events (TEAEs), and abnormal laboratory findings through 48 weeks of treatment.

[0270] Secondary Variables: Key secondary variables include the following:

- Serum concentration and pharmacokinetics (PK) of mAb
- Antitumor activities assessed using the appropriate criteria for the indication:
    - Response Evaluation Criteria in Solid Tumors (RECIST) criteria measured by computed tomography (CT) or magnetic resonance imaging (MRI)
    - Other assessment criteria should also be used for specific tumors in which RECIST measurements are not the standard.
    - Immune-Related Response Criteria (irRC) applied to RECIST measurements.

  In all cases, irRC will be the governing tool to determine progression of disease (PD), SD, CR, or PR. Standard RECIST data will also be collected for information purposes.
- Anti-mAb antibodies

Study Procedures

[0271] The following procedures will be performed at screening for the purpose of determining study eligibility or characterizing the baseline population: (i) serum β-HCG (result must be ≤72 hours before first dose); (ii) Collection of archived tumor material: After a patient has given informed consent, the patient will be asked to arrange to provide any available previously collected tumor samples; (iii) Brain MRI: Brain MRI is required at screening if not performed in the prior 60 days; and (iv) Chest x-ray: Chest is x-ray required at screening if not performed in the prior 60 days.

[0272] Efficacy Procedures: A CT or MRI for tumor assessment will be performed at the screening visit (within 28 days prior to infusion) and during every cycle (approximately every 8 weeks) on day 56±3, and when disease progression is suspected. Additionally, for patients who have not progressed on study, tumor assessment will be performed for follow-up visits 3, 5, and 7. Once the choice has been made to use CT scan or MRI, subsequent assessments will be made using the same modality.

[0273] Tumor response evaluation will be performed according to immune-related response criteria (irRC; Nishino 2013). Assessments according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (Eisenhauer 2009) will also be performed as a supportive exploration; however, the primary determination of disease progression for an individual patient will be made according to irRC. Measurable lesions selected as target lesions for RECIST assessments will also be included as index lesions for irRC assessments.

[0274] Safety Procedures: Vital signs, including temperature, resting blood pressure, pulse, and respiration, will be collected. When scheduled at the same visit as other procedures, vital signs should be measured prior to clinical laboratory assessments, PK, or exploratory sample

collection. During cycle 1, vital signs will be recorded on treatment days prior to treatment, at the end of the infusion, every 30 minutes for the first 4 hours post-infusion, and at 6 and 8 hours post study drug administration. On subsequent cycles, vital signs on treatment days will be assessed and documented prior to the infusion, every 30 minutes for the first 2 hours, and then hourly until 4 hours following study drug administration.

[0275] A thorough complete or limited physical examination will be performed at visits. Complete physical examination will include examination of skin, head, eyes, nose, throat, neck, joints, lungs, heart, pulse, abdomen (including liver and spleen), lymph nodes, and extremities, as well as a brief neurologic examination. Limited physical examination will include lungs, heart, abdomen, and skin.

[0276] A standard 12-lead ECG will be performed. Any ECG finding that is judged by the investigator as a clinically significant change (worsening) compared to the baseline value will be considered an AE, recorded, and monitored.

[0277] Immune safety assays consist of rheumatoid factor (RF), thyroid stimulating hormone (TSH), C-reactive protein (CRP), and antinuclear antibody (ANA) titer and pattern. If, during the course of the study, a 4-fold or greater increase from baseline in RF or ANA or abnormal levels of TSH or CRP are observed, the following tests may also be performed: anti-DNA antibody, anti-Sjögren's syndrome A antigen (SSA) antibody (Ro), anti-Sjögren's syndrome B antigen (SSB) antibody (La), antithyroglobulin antibody, anti-LKM antibody, antiphospholipid antibody, anti-islet cell antibody, antineutrophil cytoplasm antibody, C3, C4, CH50. Activated partial thromboplastin time (aPTT) and International Normalized Ratio (INR) will be analyzed by the site's local laboratory.

Safety

[0278] An adverse event (AE) is any untoward medical occurrence in a patient administered a study drug which may or may not have a causal relationship with the study drug. Therefore, an AE is any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease which is temporally associated with the use of a study drug, whether or not considered related to the study drug. An AE also includes any worsening (ie, any clinically significant change in frequency and/or intensity) of a pre-existing condition that is temporally associated with the use of the study drug. Progression of underlying malignancy will not be considered an AE if it is clearly consistent with the typical progression pattern of the underlying cancer (including time course, affected organs, etc.). Clinical symptoms of progression may be reported as AEs if the symptom cannot be determined as exclusively due to the progression of the underlying malignancy, or does not fit the expected pattern of progression for the disease under study.

[0279] An serious adverse event (SAE) is any untoward medical occurrence that at any dose results in death, is life-threatening, requires in-patient hospitalization or prolongation of existing

hospitalization, results in persistent or significant disability/incapacity (substantial disruption of one's ability to conduct normal life functions), is a congenital anomaly/birth defect.

[0280] Patient information on all AEs and SAEs will be recorded.

Statistical Plan

[0281] The study dose escalation is based on a traditional 3 + 3 design with 3 to 6 patients assigned per dose level. The exact number of patients enrolled in the study will depend on the number of protocol-defined DLTs observed, and the need to expand currently defined dose levels, or open additional cohorts at lower dose levels. After the required initial enrollment to the next cohort in the dose escalation has occurred, enrollment to each of the previous cohorts below the MTD for that treatment will be expanded (if not previously expanded during escalation) to a total of 6 patients.

[0282] Data will be summarized using descriptive statistics only. In general, data will be summarized by dose levels and combinations. The safety summaries and analyses will be performed on the safety analysis set (SAF). The primary analysis of safety will be based on treatment-emergent AEs (TEAEs).

[0283] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

What is claimed is:

1. An isolated antibody or antigen-binding fragment thereof that competes for binding to human programmed death-1 (PD-1) protein with a reference antibody or antigen-binding fragment thereof comprising the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR), wherein the HCVR has an amino acid sequence selected from the group consisting of HCVR sequences listed in Table 1; and the CDRs of a light chain variable region (LCVR), wherein the LCVR has an amino acid sequence selected from the group consisting of LCVR sequences listed in Table 1, and wherein the antibody has one or more of the following properties:

(a) blocks human PD-1 protein binding to PD-L1 with an IC_{50} of less than 3 nM as measured in a competition sandwich ELISA assay at 25°C;

(b) binds monomeric human PD-1 with a binding dissociation equilibrium constant (K_D) of less than about 50 nM as measured in a surface plasmon resonance assay at 37°C;

(c) binds monomeric human PD-1 with a K_D less than about 12 nM in a surface plasmon resonance assay at 25°C;

(d) binds monomeric cynomolgus PD-1 with a K_D less than about 8.5 nM in a surface plasmon resonance assay at 25°C;

(e) binds monomeric human PD-1 with a dissociative half-life (t½) of greater than about 6.3 minutes as measured in a surface plasmon resonance assay at 25°C; and

(f) binds monomeric human PD-1 with a dissociative half-life (t½) of greater than about 0.9 minutes as measured in a surface plasmon resonance assay at 37°C.

2. The isolated antibody or antigen-binding fragment of claim 1, wherein the reference antibody or antigen-binding fragment thereof comprises an HCVR/LCVR amino acid sequence pair as set forth in Table 1.

3. The isolated antibody or antigen-binding fragment of claim 2, wherein the reference antibody comprises an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 130/138, 162/170, 234/202, and 314/186.

4. An isolated human monoclonal antibody or antigen-binding fragment thereof that binds specifically to human PD-1, wherein the antibody or antigen-binding fragment thereof comprises three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within any one of the HCVR sequences listed in Table 1; and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the LCVR sequences listed in Table 1.

5. The isolated antibody or antigen-binding fragment thereof of claim 4, comprising a HCVR having an amino acid sequence selected from the group consisting of HCVR sequences listed in Table 1.

6. The isolated antibody or antigen-binding fragment thereof of either claim 4 or 5, comprising a LCVR having an amino acid sequence selected from the group consisting of LCVR sequences listed in Table 1.

7. The isolated antibody or antigen-binding fragment thereof of any one of claims 4 – 6, comprising: (a) a HCVR having an amino acid sequence selected from the group consisting of HCVR sequences listed in Table 1; and (b) a LCVR having an amino acid sequence selected from the group consisting of LCVR sequences listed in Table 1.

8. The isolated antibody or antigen-binding fragment thereof of any one of claims 1 – 7, comprising:

(a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 220, 228, 236, 244, 252, 260, 268, 276, 284, 292, 300, 308, and 316;

(b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 222, 230, 238, 246, 254, 262, 270, 278, 286, 294, 302, 310, and 318;

(c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 224, 232, 240, 248, 256, 264, 272, 280, 288, 296, 304, 312, and 320;

(d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, and 204;

(e) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, and 206; and

(f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, and 208.

9. An isolated antibody or antigen-binding fragment thereof that blocks PD-1 binding to PD-L1 comprising the CDRs of a HCVR, wherein the HCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 34, 50, 66, 82, 114, 130, 162, 178, 194, 210, 218, 226, 234, 242, 258, 266, 274, 282, 290, 298, 306 and 314; and the CDRs of a LCVR,

wherein the LCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 26, 42, 58, 74, 90, 122, 138, 170, 186, and 202.

10. The isolated antibody or antigen-binding fragment of claim 9, comprising a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 130/138, 162/170, 234/202, and 314/186.

11. An isolated antibody or antigen-binding fragment thereof that binds human PD-1, wherein the antibody or antigen-binding fragment thereof enhances PD-1 binding to PD-L1, as measured by a competition sandwich ELISA assay at 25°C.

12. The isolated antibody or antigen-binding fragment thereof of claim 11, wherein the antibody comprises the CDRs of a HCVR, wherein the HCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 98, and 250; and the CDRs of a LCVR, wherein the LCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 106, and 202.

13. The isolated antibody or antigen-binding fragment thereof of claim 12, wherein the antibody comprises an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 98/106, and 250/202.

14. The antibody or antigen-binding fragment thereof of any one of claims 1 – 13, wherein the antibody is a multi-specific antigen-binding molecule.

15. A pharmaceutical composition comprising an isolated antibody or antigen-binding fragment thereof that binds to PD-1 according to any one of claims 1 – 14 and a pharmaceutically acceptable carrier or diluent.

16. An isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a HCVR of an antibody as set forth in any one of claims 1 – 13.

17. An isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a LCVR of an antibody as set forth in any one of claims 1 – 13.

18. A vector comprising the polynucleotide sequence of claim 16 or 17.

19. A cell expressing the vector of claim 18.

20. A multi-specific antigen-binding molecule or antigen-binding fragment thereof comprising a first antigen-binding specificity that binds specifically to PD-1 and a second antigen-binding specificity that comprises an extracellular domain of PD-L1 or PD-L2, or fragment thereof.

21. A multi-specific antigen-binding molecule or antigen-binding fragment thereof comprising a first antigen-binding specificity that binds specifically to PD-1 and a second antigen-binding specificity that binds specifically to a T-cell co-inhibitor.

22. The multi-specific antigen-binding molecule or fragment thereof of claim 20 or 21, wherein the first antigen-binding specificity comprises three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within any one of the HCVR sequences listed in Table 1; and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the LCVR sequences listed in Table 1.

23. The multi-specific antigen-binding molecule or fragment thereof of any one of claims 20 – 22, wherein the first antigen-binding specificity comprises a HCVR having an amino acid sequence selected from the group consisting of HCVR sequences listed in Table 1; and a LCVR having an amino acid sequence selected from the group consisting of LCVR sequences listed in Table 1.

24. The multi-specific antigen-binding molecule or fragment thereof of claim 23, wherein the first antigen-binding specificity comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 130/138, 162/170, 234/202, and 314/186.

25. The multi-specific antigen-binding molecule or fragment thereof of claim 21, wherein the second antigen-binding specificity binds specifically to a T-cell co-inhibitor selected from the group consisting of LAG3, TIM3, B7-1, CTLA-4, BTLA, CD28, 2B4, LY108, TIGIT, ICOS, and CD160.

26. The multi-specific antigen-binding molecule or fragment thereof of any one of claims 20 – 25 for use in the treatment of a cancer selected from the group consisting of renal cell carcinoma, colorectal cancer, ovarian cancer, prostate cancer, breast cancer, colon cancer, non-small-cell lung cancer and melanoma.

27. The multi-specific antigen-binding molecule or fragment thereof any one of claims 20 – 25 for use in the treatment of a chronic viral infection caused by a virus selected from the group consisting of human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), human papilloma virus (HPV), lymphocytic choriomeningitis virus (LCMV), and simian immunodeficiency virus (SIV).

28. A multi-specific antigen-binding molecule or fragment thereof comprising a first antigen-binding specificity that binds specifically to PD-1 and a second antigen-binding specificity that binds specifically to an antigen selected from the group consisting of an autoimmune-tissue-specific antigen, a T-cell receptor and an Fc receptor.

29. The multi-specific antigen-binding molecule or fragment thereof of claim 28, wherein the first antigen-binding specificity comprises three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within any one of the HCVR sequences listed in Table 1; and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the LCVR sequences listed in Table 1.

30. The multi-specific antigen-binding molecule or fragment thereof of claim 28 or 29, wherein the first antigen-binding specificity comprises a HCVR having an amino acid sequence selected from the group consisting of HCVR sequences listed in Table 1; and a LCVR having an amino acid sequence selected from the group consisting of LCVR sequences listed in Table 1.

31. The multi-specific antigen-binding molecule or fragment thereof of claim 30, wherein the first antigen-binding specificity comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 130/138, 162/170, 234/202, and 314/186.

32. The multi-specific antigen-binding molecule or fragment thereof of claim 28, wherein the first antigen-binding specificity comprises the extracellular domain of PD-L1 and/or PD-L2 or fragment thereof.

33. The multi-specific antigen-binding molecule or fragment thereof of any one of claims 28 – 32, wherein the second antigen-binding specificity binds specifically to an autoimmune-tissue-specific antigen.

34. The multi-specific antigen-binding molecule or fragment thereof of claim 33, wherein the autoimmune-tissue-specific antigen is associated with alopecia areata, autoimmune hepatitis, celiac disease, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, hemolytic anemia, inflammatory bowel disease, inflammatory myopathies, multiple sclerosis, primary biliary cirrhosis, psoriasis, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic lupus erthyematosus, vitiligo, autoimmune pancreatitis, autoimmune urticaira, autoimmune thrombocytopenic purpura, Crohn's disease, diabetes type I, eosinophilic fasciitis, eosinophilic enterogastritis, Goodpasture's syndrome, myasthenia gravis, psoriatic arthritis, rheumatic fever, ulcerative colitis, vasculitis or Wegener's granulomatosis.

35. The multi-specific antigen-binding molecule or fragment thereof of any one of claims 28 – 32, wherein the second antigen-binding specificity binds specifically to one of a T-cell receptor, Fcα receptor, Fcγ receptor, or CD19.

36. The multi-specific antigen-binding molecule or fragment thereof of any one of claims 28 – 35 for use in the treatment of an autoimmune disease or disorder selected from the group consisting of alopecia areata, autoimmune hepatitis, celiac disease, Graves' disease, Guillain-

Barre syndrome, Hashimoto's disease, hemolytic anemia, inflammatory bowel disease, inflammatory myopathies, multiple sclerosis, primary biliary cirrhosis, psoriasis, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic lupus erthyematosus, vitiligo, autoimmune pancreatitis, autoimmune urticaria, autoimmune thrombocytopenic purpura, Crohn's disease, diabetes type I, eosinophilic fasciitis, eosinophilic enterogastritis, Goodpasture's syndrome, myasthenia gravis, psoriatic arthritis, rheumatic fever, ulcerative colitis, vasculitis and Wegener's granulomatosis.

37. A method of enhancing an immune response in a subject, the method comprising administering a pharmaceutical composition comprising an isolated antibody or antigen-binding fragment thereof according to any one of claims 1 – 13; or a multi-specific antigen-binding molecule or fragment thereof according to any one of claims 20 – 25.

38. A method of inhibiting a T-regulatory (Treg) cell in a subject comprising administering a pharmaceutical composition comprising an isolated human antibody or antigen-binding fragment thereof according to any one of claims 1 – 13; or a multi-specific antigen-binding molecule or fragment thereof according to any one of claims 20 – 25.

39. A method of enhancing T-cell activation in a subject, the method comprising administering a pharmaceutical composition comprising an antibody or antigen-binding fragment thereof according to any one of claims 1 – 13; or a multi-specific antigen-binding molecule or fragment thereof according to any one of claims 20 – 25.

40. The method of any one of claims 37 – 39, wherein the subject has a disease or disorder selected from the group consisting of brain cancer, renal cell carcinoma, ovarian cancer, prostate cancer, colon cancer, non-small-cell lung cancer, squamous cell carcinoma of head and neck, colorectal cancer, and melanoma.

41. The method of any one of claims 37 – 39, wherein the subject has a chronic viral infection caused by a virus selected from the group consisting of HIV, HCV, HBV, HPV, LCMV and SIV.

42. A method of inhibiting growth of a tumor or a tumor cell comprising contacting the tumor or tumor cell with a therapeutically effective amount of the antibody of any one of claims 1 – 13 or 20 – 25.

43. A method of inhibiting T-cell activation in a subject, the method comprising administering a therapeutically effective amount of an antibody or antigen-binding fragment thereof according to any one of claims 1 – 13; or a multi-specific antigen-binding molecule or fragment thereof according to any one of claims 28 – 35.

44. The method of claim 43, wherein the subject has an autoimmune disease or disorder selected from the group consisting of alopecia areata, autoimmune hepatitis, celiac disease, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, hemolytic anemia, inflammatory bowel disease, inflammatory myopathies, multiple sclerosis, primary biliary cirrhosis, psoriasis, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic lupus erthyematosus, vitiligo, autoimmune pancreatitis, autoimmune urticaria, autoimmune thrombocytopenic purpura, Crohn's disease, diabetes type I, eosinophilic fasciitis, eosinophilic enterogastritis, Goodpasture's syndrome, myasthenia gravis, psoriatic arthritis, rheumatic fever, ulcerative colitis, vasculitis and Wegener's granulomatosis.

45. The method of any one of claims 37 – 44, wherein the antibody or antigen-binding fragment thereof, or the pharmaceutical composition comprising the antibody or antigen-binding fragment thereof, is administered to the subject in combination with a second therapeutic agent.

46. The method of claim 45, wherein the second therapeutic agent is selected from the group consisting of a NSAID, a corticosteroid, an antibody to a different T-cell co-inhibitor, an antibody to a tumor specific antigen, an antibody to an autoimmune tissue antigen, an antibody to a virally-infected-cell antigen, an antibody to PD-L1, a dietary supplement such an antioxidant, a VEGF antagonist, a chemotherapeutic agent, a cytotoxic agent, an anti-viral drug, radiation, and any other therapy useful for ameliorating at least one symptom associated with the disease or disorder.

47. The method of any one of claims 37 – 46, wherein the antibody or antigen-binding fragment thereof is administered subcutaneously, intravenously, intradermally, intraperitoneally, orally, intramuscularly or intracranially.

48. The method of any one of claims 37 – 47, wherein the antibody or antigen-binding fragment is administered at a dose of about 0.1 mg/kg of body weight to about 60 mg/kg of body weight of the subject.

49. The isolated antibody or antigen-binding fragment of claim 1, wherein the antibody or antigen-binding fragment thereof competes for binding to human PD-1 with a reference antibody or antigen-binding fragment thereof comprising a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 330, 332, 334 and 336.

50. The isolated antibody or antigen-binding fragment of claim 1, wherein the antibody or antigen-binding fragment thereof competes for binding to human PD-1 with a reference antibody or antigen-binding fragment thereof comprising a heavy chain and a light chain,

wherein the light chain chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 331, 333, 335 and 337.

51. The isolated antibody or antigen-binding fragment of claim 1, wherein the antibody or antigen-binding fragment thereof competes for binding to human PD-1 with a reference antibody or antigen-binding fragment thereof comprising a heavy chain/light chain amino acid sequence pair selected from the group consisting of SEQ ID NOs: 330/331, 332/333, 334/335 and 336/337.

52. A pharmaceutical composition comprising an isolated monoclonal antibody or antigen-binding fragment thereof that binds PD-1 according to any one of claims 49 – 51, and a pharmaceutically acceptable carrier or diluent.

53. An isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a heavy chain of an antibody as set forth in any one of claims 49 – 51.

54. An isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a light chain of an antibody as set forth in any one of claims 49 – 51.

55. A vector comprising the polynucleotide sequence of claim 53 or 54.

56. A cell expressing the vector of claim 55.

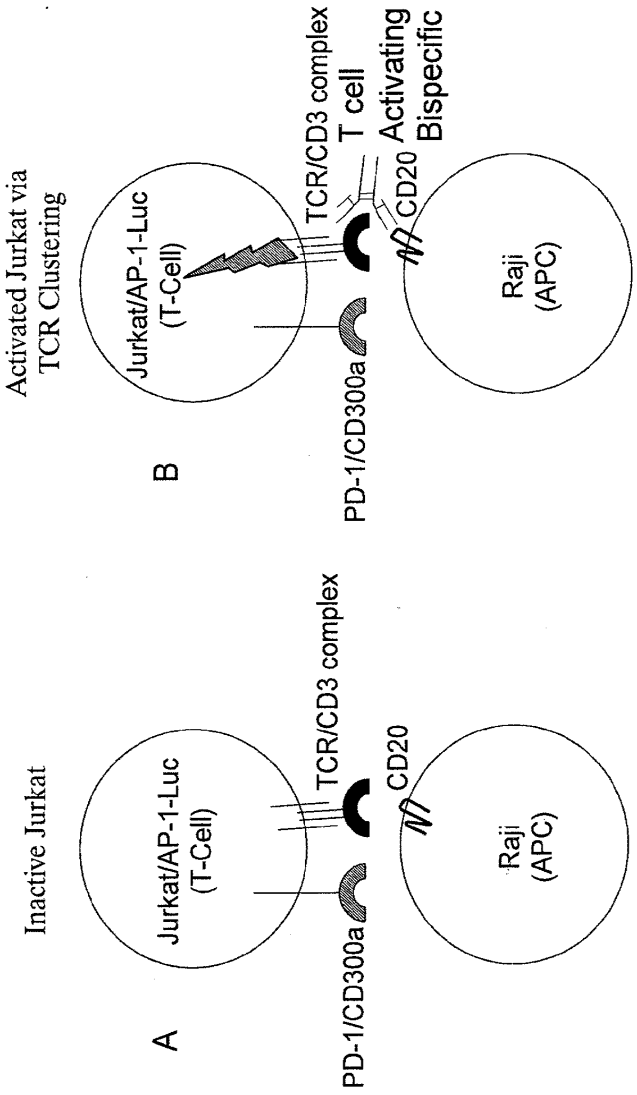


FIG. 1

EX1002 - Page 962

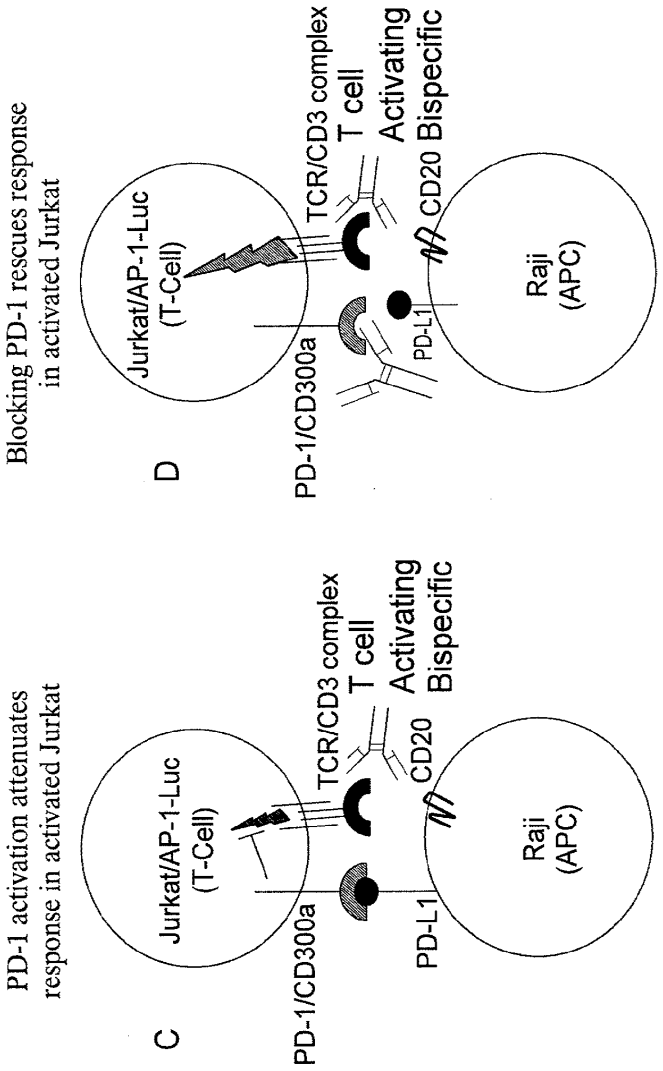


FIG. 1 (Contd.)

EX1002 - Page 963

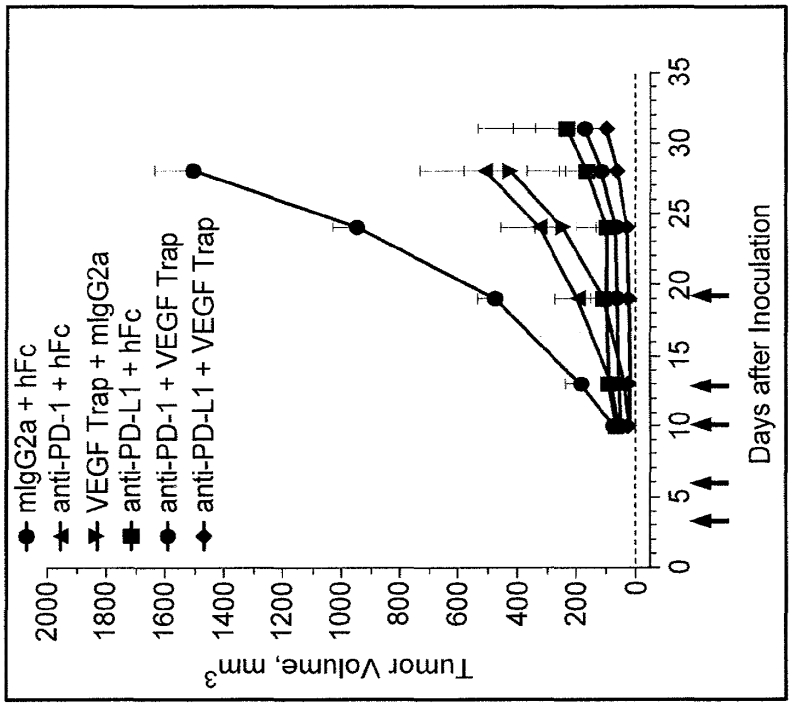

mIgG2a + hFc
anti-PD-1 + hFc
VEGF Trap + mIgG2a
anti-PD-L1 + hFc
anti-PD-1 + VEGF Trap
anti-PD-L1 + VEGF Trap
Tumor Volume, mm3
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
35
Days after Inoculation

FIG. 2

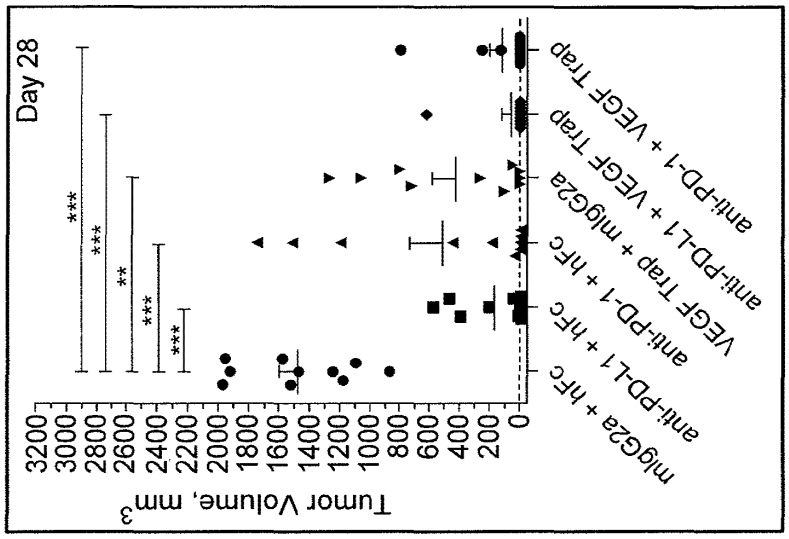

Day 28
Tumor Volume, mm3
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
***
***
**
***
***
mIgG2a + hFc
anti-PD-L1 + hFc
anti-PD-1 + hFc
VEGF Trap + mIgG2a
anti-PD-L1 + VEGF Trap
anti-PD-1 + VEGF Trap

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/012589

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, SCISEARCH, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/121168 A1 (ONO PHARMACEUTICAL CO [JP]; MEDAREX INC [US]; KORMAN ALAN J; SRINIVASA) 16 November 2006 (2006-11-16) examples 4-6,11-22 page 49 - page 51 page 52 - page 57 page 59 - page 73; claim 50 ----- -/--	1-3, 14-19, 37-40, 42,45-56

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 July 2015	10/07/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Domingues, Helena

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/110621 A1 (UCB PHARMA SA [BE]; TYSON KERRY LOUISE [GB]) 15 September 2011 (2011-09-15) figure 13; examples 2,4 page 12, line 25 - line 27 page 20 - page 21 claims 23,24 page 25, line 13 - line 15 page 3, line 15 - line 21	1,2,11, 14-19, 37,43, 45,47
Y	WO 2004/056875 A1 (WYETH CORP [US]; CAMBRIDGE ANTIBODY TECH [GB]; COLLINS MARY [US]; WOOD) 8 July 2004 (2004-07-08) page 1, paragraph 10; example 6	1,2,4-8, 11-19, 37-56
A	EP 1 591 527 A1 (ONO PHARMACEUTICAL CO [JP]; HONJO TASUKU [JP]) 2 November 2005 (2005-11-02) figure 3; example 4	1,2,4-8, 11-19, 37-56
Y	KEIR MARY E ET AL: "Programmed death-1 (PD-1):PD-ligand 1 interactions inhibit TCR-mediated positive selection of thymocytes", THE JOURNAL OF IMMUNOLOGY, THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS, US, vol. 175, no. 11, 1 December 2005 (2005-12-01), pages 7372-7379, XP002636167, ISSN: 0022-1767 page 7373, left-hand column, paragraph final	1,2,4-8, 11-19, 37-56
A	RIELLA L V ET AL: "Role of the PD-1 pathway in the immune response.", AMERICAN JOURNAL OF TRANSPLANTATION : OFFICIAL JOURNAL OF THE AMERICAN SOCIETY OF TRANSPLANTATION AND THE AMERICAN SOCIETY OF TRANSPLANT SURGEONS OCT 2012, vol. 12, no. 10, October 2012 (2012-10), pages 2575-2587, XP002738626, ISSN: 1600-6143 page 2584, left-hand column, paragraph final	1,2,4-8, 11-19, 37-56

-/--

2

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	ZORAN GATALICA, CARRIE L. SNYDER, KIMBERLY YEATTS, NIANQING XIAO, DANIEL HOLTERMAN, HENRY T. LYNCH: "Programmed death 1 (PD-1) lymphocytes and ligand (PD-L1) in colorectal cancer and their relationship to microsatellite instability status.", J CLIN ONCOL, vol. 32, no. 5s, 30 May 2014 (2014-05-30), XP0055199884, the whole document -----	1-10, 14-19, 37-56
Y	DA SILVA R MOREIRA: "Anti-PD-1 monoclonal antibody Cancer immunotherapy", DRUGS OF THE FUTURE, vol. 39, no. 1, 1 January 2014 (2014-01-01), pages 15-24, XP055199597, ISSN: 0377-8282 the whole document -----	1-19, 37-56

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/012589

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

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

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

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

see additional sheet

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

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

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

1-10, 14-19, 37-56

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 2, 4-8, 11-19, 37-56(all partially)

   Concerns aspects related to an isolated antibody or antigen-binding fragment thereof that competes for binding to hPD-1 with a reference antibody or antigen-binding fragment thereof listed in Table 1, wherein the antibody is H1M7789N; and to aspects related to H1M7789N per se.

   ---

2. claims: 1, 2, 4-8, 11-19, 37-56(all partially)

   Concerns aspects related to an isolated antibody or antigen-binding fragment thereof that competes for binding to hPD-1 with a reference antibody or antigen-binding fragment thereof listed in Table 1, wherein the antibody is H2M7791N; and to aspects related to H2M7791N per se.

   ---

3. claims: 1, 2, 4-8, 11-19, 37-56(all partially)

   Concerns aspects related to an isolated antibody or antigen-binding fragment thereof that competes for binding to hPD-1 with a reference antibody or antigen-binding fragment thereof listed in Table 1, wherein the antibody is H4H9068P2; and to aspects related to H4H9068P2 per se.

   ---

4. claims: 1, 2, 4-9, 14-19, 37-56(all partially)

   Concerns aspects related to an isolated antibody or antigen-binding fragment thereof that competes for binding to hPD-1 with a reference antibody or antigen-binding fragment thereof listed in Table 1, wherein the antibody is H1M7799N; and to aspects related to H1M7799N per se.

   ---

5. claims: 1, 2, 4-9, 14-19, 37-56(all partially)

   Concerns aspects related to an isolated antibody or antigen-binding fragment thereof that competes for binding to hPD-1 with a reference antibody or antigen-binding fragment thereof listed in Table 1, wherein the antibody is H1M7800N; and to aspects related to H1M7800N per se .

   ---

6. claims: 1, 2, 4-9, 14-19, 37-56(all partially)

   Concerns aspects related to an isolated antibody or antigen-binding fragment thereof that competes for binding to hPD-1 with a reference antibody or antigen-binding fragment thereof listed in Table 1, wherein the antibody is H2M7780N; and to aspects related to H2M7780N per se.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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7. claims: 1, 2, 4-9, 14-19, 37-56(all partially)

Concerns aspects related to an isolated antibody or antigen-binding fragment thereof that competes for binding to hPD-1 with a reference antibody or antigen-binding fragment thereof listed in Table 1, wherein the antibody is H2M7788N; and to aspects related to H2M7788N per se.

---

8. claims: 1, 2, 4-9, 14-19, 37-56(all partially)

Concerns aspects related to an isolated antibody or antigen-binding fragment thereof that competes for binding to hPD-1 with a reference antibody or antigen-binding fragment thereof listed in Table 1, wherein the antibody is H2M7790N; and to aspects related to H2M7790N per se.

---

9. claims: 1, 2, 4-9, 14-19, 37-56(all partially)

Concerns aspects related to an isolated antibody or antigen-binding fragment thereof that competes for binding to hPD-1 with a reference antibody or antigen-binding fragment thereof listed in Table 1, wherein the antibody is H2M7794N; and to aspects related to H2M7794N per se.

---

10. claims: 1-10, 14-19, 37-56(all partially)

Concerns aspects related to an isolated antibody or antigen-binding fragment thereof that competes for binding to hPD-1 with a reference antibody or antigen-binding fragment thereof listed in Table 1, wherein the antibody is H2M7795N; and to aspects related to H2M7795N per se.

---

11. claims: 1, 2, 4-9, 14-19, 37-56(all partially)

Concerns aspects related to an isolated antibody or antigen-binding fragment thereof that competes for binding to hPD-1 with a reference antibody or antigen-binding fragment thereof listed in Table 1, wherein the antibody is H2M7796N; and to aspects related to H2M7796N per se.

---

12. claims: 1-10, 14-19, 37-56(all partially)

Concerns aspects related to an isolated antibody or antigen-binding fragment thereof that competes for binding to hPD-1 with a reference antibody or antigen-binding fragment thereof listed in Table 1, wherein the antibody is H2M7798N; and to aspects related to H2M7798N per se.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

13-19. claims: 1, 2, 4-9, 14-19, 37-56(all partially)

Each invention relates to aspects of an isolated antibody or antigen-binding fragment thereof that competes for binding to hPD-1 with a reference antibody or antigen-binding fragment thereof listed in Table 1, wherein the antibody corresponds to each of the antibodies listed in Table 1 starting with antibody H4H9019P (invention 13) and finishing with antibody H4H9057P2 (invention 19); each invention also relates to aspects of the antibodies per se.

20-26. claims: 1, 2, 4-9, 14-19, 37-56(all partially)

Each invention relates to aspects of an isolated antibody or antigen-binding fragment thereof that competes for binding to hPD-1 with a reference antibody or antigen-binding fragment thereof listed in Table 1, wherein the antibody corresponds to each of the antibodies listed in Table 1 starting with antibody H4xH9119P2 (invention 20) and finishing with antibody H4XH8999P (invention 26); each invention also relates to aspects of the antibodies per se.

---

27. claims: 1-10, 14-19, 37-56(all partially)

Concerns aspects related to an isolated antibody or antigen-binding fragment thereof that competes for binding to hPD-1 with a reference antibody or antigen-binding fragment thereof listed in Table 1, wherein the antibody is H4xH9008P; and to aspects related to H4xH9008Pper se.

---

28. claims: 20(completely); 26, 27, 37-42, 45-48(partially)

Concerns aspects related to a multi-specific antigen-binding molecule or antigen-binding fragment thereof comprising a first antigen-binding specificity that binds specifically to PD-1 and a second antigen-binding specificity that comprises an extracellular domain of PD-L1 or PD-L2, or fragment thereof.

---

29. claims: 21-25(completely); 26, 27, 37-42, 45-48(partially)

Concerns aspects related to a multi-specific antigen-binding molecule or antigen-binding fragment thereof comprising a first antigen-binding specificity that binds specifically to PD-1 and a second antigen-binding specificity that binds specifically to a T-cell co-inhibitor.

---

30. claims: 28-36(completely); 43-48(partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Concerns aspects related to a multi-specific antigen-binding molecule or fragment thereof comprising a first antigen-binding specificity that binds specifically to PD-1 and a second antigen-binding specificity that binds specifically to an antigen selected from the group consisting of an autoimmune-tissue-specific antigen, a T-cell receptor and an Fc receptor.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/012589

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/012589

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

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(30) Priority Data:

61/931,512	24 January 2014 (24.01.2014)	US
62/059,676	3 October 2014 (03.10.2014)	US
62/094,834	19 December 2014 (19.12.2014)	US

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(72) Inventors: **FREEMAN, Gordon, James**; 305 Walnut Street, Brookline, MA 02445 (US). **SHARPE, Arlene, Helen**; 305 Walnut Street, Brookline, MA 02445 (US). **BLATTLER, Walter, A.**; 197 Mason Terrace, Brookline, MA 02446-2768 (US). **MATARAZA, Jennifer, Marie**; 631 Chestnut Hill Avenue, Brookline, MA 02445 (US). **SABATOS-PEYTON, Catherine, Anne**; 17 Perkins Street, Newton, MA 02465 (US). **CHANG, Hwai, Wen**; 1318 Shadow Hills Drive, San Marcos, CA 92069 (US). **FREY, Gerhard, Johann**; 13768 Via Cima Bella, San Diego, CA 92129 (US).

(74) Agent: **LU, Shihua**; Lando & Anastasi LLP, Riverfront Office Park, One Main Street, Suite 1100, Cambridge, MA 02142 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

[Continued on next page]

(54) Title: ANTIBODY MOLECULES TO PD-1 AND USES THEREOF

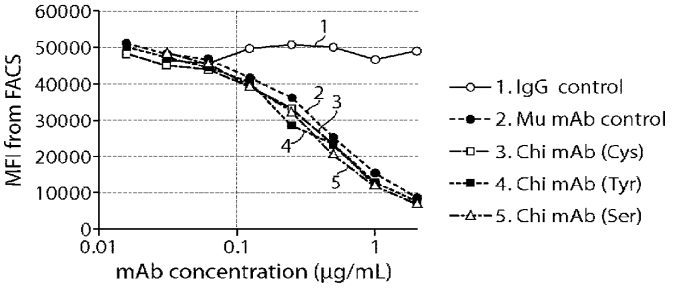


FIGURE 3A

(57) Abstract: Antibody molecules that specifically bind to PD-1 are disclosed. The anti-PD-1 antibody molecules can be used to treat, prevent and/or diagnose cancerous or infectious conditions and disorders.

WO 2015/112900 A1

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

ANTIBODY MOLECULES TO PD-1 AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/931,512, filed January 24, 2014, U.S. Provisional Application No. 62/059,676, filed October 3, 2014, and U.S. Provisional Application No. 62/094,834, filed December 19, 2014, the contents of the aforementioned applications are hereby incorporated by reference in their entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on, January 12, 2015, is named C2160-7000WO_SL.txt and is 191,589 bytes in size.

BACKGROUND

The ability of T cells to mediate an immune response against an antigen requires two distinct signaling interactions (Viglietta, V. *et al.* (2007) *Neurotherapeutics* 4:666-675; Korman, A. J. *et al.* (2007) *Adv. Immunol.* 90:297-339). First, an antigen that has been arrayed on the surface of antigen-presenting cells (APC) is presented to an antigen-specific naive $CD4^+$ T cell. Such presentation delivers a signal via the T cell receptor (TCR) that directs the T cell to initiate an immune response specific to the presented antigen. Second, various co-stimulatory and inhibitory signals mediated through interactions between the APC and distinct T cell surface molecules trigger the activation and proliferation of the T cells and ultimately their inhibition.

The immune system is tightly controlled by a network of costimulatory and co-inhibitory ligands and receptors. These molecules provide the second signal for T cell activation and provide a balanced network of positive and negative signals to maximize immune responses against infection, while limiting immunity to self (Wang, L. *et al.* (Epub Mar. 7, 2011) *J. Exp. Med.* 208(3):577-92; Lepenies, B. *et al.* (2008) *Endocrine, Metabolic & Immune Disorders--Drug Targets* 8:279-288). Examples of costimulatory signals include the binding between the B7.1 (CD80) and B7.2 (CD86) ligands of the APC and the CD28 and CTLA-4 receptors of the $CD4^+$ T-lymphocyte (Sharpe, A. H. *et al.* (2002) *Nature Rev. Immunol.* 2:116-126; Lindley, P. S.

et al. (2009) *Immunol. Rev.* 229:307-321). Binding of B7.1 or B7.2 to CD28 stimulates T cell activation, whereas binding of B7.1 or B7.2 to CTLA-4 inhibits such activation (Dong, C. *et al.* (2003) *Immunolog. Res.* 28(1):39-48; Greenwald, R. J. *et al.* (2005) *Ann. Rev. Immunol.* 23:515-548). CD28 is constitutively expressed on the surface of T cells (Gross, J., *et al.* (1992) *J. Immunol.* 149:380-388), whereas CTLA-4 expression is rapidly up-regulated following T-cell activation (Linsley, P. *et al.* (1996) *Immunity* 4:535-543).

Other ligands of the CD28 receptor include a group of related B7 molecules, also known as the "B7 Superfamily" (Coyle, A. J. *et al.* (2001) *Nature Immunol.* 2(3):203-209; Sharpe, A. H. *et al.* (2002) *Nature Rev. Immunol.* 2:116-126; Collins, M. *et al.* (2005) *Genome Biol.* 6:223.1-223.7; Korman, A. J. *et al.* (2007) *Adv. Immunol.* 90:297-339). Several members of the B7 Superfamily are known, including B7.1 (CD80), B7.2 (CD86), the inducible co-stimulator ligand (ICOS-L), the programmed death-1 ligand (PD-L1; B7-H1), the programmed death-2 ligand (PD-L2; B7-DC), B7-H3, B7-H4 and B7-H6 (Collins, M. *et al.* (2005) *Genome Biol.* 6:223.1-223.7).

The Programmed Death 1 (PD-1) protein is an inhibitory member of the extended CD28/CTLA-4 family of T cell regulators (Okazaki *et al.* (2002) *Curr Opin Immunol* 14: 391779-82; Bennett *et al.* (2003) *J. Immunol.* 170:711-8). Other members of the CD28 family include CD28, CTLA-4, ICOS and BTLA. PD-1 is suggested to exist as a monomer, lacking the unpaired cysteine residue characteristic of other CD28 family members. PD-1 is expressed on activated B cells, T cells, and monocytes.

The PD-1 gene encodes a 55 kDa type I transmembrane protein (Agata *et al.* (1996) *Int Immunol.* 8:765-72). Although structurally similar to CTLA-4, PD-1 lacks the MYPPY motif (SEQ ID NO: 236) that is important for B7-1 and B7-2 binding. Two ligands for PD-1 have been identified, PD-L1 (B7-H1) and PD-L2 (B7-DC), that have been shown to downregulate T cell activation upon binding to PD-1 (Freeman *et al.* (2000) *J. Exp. Med.* 192:1027-34; Carter *et al.* (2002) *Eur. J. Immunol.* 32:634-43). Both PD-L1 and PD-L2 are B7 homologs that bind to PD-1, but do not bind to other CD28 family members. PD-L1 is abundant in a variety of human cancers (Dong *et al.* (2002) *Nat. Med.* 8:787-9).

PD-1 is known as an immunoinhibitory protein that negatively regulates TCR signals (Ishida, Y. *et al.* (1992) *EMBO J.* 11:3887-3895; Blank, C. *et al.* (Epub 2006 Dec. 29) *Immunol. Immunother.* 56(5):739-745). The interaction between PD-1 and PD-L1 can act as an immune

checkpoint, which can lead to, *e.g.*, a decrease in tumor infiltrating lymphocytes, a decrease in T-cell receptor mediated proliferation, and/or immune evasion by cancerous cells (Dong *et al.* (2003) *J. Mol. Med.* 81:281-7; Blank *et al.* (2005) *Cancer Immunol. Immunother.* 54:307-314; Konishi *et al.* (2004) *Clin. Cancer Res.* 10:5094-100). Immune suppression can be reversed by inhibiting the local interaction of PD-1 with PD-L1 or PD-L2; the effect is additive when the interaction of PD-1 with PD-L2 is blocked as well (Iwai *et al.* (2002) *Proc. Nat'l. Acad. Sci.* USA 99:12293-7; Brown *et al.* (2003) *J. Immunol.* 170:1257-66).

Given the importance of immune checkpoint pathways in regulating an immune response, the need exists for developing novel agents that modulate the activity of immunoinhibitory proteins, such as PD-1, thus leading to activation of the immune system. Such agents can be used, *e.g.*, for cancer immunotherapy and treatment of other conditions, such as chronic infection.

SUMMARY

Disclosed herein are antibody molecules *(e.g.*, humanized antibody molecules) that bind to Programmed Death 1 (PD-1) with high affinity and specificity. In one embodiment, the anti-PD-1 antibody molecules comprise a novel combination of framework regions (*e.g.*, FW1, FW2, FW3 and/or FW4), *e.g.*, novel combinations of a heavy chain framework regions and/or light chain framework regions. Nucleic acid molecules encoding the antibody molecules, expression vectors, host cells and methods for making the antibody molecules are also provided. Immunoconjugates, multi- or bispecific antibody molecules and pharmaceutical compositions comprising the antibody molecules are also provided. The anti-PD-1 antibody molecules disclosed herein can be used (alone or in combination with other agents or therapeutic modalities) to treat, prevent and/or diagnose disorders, such as cancerous disorders (*e.g.*, solid and soft-tissue tumors), as well as infectious diseases (*e.g.*, chronic infectious disorders or sepsis). Thus, compositions and methods for detecting PD-1, as well as methods for treating various disorders including cancer and/or infectious diseases, using the anti-PD-1 antibody molecules are disclosed herein.

Accordingly, in one aspect, the invention features an antibody molecule (*e.g.*, an isolated or recombinant antibody molecule) having one or more of the following properties:

(i) binds to PD-1, *e.g.*, human PD-1, with high affinity, *e.g.*, with an affinity constant of at least about 10^7 M^{-1}, typically about 10^8 M^{-1}, and more typically, about 10^9 M^{-1} to 10^{10} M^{-1} or stronger;

(ii) does not substantially bind to CD28, CTLA-4, ICOS or BTLA;

(iii) inhibits or reduces binding of PD-1 to a PD-1 ligand, *e.g.*, PD-L1 or PD-L2, or both;

(iv) binds specifically to an epitope on PD-1, *e.g.*, the same or similar epitope as the epitope recognized by murine monoclonal antibody BAP049 or a chimeric antibody BAP049, *e.g.*, BAP049-chi or BAP049-chi-Y;

(v) shows the same or similar binding affinity or specificity, or both, as any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E;

(vi) shows the same or similar binding affinity or specificity, or both, as an antibody molecule (*e.g.*, an heavy chain variable region and light chain variable region) described in Table 1;

(vii) shows the same or similar binding affinity or specificity, or both, as an antibody molecule (*e.g.*, an heavy chain variable region and light chain variable region) having an amino acid sequence shown in Table 1;

(viii) shows the same or similar binding affinity or specificity, or both, as an antibody molecule (*e.g.*, an heavy chain variable region and light chain variable region) encoded by the nucleotide sequence shown in Table 1;

(ix) inhibits, *e.g.*, competitively inhibits, the binding of a second antibody molecule to PD-1, wherein the second antibody molecule is an antibody molecule described herein, *e.g.*, an antibody molecule chosen from, *e.g.*, any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E;

(x) binds the same or an overlapping epitope with a second antibody molecule to PD-1, wherein the second antibody molecule is an antibody molecule described herein, *e.g.*, an antibody molecule chosen from, *e.g.*, any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E;

(xi) competes for binding, and/or binds the same epitope, with a second antibody molecule to PD-1, wherein the second antibody molecule is an antibody molecule described herein, *e.g.*, an antibody molecule chosen from, *e.g.*, any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E;

(xii) has one or more biological properties of an antibody molecule described herein, *e.g.*, an antibody molecule chosen from, *e.g.*, any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E;

(xiii) has one or more pharmacokinetic properties of an antibody molecule described herein, *e.g.*, an antibody molecule chosen from, *e.g.*, any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E;

(xiv) inhibits one or more activities of PD-1, *e.g.*, results in one or more of: an increase in tumor infiltrating lymphocytes, an increase in T-cell receptor mediated proliferation, or a decrease in immune evasion by cancerous cells;

(xv) binds human PD-1 and is cross-reactive with cynomolgus PD-1;

(xvi) binds to one or more residues within the C strand, CC' loop, C' strand, or FG loop of PD-1, or a combination two, three or all of the C strand, CC' loop, C' strand or FG loop of PD-1, *e.g.*, wherein the binding is assayed using ELISA or Biacore; or

(xvii) has a VL region that contributes more to binding to PD-1 than a VH region.

In some embodiments, the antibody molecule binds to PD-1 with high affinity, *e.g.*, with a K_D that is about the same, or at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% higher or lower than the K_D of a murine or chimeric anti-PD-1 antibody molecule, *e.g.*, a murine or chimeric anti-PD-1 antibody molecule described herein. In some embodiments, the K_D of the murine or chimeric anti-PD-1 antibody molecule is less than about 0.4, 0.3, 0.2, 0.1, or 0.05 nM, *e.g.*, measured by a Biacore method. In some embodiments, the K_D of the murine or chimeric anti-PD-1 antibody molecule is less than about 0.2 nM, *e.g.*, about 0.135 nM. In other embodiments, the K_D of the murine or chimeric anti PD-1 antibody molecule is less than about 10, 5, 3, 2, or 1 nM, *e.g.*, measured by binding on cells expressing PD-1 (*e.g.*, 300.19 cells). In some embodiments, the K_D of the murine or chimeric anti PD-1 antibody molecule is less than about 5 nM, *e.g.*, about 4.60 nM (or about 0.69 μg/mL).

In some embodiments, the anti-PD-1 antibody molecule binds to PD-1 with a K_{off} slower than 1×10^{-4}, 5×10^{-5}, or 1×10^{-5} s^{-1}, *e.g.*, about 1.65×10^{-5} s^{-1}. In some embodiments, the the anti-PD-1 antibody molecule binds to PD-1 with a K_{on} faster than 1×10^{4}, 5×10^{4}, 1×10^{5}, or 5×10^{5} $M^{-1}s^{-1}$, *e.g.*, about 1.23×10^{5} $M^{-1}s^{-1}$.

In some embodiments, the expression level of the antibody molecule is higher, *e.g.*, at least about 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10-fold higher, than the expression level of a murine or chimeric antibody molecule, *e.g.*, a murine or chimeric anti-PD-1 antibody molecule described herein. In some embodiments, the antibody molecule is expressed in CHO cells.

In some embodiments, the anti-PD-1 antibody molecule reduces one or more PD-1-associated activities with an IC_{50} (concentration at 50% inhibition) that is about the same or lower, *e.g.*, at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% lower, than the IC_{50} of a murine or chimeric anti-PD-1 antibody molecule, *e.g.*, a murine or chimeric anti-PD-1 antibody molecule described herein. In some embodiments, the IC_{50} of the murine or chimeric anti-PD-1 antibody molecule is less than about 6, 5, 4, 3, 2, or 1 nM, *e.g.*, measured by binding on cells expressing PD-1 (*e.g.*, 300.19 cells). In some embodiments, the IC_{50} of the murine or chimeric anti-PD-1 antibody molecule is less than about 4 nM, *e.g.*, about 3.40 nM (or about

0.51 μg/mL). In some embodiments, the PD-1-associated activity reduced is the binding of PD-L1 and/or PD-L2 to PD-1. In some embodiments, the anti-PD-1 antibody molecule binds to peripheral blood mononucleated cells (PBMCs) activated by Staphylococcal enterotoxin B (SEB). In other embodiments, the anti-PD-1 antibody molecule increases the expression of IL-2 on whole blood activated by SEB. For example, the anti-PD-1 antibody increases the expression of IL-2 by at least about 2, 3, 4, or 5-fold, compared to the expression of IL-2 when an isotype control (*e.g.*, IgG4) is used.

In some embodiments, the anti-PD-1 antibody molecule has improved stability, *e.g.*, at least about 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10-fold more stable *in vivo* or *in vitro*, than a murine or chimeric anti-PD-1 antibody molecule, *e.g.*, a murine or chimeric anti-PD-1 antibody molecule described herein.

In one embodiment, the anti PD-1 antibody molecule is a humanized antibody molecule and has a risk score based on T cell epitope analysis of 300 to 700, 400 to 650, 450 to 600, or a risk score as described herein.

In another embodiment, the anti-PD-1 antibody molecule comprises at least one antigen-binding region, *e.g.*, a variable region or an antigen-binding fragment thereof, from an antibody described herein, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1, or encoded by the nucleotide sequence in Table 1; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

In yet another embodiment, the anti-PD-1 antibody molecule comprises at least one, two, three or four variable regions from an antibody described herein, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1, or encoded by the nucleotide sequence in Table 1;

or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

In yet another embodiment, the anti-PD-1 antibody molecule comprises at least one or two heavy chain variable regions from an antibody described herein, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1, or encoded by the nucleotide sequence in Table 1; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

In yet another embodiment, the anti-PD-1 antibody molecule comprises at least one or two light chain variable regions from an antibody described herein, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1, or encoded by the nucleotide sequence in Table 1; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

In yet another embodiment, the anti-PD-1 antibody molecule includes a heavy chain constant region for an IgG4, *e.g.*, a human IgG4. In one embodiment, the human IgG4 includes a substitution at position 228 according to EU numbering (*e.g.*, a Ser to Pro substitution). In still another embodiment, the anti-PD-1 antibody molecule includes a heavy chain constant region for an IgG1, *e.g.*, a human IgG1. In one embodiment, the human IgG1 includes a substitution at position 297 according to EU numbering (*e.g.*, an Asn to Ala substitution). In one embodiment, the human IgG1 includes a substitution at position 265 according to EU numbering, a substitution at position 329 according to EU numbering, or both (*e.g.*, an Asp to Ala substitution at position 265 and/or a Pro to Ala substitution at position 329). In one embodiment, the human IgG1 includes a substitution at position 234 according to EU numbering, a substitution at position 235 according to EU numbering, or both (*e.g.*, a Leu to Ala substitution at position 234

and/or a Leu to Ala substitution at position 235). In one embodiment, the heavy chain constant region comprises an amino sequence set forth in Table 3, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) thereto.

In yet another embodiment, the anti-PD-1 antibody molecule includes a kappa light chain constant region, *e.g.*, a human kappa light chain constant region. In one embodiment, the light chain constant region comprises an amino sequence set forth in Table 3, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) thereto.

In another embodiment, the anti-PD-1 antibody molecule includes a heavy chain constant region for an IgG4, *e.g.*, a human IgG4, and a kappa light chain constant region, *e.g.*, a human kappa light chain constant region, *e.g.*, a heavy and light chain constant region comprising an amino sequence set forth in Table 3, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) thereto. In one embodiment, the human IgG4 includes a substitution at position 228 according to EU numbering (*e.g.*, a Ser to Pro substitution). In yet another embodiment, the anti-PD-1 antibody molecule includes a heavy chain constant region for an IgG1, *e.g.*, a human IgG1, and a kappa light chain constant region, *e.g.*, a human kappa light chain constant region, *e.g.*, a heavy and light chain constant region comprising an amino sequence set forth in Table 3, or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) thereto. In one embodiment, the human IgG1 includes a substitution at position 297 according to EU numbering (*e.g.*, an Asn to Ala substitution). In one embodiment, the human IgG1 includes a substitution at position 265 according to EU numbering, a substitution at position 329 according to EU numbering, or both (*e.g.*, an Asp to Ala substitution at position 265 and/or a Pro to Ala substitution at position 329). In one embodiment, the human IgG1 includes a substitution at position 234 according to EU numbering, a substitution at position 235 according to EU numbering, or both (*e.g.*, a Leu to Ala substitution at position 234 and/or a Leu to Ala substitution at position 235).

In another embodiment, the anti-PD-1 antibody molecule includes a heavy chain variable domain and a constant region, a light chain variable domain and a constant region, or both, comprising the amino acid sequence of BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1, or encoded by the nucleotide

sequence in Table 1; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences. The anti-PD-1 antibody molecule, optionally, comprises a leader sequence from a heavy chain, a light chain, or both, as showin in Table 4; or a sequence substantially identical thereto.

In yet another embodiment, the anti-PD-1 antibody molecule includes at least one, two, or three complementarity determining regions (CDRs) from a heavy chain variable region of an antibody described herein, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1, or encoded by the nucleotide sequence in Table 1; or a sequence substantially identical (*e.g.,* at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

In yet another embodiment, the anti-PD-1 antibody molecule includes at least one, two, or three CDRs (or collectively all of the CDRs) from a heavy chain variable region comprising an amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, *e.g.*, amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1.

In yet another embodiment, the anti-PD-1 antibody molecule includes at least one, two, or three CDRs from a light chain variable region of an antibody described herein, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1, or encoded by the nucleotide sequence in Table 1; or a sequence substantially identical (*e.g.,* at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequence.

In yet another embodiment, the anti-PD-1 antibody molecule includes at least one, two, or three CDRs (or collectively all of the CDRs) from a light chain variable region comprising an

amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, *e.g.*, amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1. In certain embodiments, the anti-PD-1 antibody molecule includes a substitution in a light chain CDR, *e.g.*, one or more substitutions in a CDR1, CDR2 and/or CDR3 of the light chain. In one embodiment, the anti-PD-1 antibody molecule includes a substitution in the light chain CDR3 at position 102 of the light variable region, *e.g.*, a substitution of a cysteine to tyrosine, or a cysteine to serine residue, at position 102 of the light variable region according to Table 1 (*e.g.*, SEQ ID NO: 16 or 24 for murine or chimeric, unmodified; or any of SEQ ID NOs: 34, 42, 46, 54, 58, 62, 66, 70, 74, or 78 for a modified sequence).

In another embodiment, the anti-PD-1 antibody molecule includes at least one, two, three, four, five or six CDRs (or collectively all of the CDRs) from a heavy and light chain variable region comprising an amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, *e.g.*, amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1.

In one embodiment, the anti-PD-1 antibody molecule includes all six CDRs from an antibody described herein, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1, or encoded by the nucleotide sequence in Table 1, or closely related CDRs, *e.g.*, CDRs which are identical or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions). In one embodiment, the anti-PD-1 antibody molecule may include any CDR described herein. In certain embodiments, the anti-PD-1 antibody molecule includes a substitution in a light chain CDR, *e.g.*, one or more substitutions in a CDR1, CDR2 and/or CDR3 of the light chain. In one embodiment, the anti-PD-1 antibody molecule includes a substitution in the light chain CDR3 at

position 102 of the light variable region, *e.g.*, a substitution of a cysteine to tyrosine, or a cysteine to serine residue, at position 102 of the light variable region according to Table 1 (*e.g.*, SEQ ID NO: 16 or 24 for murine or chimeric, unmodified; or any of SEQ ID NOs: 34, 42, 46, 54, 58, 62, 66, 70, 74, or 78 for a modified sequence).

In another embodiment, the anti-PD-1 antibody molecule includes at least one, two, or three CDRs according to Kabat *et al.* (*e.g.*, at least one, two, or three CDRs according to the Kabat definition as set out in Table 1) from a heavy chain variable region of an antibody described herein, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1, or encoded by the nucleotide sequence in Table 1; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, or three CDRs according to Kabat *et al.* shown in Table 1.

In another embodiment, the anti-PD-1 antibody molecule includes at least one, two, or three CDRs according to Kabat *et al.* (*e.g.*, at least one, two, or three CDRs according to the Kabat definition as set out in Table 1) from a light chain variable region of an antibody described herein, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1, or encoded by the nucleotide sequence in Table 1; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, or three CDRs according to Kabat *et al.* shown in Table 1.

In yet another embodiment, the anti-PD-1 antibody molecule includes at least one, two, three, four, five, or six CDRs according to Kabat *et al.* (*e.g.*, at least one, two, three, four, five, or six CDRs according to the Kabat definition as set out in Table 1) from the heavy and light chain variable regions of an antibody described herein, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1, or encoded by the nucleotide sequence in Table 1; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, three, four, five, or six CDRs according to Kabat *et al.* shown in Table 1.

In yet another embodiment, the anti-PD-1 antibody molecule includes all six CDRs according to Kabat *et al.* (*e.g.*, all six CDRs according to the Kabat definition as set out in Table 1) from the heavy and light chain variable regions of an antibody described herein, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1, or encoded by the nucleotide sequence in Table 1; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to all six CDRs according to Kabat *et al.* shown in Table 1. In one embodiment, the anti-PD-1 antibody molecule may include any CDR described herein.

In another embodiment, the anti-PD-1 antibody molecule includes at least one, two, or three Chothia hypervariable loops (*e.g.*, at least one, two, or three hypervariable loops according to the Chothia definition as set out in Table 1) from a heavy chain variable region of an antibody

described herein, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1, or encoded by the nucleotide sequence in Table 1; or at least the amino acids from those hypervariable loops that contact PD-1; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, or three hypervariable loops according to Chothia *et al.* shown in Table 1.

In another embodiment, the anti-PD-1 antibody molecule includes at least one, two, or three Chothia hypervariable loops (*e.g.*, at least one, two, or three hypervairalbe loops according to the Chothia definition as set out in Table 1) of a light chain variable region of an antibody described herein, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1, or encoded by the nucleotide sequence in Table 1; or at least the amino acids from those hypervariable loops that contact PD-1; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, or three hypervariable loops according to Chothia *et al.* shown in Table 1.

In yet another embodiment, the anti-PD-1 antibody molecule includes at least one, two, three, four, five, or six hypervariable loops (*e.g.*, at least one, two, three, four, five, or six hypervariable loops according to the Chothia definition as set out in Table 1) from the heavy and light chain variable regions of an antibody described herein, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or

BAP049-Clone-E; or as described in Table 1, or encoded by the nucleotide sequence in Table 1; or at least the amino acids from those hypervariable loops that contact PD-1; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to one, two, three, four, five or six hypervariable loops according to Chothia *et al.* shown in Table 1.

In one embodiment, the anti-PD-1 antibody molecule includes all six hypervariable loops (*e.g.*, all six hypervariable loops according to the Chothia definition as set out in Table 1) of an antibody described herein, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E, or closely related hypervariable loops, *e.g.*, hypervariable loops which are identical or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions); or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) relative to all six hypervariable loops according to Chothia *et al.* shown in Table 1. In one embodiment, the anti-PD-1 antibody molecule may include any hypervariable loop described herein.

In still another embodiment, the anti-PD-1 antibody molecule includes at least one, two, or three hypervariable loops that have the same canonical structures as the corresponding hypervariable loop of an antibody described herein, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E, *e.g.*, the same canonical structures as at least loop 1 and/or loop 2 of the heavy and/or light chain variable domains of an antibody described herein. *See, e.g.*, Chothia *et al.*, (1992) *J. Mol. Biol.* 227:799-817; Tomlinson et *al.*, (1992) *J. Mol. Biol.* 227:776-798 for descriptions of hypervariable loop canonical structures. These structures can be determined by inspection of the tables described in these references.

In certain embodiments, the anti-PD-1 antibody molecule includes a combination of CDRs or hypervariable loops defined according to the Kabat *et al.* and Chothia *et al.*

In one embodiment, the anti-PD-1 antibody molecule includes at least one, two or three CDRs or hypervariable loops from a heavy chain variable region of an antibody described herein, *e.g.,* an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E, according to the Kabat and Chothia definition (*e.g.,* at least one, two, or three CDRs or hypervariable loops according to the Kabat and Chothia definition as set out in Table 1); or encoded by the nucleotide sequence in Table 1; or a sequence substantially identical (*e.g.,* at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences; or which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.,* substitutions, deletions, or insertions, *e.g.,* conservative substitutions) relative to one, two, or three CDRs or hypervariable loops according to Kabat and/or Chothia shown in Table 1.

For example, the anti-PD-1 antibody molecule can include VH CDR1 according to Kabat *et al.* or VH hypervariable loop 1 according to Chothia *et al.*, or a combination thereof, *e.g.*, as shown in Table 1. In one embodiment, the combination of Kabat and Chothia CDR of VH CDR1 comprises the amino acid sequence GYTFTTYWMH (SEQ ID NO: 224), or an amino acid sequence substantially identical thereto (*e.g.*, having at least one amino acid alteration, but not more than two, three or four alterations (*e.g.,* substitutions, deletions, or insertions, *e.g.,* conservative substitutions)). The anti-PD-1 antibody molecule can further include, *e.g.,* VH CDRs 2-3 according to Kabat *et al.* and VL CDRs 1-3 according to Kabat *et al., e.g.*, as shown in Table 1. Accordingly, in some embodiments, framework regions are defined based on a combination of CDRs defined according to Kabat *et al.* and hypervariable loops defined according to Chothia *et al.* For example, the anti-PD-1 antibody molecule can include VH FR1 defined based on VH hypervariable loop 1 according to Chothia *et al.* and VH FR2 defined based on VH CDRs 1-2 according to Kabat *et al.*, *e.g.*, as shown in Table 1. The anti-PD-1 antibody molecule can further include, *e.g.,* VH FRs 3-4 defined based on VH CDRs 2-3

according to Kabat *et al.* and VL FRs 1-4 defined based on VL CDRs 1-3 according to Kabat *et al.*

The anti-PD-1 antibody molecule can contain any combination of CDRs or hypervariable loops according to the Kabat and Chothia definitions. In one embodiment, the anti-PD-1 antibody molecule includes at least one, two or three CDRs from a light chain variable region of an antibody described herein, *e.g.,* an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E, according to the Kabat and Chothia definition (*e.g.,* at least one, two, or three CDRs according to the Kabat and Chothia definition as set out in Table 1).

In an embodiment, *e.g.*, an embodiment comprising a variable region, a CDR (*e.g.,* Chothia CDR or Kabat CDR), or other sequence referred to herein, *e.g.,* in Table 1, the antibody molecule is a monospecific antibody molecule, a bispecific antibody molecule, or is an antibody molecule that comprises an antigen binding fragment of an antibody, *e.g.,* a half antibody or antigen binding fragment of a half antibody. In certain embodiments the antibody molecule is a bispecific antibody molecule having a first binding specificity for PD-1 and a second binding specificity for TIM-3, LAG-3, CEACAM (*e.g.*, CEACAM-1 and/or CEACAM-5), PD-L1 or PD-L2.

In one embodiment, the anti-PD-1 antibody molecule includes:

(a) a heavy chain variable region (VH) comprising a VHCDR1 amino acid sequence of SEQ ID NO: 4, a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a light chain variable region (VL) comprising a VLCDR1 amino acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 33;

(b) a VH comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 32;

(c) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 224, a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 33; or

(d) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 224; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 32.

In one embodiment, the anti-PD-1 antibody molecule comprises a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 4, a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 33.

In one embodiment, the anti-PD-1 antibody molecule comprises a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 1; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 32.

In one embodiment, the anti-PD-1 antibody molecule comprises a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 224, a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 33.

In one embodiment, the anti-PD-1 antibody molecule comprises a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 224; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 32.

In one embodiment, the antibody molecule is a humanized antibody molecule. In another embodiment, the antibody molecule is a monospecific antibody molecule. In yet another embodiment, the antibody molecule is a bispecific antibody molecule.

In one embodiment, the anti-PD-1 antibody molecule includes:

(i) a heavy chain variable region (VH) including a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1, SEQ ID NO: 4 or SEQ ID NO: 224; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and

(ii) a light chain variable region (VL) including a VLCDR1 amino acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 32.

In another embodiment, the anti-PD-1 antibody molecule includes:

(i) a heavy chain variable region (VH) including a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1, SEQ ID NO: 4 or SEQ ID NO: 224; a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and

(ii) a light chain variable region (VL) including a VLCDR1 amino acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 33.

In one embodiment, the anti-PD-1 antibody molecule comprises the VHCDR1 amino acid sequence of SEQ ID NO: 1. In another embodiment, the anti-PD-1 antibody molecule comprises the VHCDR1 amino acid sequence of SEQ ID NO: 4. In yet another embodiment, the anti-PD-1 antibody molecule comprises the VHCDR1 amino acid sequence of SEQ ID NO: 224.

In one embodiment, the light or the heavy chain variable framework (*e.g.*, the region encompassing at least FR1, FR2, FR3, and optionally FR4) of the anti-PD-1 antibody molecule can be chosen from: (a) a light or heavy chain variable framework including at least 80%, 85%, 87% 90%, 92%, 93%, 95%, 97%, 98%, or preferably 100% of the amino acid residues from a human light or heavy chain variable framework, *e.g.*, a light or heavy chain variable framework residue from a human mature antibody, a human germline sequence, or a human consensus sequence; (b) a light or heavy chain variable framework including from 20% to 80%, 40% to 60%, 60% to 90%, or 70% to 95% of the amino acid residues from a human light or heavy chain variable framework, *e.g.*, a light or heavy chain variable framework residue from a human mature antibody, a human germline sequence, or a human consensus sequence; (c) a non-human framework (*e.g.*, a rodent framework); or (d) a non-human framework that has been modified, *e.g.,* to remove antigenic or cytotoxic determinants, *e.g.*, deimmunized, or partially humanized. In one embodiment, the light or heavy chain variable framework region (particularly FR1, FR2

and/or FR3) includes a light or heavy chain variable framework sequence at least 70, 75, 80, 85, 87, 88, 90, 92, 94, 95, 96, 97, 98, 99% identical or identical to the frameworks of a VL or VH segment of a human germline gene.

In certain embodiments, the anti-PD-1 antibody molecule comprises a heavy chain variable domain having at least one, two, three, four, five, six, seven, ten, fifteen, twenty or more changes, *e.g.*, amino acid substitutions or deletions, from an amino acid sequence of BAP049-chi-HC, *e.g.*, the amino acid sequence of the FR region in the entire variable region, *e.g.*, shown in FIGs. 9A-9B, or SEQ ID NO: 18, 20, 22 or 30. In one embodiment, the anti-PD-1 antibody molecule comprises a heavy chain variable domain having one or more of: E at position 1, V at position 5, A at position 9, V at position 11, K at position 12, K at position 13, E at position 16, L at position 18, R at position 19, I or V at position 20, G at position 24, I at position 37, A or S at position 40, T at position 41, S at position 42, R at position 43, M or L at position 48, V or F at position 68, T at position 69, I at position 70, S at position 71, A or R at position 72, K or N at position 74, T or K at position 76, S or N at position 77, L at position 79, L at position 81, E or Q at position 82, M at position 83, S or N at position 84, R at position 87, A at position 88, or T at position 91 of amino acid sequence of BAP049-chi-HC, *e.g.*, the amino acid sequence of the FR in the entire variable region, *e.g.*, shown in FIGs. 9A-9B, or SEQ ID NO: 18, 20, 22 or 30.

Alternatively, or in combination with the heavy chain substitutions of BAP049-chi-HC described herein, the anti-PD-1 antibody molecule comprises a light chain variable domain having at least one, two, three, four, five, six, seven, ten, fifteen, twenty or more amino acid changes, *e.g.*, amino acid substitutions or deletions, from an amino acid sequence of BAP049-chi-LC, *e.g.*, the amino acid sequence shown in FIGs. 10A-10B, or SEQ ID NO: 24 or 26. In one embodiment, the anti-PD-1 antibody molecule comprises a heavy chain variable domain having one or more of: E at position 1, V at position 2, Q at position 3, L at position 4, T at position 7, D or L or A at position 9, F or T at position 10, Q at position 11, S or P at position 12, L or A at position 13, S at position 14, P or L or V at position 15, K at position 16, Q or D at position 17, R at position 18, A at position 19, S at position 20, I or L at position 21, T at position 22, L at position 43, K at position 48, A or S at position 49, R or Q at position 51, Y at position 55, I at position 64, S or P at position 66, S at position 69, Y at position 73, G at position 74, E at position 76, F at position 79, N at position 82, N at position 83, L or I at position 84, E at position 85, S or P at position 86, D at position 87, A or F or I at position 89, T or Y at position

91, F at position 93, or Y at position 102 of the amino acid sequence of BAP049-chi-LC, *e.g.*, the amino acid sequence shown in FIGs. 10A-10B, or SEQ ID NO: 24 or 26.

In other embodiments, the anti-PD-1 antibody molecule includes one, two, three, or four heavy chain framework regions (*e.g.*, a VHFW amino acid sequence shown in Table 2, or encoded by the nucleotide sequence shown in Table 2), or a sequence substantially identical thereto.

In yet other embodiments, the anti-PD-1 antibody molecule includes one, two, three, or four light chain framework regions (*e.g.*, a VLFW amino acid sequence shown in Table 2, or encoded by the nucleotide sequence shown in Table 2), or a sequence substantially identical thereto.

In other embodiments, the anti-PD-1 antibody molecule includes one, two, three, or four heavy chain framework regions (*e.g.*, a VHFW amino acid sequence shown in Table 2, or encoded by the nucleotide sequence shown in Table 2), or a sequence substantially identical thereto; and one, two, three, or four light chain framework regions (*e.g.*, a VLFW amino acid equence shown in Table 2, or encoded by the nucleotide sequence shown in Table 2), or a sequence substantially identical thereto.

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework region 1 (VHFW1) of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E (*e.g.,* SEQ ID NO: 147). In some embodiments, the antibody molecule comprises the heavy chain framework region 1 (VHFW1) of BAP049-hum14 or BAP049-hum15 (*e.g.,* SEQ ID NO: 151).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework region 2 (VHFW2) of BAP049-hum01, BAP049-hum02, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum09, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, or BAP049-Clone-E (*e.g.,* SEQ ID NO: 153). In some embodiments, the antibody molecule comprises the heavy chain framework region 2 (VHFW2) of BAP049-hum03, BAP049-hum04, BAP049-hum08, BAP049-hum10, BAP049-hum14, BAP049-hum15, or BAP049-Clone-D (*e.g.,* SEQ ID NO: 157). In some

embodiments, the antibody molecule comprises the heavy chain framework region 2 (VHFW2) of BAP049-hum16 (*e.g.,* SEQ ID NO: 160).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework region 3 (VHFW3) of BAP049-hum01, BAP049-hum02, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum09, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, or BAP049-Clone-E (*e.g.,* SEQ ID NO: 162). In some embodiments, the antibody molecule comprises the heavy chain framework region 3 (VHFW3) of BAP049-hum03, BAP049-hum04, BAP049-hum08, BAP049-hum10, BAP049-hum14, BAP049-hum15, BAP049-hum16, or BAP049-Clone-D (*e.g.,* SEQ ID NO: 166).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework region 4 (VHFW4) of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E (*e.g.,* SEQ ID NO: 169).

In some embodiments, the anti-PD-1 antibody molecule comprises the light chain framework region 1 (VLFW1) of BAP049-hum08, BAP049-hum09, BAP049-hum15, BAP049-hum16, or BAP049-Clone-C (*e.g.,* SEQ ID NO: 174). In some embodiments, the antibody molecule comprises the light chain framework region 1 (VLFW1) of BAP049-hum01, BAP049-hum04, BAP049-hum05, BAP049-hum07, BAP049-hum10, BAP049-hum11, BAP049-hum14, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-D, or BAP049-Clone-E (*e.g.,* SEQ ID NO: 177). In some embodiments, the antibody molecule comprises the light chain framework region 1 (VLFW1) of BAP049-hum06 (*e.g.,* SEQ ID NO: 181). In some embodiments, the antibody molecule comprises the light chain framework region 1 (VLFW1) of BAP049-hum13 (*e.g.,* SEQ ID NO: 183). In some embodiments, the antibody molecule comprises the light chain framework region 1 (VLFW1) of BAP049-hum02, BAP049-hum03, or BAP049-hum12 (*e.g.,* SEQ ID NO: 185).

In some embodiments, the anti-PD-1 antibody molecule comprises the light chain framework region 2 (VLFW2) of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum06, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum14,

BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-D, or BAP049-Clone-E (*e.g.,* SEQ ID NO: 187). In some embodiments, the antibody molecule comprises the light chain framework region 2 (VLFW2) of BAP049-hum04, BAP049-hum05, BAP049-hum07, BAP049-hum13, or BAP049-Clone-C (*e.g.,* SEQ ID NO: 191). In some embodiments, the antibody molecule comprises the light chain framework region 2 (VLFW2) of BAP049-hum12 (*e.g.,* SEQ ID NO: 194).

In some embodiments, the anti-PD-1 antibody molecule comprises the light chain framework region 3 (VLFW3) of BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E (*e.g.,* SEQ ID NO: 196). In some embodiments, the antibody molecule comprises the light chain framework region 3 (VLFW3) of BAP049-hum02 or BAP049-hum03 (*e.g.,* SEQ ID NO: 200). In some embodiments, the antibody molecule comprises the light chain framework region 3 (VLFW3) of BAP049-hum01 or BAP049-Clone-A (*e.g.,* SEQ ID NO: 202). In some embodiments, the antibody molecule comprises the light chain framework region 3 (VLFW3) of BAP049-hum04, BAP049-hum05, or BAP049-Clone-B (*e.g.,* SEQ ID NO: 205).

In some embodiments, the anti-PD-1 antibody molecule comprises the light chain framework region 4 (VLFW4) of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E (*e.g.,* SEQ ID NO: 208).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework regions 1-3 of BAP049-hum01, BAP049-hum02, BAP049-hum05, BAP049-hum06, BAP-hum07, BAP049-hum09, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, or BAP049-Clone-E (*e.g.,* SEQ ID NO: 147 (VHFW1), SEQ ID NO: 153 (VHFW2), and SEQ ID NO: 162 (VHFW3)). In some embodiments, the antibody molecule comprises the heavy chain framework regions 1-3 of BAP049-hum03, BAP049-hum04, BAP049-hum08, BAP049-hum10, or BAP049-Clone-D (*e.g.,* SEQ ID NO: 147 (VHFW1), SEQ ID NO: 157 (VHFW2), and SEQ ID NO: 166 (VHFW3)). In some embodiments, the antibody molecule comprises the heavy chain framework regions 1-3 of

BAP049-hum14 or BAP049-hum15 (*e.g.*, SEQ ID NO: 151 (VHFW1), SEQ ID NO: 157 (VHFW2), and SEQ ID NO: 166 (VHFW3)). In some embodiments, the antibody molecule comprises the heavy chain framework regions 1-3 of BAP049-hum16 (*e.g.*, SEQ ID NO: 147 (VHFW1), SEQ ID NO: 160 (VHFW2), and SEQ ID NO: 166 (VHFW3)). In some embodiments, the antibody molecule further comprises the heavy chain framework region 4 (VHFW4) of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E (*e.g.*, SEQ ID NO: 169).

In some embodiments, the anti-PD-1 antibody molecule comprises the light chain framework regions 1-3 of BAP049-hum01 or BAP049-Clone-A (*e.g.*, SEQ ID NO: 177 (VLFW1), SEQ ID NO: 187 (VLFW2), and SEQ ID NO: 202 (VLFW3)). In some embodiments, the antibody molecule comprises the light chain framework regions 1-3 of BAP049-hum02 or BAP049-hum03 (*e.g.*, SEQ ID NO: 185 (VLFW1), SEQ ID NO: 187 (VLFW2), and SEQ ID NO: 200 (VLFW3)). In some embodiments, the antibody molecule comprises the light chain framework regions 1-3 of BAP049-hum04, BAP049-hum05, or BAP049-Clone-B (*e.g.*, SEQ ID NO: 177 (VLFW1), SEQ ID NO: 191 (VLFW2), and SEQ ID NO: 205 (VLFW3)). In some embodiments, the antibody molecule comprises the light chain framework regions 1-3 of BAP049-hum06 (*e.g.*, SEQ ID NO: 181 (VLFW1), SEQ ID NO: 187 (VLFW2), and SEQ ID NO: 196 (VLFW3)). In some embodiments, the antibody molecule comprises the light chain framework regions 1-3 of BAP049-hum07 (*e.g.*, SEQ ID NO: 177 (VLFW1), SEQ ID NO: 191 (VLFW2), and SEQ ID NO: 196 (VLFW3)). In some embodiments, the antibody molecule comprises the light chain framework regions 1-3 of BAP049-hum08, BAP049-hum09, BAP049-hum15, BAP049-hum16, or BAP049-Clone-C (*e.g.*, SEQ ID NO: 174 (VLFW1), SEQ ID NO: 187 (VLFW2), and SEQ ID NO: 196 (VLFW3)). In some embodiments, the antibody molecule comprises the light chain framework regions 1-3 of BAP049-hum10, BAP049-hum11, BAP049-hum14, BAP049-Clone-D, or BAP049-Clone-E (*e.g.*, SEQ ID NO: 177 (VLFW1), SEQ ID NO: 187 (VLFW2), and SEQ ID NO: 196 (VLFW3)). In some embodiments, the antibody molecule comprises the light chain framework regions 1-3 of BAP049-hum12 (*e.g.*, SEQ ID NO: 185 (VLFW1), SEQ ID NO: 194 (VLFW2),

and SEQ ID NO: 196 (VLFW3)). In some embodiments, the antibody molecule comprises the light chain framework regions 1-3 of BAP049-hum13 (*e.g.,* SEQ ID NO: 183 (VLFW1), SEQ ID NO: 191 (VLFW2), and SEQ ID NO: 196 (VLFW3)). In some embodiments, the antibody molecule further comprises the light chain framework region 4 (VLFW4) of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E (*e.g.,* SEQ ID NO: 208).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework regions 1-3 of BAP049-hum01 or BAP049-Clone-A (*e.g.,* SEQ ID NO: 147 (VHFW1), SEQ ID NO: 153 (VHFW2), and SEQ ID NO: 162 (VHFW3)) and the light chain framework regions 1-3 of BAP049-hum01 or BAP049-Clone-A (*e.g.,* SEQ ID NO: 177 (VLFW1), SEQ ID NO: 187 (VLFW2), and SEQ ID NO: 202 (VLFW3)).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework regions 1-3 of BAP049-hum02 (*e.g.,* SEQ ID NO: 147 (VHFW1), SEQ ID NO: 153 (VHFW2), and SEQ ID NO: 162 (VHFW3)) and the light chain framework regions 1-3 of BAP049-hum02 (*e.g.,* SEQ ID NO: 185 (VLFW1), SEQ ID NO: 187 (VLFW2), and SEQ ID NO: 200 (VLFW3)).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework regions 1-3 of BAP049-hum03 (*e.g.,* SEQ ID NO: 147 (VHFW1), SEQ ID NO: 157 (VHFW2), and SEQ ID NO: 166 (VHFW3)) and the light chain framework regions 1-3 of BAP049-hum03 (*e.g.,* SEQ ID NO: 185 (VLFW1), SEQ ID NO: 187 (VLFW2), and SEQ ID NO: 200 (VLFW3)).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework regions 1-3 of BAP049-hum04 (*e.g.,* SEQ ID NO: 147 (VHFW1), SEQ ID NO: 157 (VHFW2), and SEQ ID NO: 166 (VHFW3)) and the light chain framework regions 1-3 of BAP049-hum04 (*e.g.,* SEQ ID NO: 177 (VLFW1), SEQ ID NO: 191 (VLFW2), and SEQ ID NO: 205 (VLFW3)).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework regions 1-3 of BAP049-hum05 or BAP049-Clone-B (*e.g.,* SEQ ID NO: 147

(VHFW1), SEQ ID NO: 153 (VHFW2), and SEQ ID NO: 162 (VHFW3)) and the light chain framework regions 1-3 of BAP049-hum05 or BAP049-Clone-B (*e.g.,* SEQ ID NO: 177 (VLFW1), SEQ ID NO: 191 (VLFW2), and SEQ ID NO: 205 (VLFW3)).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework regions 1-3 of BAP049-hum06 (*e.g.,* SEQ ID NO: 147 (VHFW1), SEQ ID NO: 153 (VHFW2), and SEQ ID NO: 162 (VHFW3)) and the light chain framework regions 1-3 of BAP049-hum06 (*e.g.,* SEQ ID NO: 181 (VLFW1), SEQ ID NO: 187 (VLFW2), and SEQ ID NO: 196 (VLFW3)).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework regions 1-3 of BAP049-hum07 (*e.g.,* SEQ ID NO: 147 (VHFW1), SEQ ID NO: 153 (VHFW2), and SEQ ID NO: 162 (VHFW3)) and the light chain framework regions 1-3 of BAP049-hum07 (*e.g.,* SEQ ID NO: 177 (VLFW1), SEQ ID NO: 191 (VLFW2), and SEQ ID NO: 196 (VLFW3)).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework regions 1-3 of BAP049-hum08 (*e.g.,* SEQ ID NO: 147 (VHFW1), SEQ ID NO: 157 (VHFW2), and SEQ ID NO: 166 (VHFW3)) and the light chain framework regions 1-3 of BAP049-hum08 (*e.g.,* SEQ ID NO: 174 (VLFW1), SEQ ID NO: 187 (VLFW2), and SEQ ID NO: 196 (VLFW3)).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework regions 1-3 of BAP049-hum09 or BAP049-Clone-C (*e.g.,* SEQ ID NO: 147 (VHFW1), SEQ ID NO: 153 (VHFW2), and SEQ ID NO: 162 (VHFW3)) and the light chain framework regions 1-3 of BAP049-hum09 or BAP049-Clone-C (*e.g.,* SEQ ID NO: 174 (VLFW1), SEQ ID NO: 187 (VLFW2), and SEQ ID NO: 196 (VLFW3)).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework regions 1-3 of BAP049-hum10 or BAP049-Clone-D (*e.g.,* SEQ ID NO: 147 (VHFW1), SEQ ID NO: 157 (VHFW2), and SEQ ID NO: 166 (VHFW3)) and the light chain framework regions 1-3 of BAP049-hum10 or BAP049-Clone-D (*e.g.,* SEQ ID NO: 177 (VLFW1), SEQ ID NO: 187 (VLFW2), and SEQ ID NO: 196 (VLFW3)).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework regions 1-3 of BAP049-hum11 or BAP049-Clone-E (*e.g.,* SEQ ID NO: 147 (VHFW1), SEQ ID NO: 153 (VHFW2), and SEQ ID NO: 162 (VHFW3)) and the light chain

framework regions 1-3 of BAP049-hum11 or BAP049-Clone-E (*e.g.,* SEQ ID NO: 177 (VLFW1), SEQ ID NO: 187 (VLFW2), and SEQ ID NO: 196 (VLFW3)).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework regions 1-3 of BAP049-hum12 (*e.g.,* SEQ ID NO: 147 (VHFW1), SEQ ID NO: 153 (VHFW2), and SEQ ID NO: 162 (VHFW3)) and the light chain framework regions 1-3 of BAP049-hum12 (*e.g.,* SEQ ID NO: 185 (VLFW1), SEQ ID NO: 194 (VLFW2), and SEQ ID NO: 196 (VLFW3)).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework regions 1-3 of BAP049-hum13 (*e.g.,* SEQ ID NO: 147 (VHFW1), SEQ ID NO: 153 (VHFW2), and SEQ ID NO: 162 (VHFW3)) and the light chain framework regions 1-3 of BAP049-hum13 (*e.g.,* SEQ ID NO: 183 (VLFW1), SEQ ID NO: 191 (VLFW2), and SEQ ID NO: 196 (VLFW3)).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework regions 1-3 of BAP049-hum14 (*e.g.,* SEQ ID NO: 151 (VHFW1), SEQ ID NO: 157 (VHFW2), and SEQ ID NO: 166 (VHFW3)) and the light chain framework regions 1-3 of BAP049-hum14 (*e.g.,* SEQ ID NO: 177 (VLFW1), SEQ ID NO: 187 (VLFW2), and SEQ ID NO: 196 (VLFW3)).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework regions 1-3 of BAP049-hum15 (*e.g.,* SEQ ID NO: 151 (VHFW1), SEQ ID NO: 157 (VHFW2), and SEQ ID NO: 166 (VHFW3)) and the light chain framework regions 1-3 of BAP049-hum15 (*e.g.,* SEQ ID NO: 174 (VLFW1), SEQ ID NO: 187 (VLFW2), and SEQ ID NO: 196 (VLFW3)).

In some embodiments, the anti-PD-1 antibody molecule comprises the heavy chain framework regions 1-3 of BAP049-hum16 (*e.g.,* SEQ ID NO: 147 (VHFW1), SEQ ID NO: 160 (VHFW2), and SEQ ID NO: 166 (VHFW3)) and the light chain framework regions 1-3 of BAP049-hum16 (*e.g.,* SEQ ID NO: 174 (VLFW1), SEQ ID NO: 187 (VLFW2), and SEQ ID NO: 196 (VLFW3)).

In some embodiments, the anti-PD-1 antibody molecule further comprises the heavy chain framework region 4 (VHFW4) of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13,

BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E (*e.g.*, SEQ ID NO: 169) and the light chain framework region 4 (VLFW4) of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E (*e.g.*, SEQ ID NO: 208).

In some embodiments, the anti-PD-1 antibody molecule comprises a heavy chain framework region having a combination of framework regions FW1, FW2 and FW3 as showin in FIGs. 5 or 7. In other embodiment, the antibody molecule comprises a light chain framework region having a combination of framework regions FW1, FW2 and FW3 as showin in FIGs. 5 or 7. In yet other embodiments, the antibody molecule comprises a heavy chain framework region having a combination of framework regions FW1, FW2 and FW3 as showin in FIGs. 5 or 7, and a light chain framework region having a combination of framework regions FW1, FW2 and FW3 as showin in FIGs. 5 or 7.

In one embodiment, the heavy or light chain variable domain, or both, of the anti-PD-1 antibody molecule includes an amino acid sequence, which is substantially identical to an amino acid disclosed herein, *e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical to a variable region of an antibody described herein, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1, or encoded by the nucleotide sequence in Table 1; or which differs at least 1 or 5 residues, but less than 40, 30, 20, or 10 residues, from a variable region of an antibody described herein.

In one embodiment, the heavy or light chain variable region, or both, of the anti-PD-1 antibody molecule includes an amino acid sequence encoded by a nucleic acid sequence described herein or a nucleic acid that hybridizes to a nucleic acid sequence described herein (*e.g.*, a nucleic acid sequence as shown in Tables 1 and 2) or its complement, *e.g.*, under low

stringency, medium stringency, or high stringency, or other hybridization condition described herein.

In another embodiment, the anti-PD-1 antibody molecule comprises at least one, two, three, or four antigen-binding regions, *e.g.*, variable regions, having an amino acid sequence as set forth in Table 1, or a sequence substantially identical thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, or which differs by no more than 1, 2, 5, 10, or 15 amino acid residues from the sequences shown in Table 1. In another embodiment, the anti-PD-1 antibody molecule includes a VH and/or VL domain encoded by a nucleic acid having a nucleotide sequence as set forth in Table 1, or a sequence substantially identical thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, or which differs by no more than 3, 6, 15, 30, or 45 nucleotides from the sequences shown in Table 1.

In yet another embodiment, the anti-PD-1 antibody molecule comprises at least one, two, or three CDRs from a heavy chain variable region having an amino acid sequence as set forth in Table 1, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions). In yet another embodiment, the anti-PD-1 antibody molecule comprises at least one, two, or three CDRs from a light chain variable region having an amino acid sequence as set forth in Table 1, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions). In yet another embodiment, the anti-PD-1 antibody molecule comprises at least one, two, three, four, five or six CDRs from heavy and light chain variable regions having an amino acid sequence as set forth in Table 1), or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one embodiment, the anti-PD-1 antibody molecule comprises at least one, two, or three CDRs and/or hypervariable loops from a heavy chain variable region having an amino acid sequence of an antibody described herein, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16,

BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E, as summarized in Table 1, or a sequence substantially identical thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions). In another embodiment, the anti-PD-1 antibody molecule comprises at least one, two, or three CDRs and/or hypervariable loops from a light chain variable region having an amino acid sequence of of an antibody described herein, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E, as summarized in Table 1, or a sequence substantially identical thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions). In one embodiment, the anti-PD-1 antibody molecule comprises all six CDRs and/or hypervariable loops described herein, *e.g.*, described in Table 1.

In one embodiment, the anti-PD-1 antibody molecule has a variable region that is identical in sequence, or which differs by 1, 2, 3, or 4 amino acids from a variable region described herein (*e.g.*, an FR region disclosed herein).

In one embodiment, the anti-PD-1 antibody molecule is a full antibody or fragment thereof (*e.g.*, a Fab, $F(ab')_2$, Fv, or a single chain Fv fragment (scFv)). In certain embodiments, the anti-PD-1 antibody molecule is a monoclonal antibody or an antibody with single specificity. The anti-PD-1 antibody molecule can also be a humanized, chimeric, camelid, shark, or an *in vitro*-generated antibody molecule. In one embodiment, the anti-PD-1 antibody molecule thereof is a humanized antibody molecule. The heavy and light chains of the anti-PD-1 antibody molecule can be full-length (*e.g.*, an antibody can include at least one, and preferably two, complete heavy chains, and at least one, and preferably two, complete light chains) or can include an antigen-binding fragment (*e.g.*, a Fab, $F(ab')_2$, Fv, a single chain Fv fragment, a single domain antibody, a diabody (dAb), a bivalent antibody, or bispecific antibody or fragment thereof, a single domain variant thereof, or a camelid antibody).

In certain embodiments, the anti-PD-1 antibody molecule is in the form of a bispecific or a multispecific antibody molecule. In one embodiment, the bispecific antibody molecule has a first binding specificity for PD-1 and a second binding specifity for TIM-3, LAG-3, CEACAM (*e.g.*, CEACAM-1, CEACAM-3, and/or CEACAM-5), PD-L1 or PD-L2. In one embodiment, the bispecific antibody molecule binds to PD-1 and TIM-3. In another embodiment, the bispecific antibody molecule binds to PD-1 and LAG-3. In another embodiment, the bispecific antibody molecule binds to PD-1 and CEACAM (*e.g.*, CEACAM-1, CEACAM-3, and/or CEACAM-5). In another embodiment, the bispecific antibody molecule binds to PD-1 and CEACAM-1. In yet another embodiment, the bispecific antibody molecule binds to PD-1 and CEACAM-5. In another embodiment, the bispecific antibody molecule binds to PD-1 and PD-L1. In yet another embodiment, the bispecific antibody molecule binds to PD-1 and PD-L2. Any combination of the aforesaid molecules can be made in a multispecific antibody molecule, *e.g.*, a trispecific antibody that includes a first binding specificity to PD-1, and a second and third binding specificity to one or more of: TIM-3, LAG-3, CEACAM (*e.g.*, CEACAM-1, CEACAM-3, or CEACAM-5), PD-L1 or PD-L2.

In other embodiments, the anti-PD-1 antibody molecule is used in combination with a bispecific molecule comprising one or more of: TIM-3, LAG-3, CEACAM (*e.g.*, CEACAM-1, CEACAM-3, or CEACAM-5), PD-L1 or PD-L2. In one embodiment, the bispecific antibody molecule used in combination binds to CEACAM (*e.g.*, CEACAM-1, CEACAM-3, and/or CEACAM-5) and LAG-3. In another embodiment, the bispecific antibody molecule used in combination binds to CEACAM (*e.g.*, CEACAM-1, CEACAM-3, and/or CEACAM-5) and TIM-3. In another embodiment, the bispecific antibody molecule used in combination binds to LAG-3 and TIM-3.

In yet other embodiments, the anti-PD-1 antibody molecule has a heavy chain constant region (Fc) chosen from, *e.g.*, the heavy chain constant regions of IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE; particularly, chosen from, *e.g.*, the heavy chain constant regions of IgG1, IgG2, IgG3, and IgG4, more particularly, the heavy chain constant region of IgG1 or IgG2 (*e.g.*, human IgG1, IgG2 or IgG4). In one embodiment, the heavy chain constant region is human IgG1. In another embodiment, the anti-PD-1 antibody molecule has a light chain constant region chosen from, *e.g.*, the light chain constant regions of kappa or lambda, preferably kappa (*e.g.*, human kappa). In one embodiment, the constant region is altered, *e.g.*, mutated, to modify the

properties of the anti-PD-1 antibody molecule (*e.g.*, to increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, effector cell function, or complement function). For example, the constant region is mutated at positions 296 (M to Y), 298 (S to T), 300 (T to E), 477 (H to K) and 478 (N to F) to alter Fc receptor binding (*e.g.*, the mutated positions correspond to positions 132 (M to Y), 134 (S to T), 136 (T to E), 313 (H to K) and 314 (N to F) of SEQ ID NOs: 212 or 214; or positions 135 (M to Y), 137 (S to T), 139 (T to E), 316 (H to K) and 317 (N to F) of SEQ ID NOs: 215, 216, 217 or 218). In another embodiment, the heavy chain constant region of an IgG4, *e.g.*, a human IgG4, is mutated at position 228 according to EU numbering (*e.g.*, S to P), *e.g.*, as shown in Table 3. In certain embodiments, the anti-PD-1 antibody molecules comprises a human IgG4 mutated at position 228 according to EU numbering (*e.g.*, S to P), *e.g.*, as shown in Table 3; and a kappa light chain constant region, *e.g.*, as shown in Table 3. In still another embodiment, the heavy chain constant region of an IgG1, *e.g.*, a human IgG1, is mutated at one or more of position 297 according to EU numbering (*e.g.*, N to A), position 265 according to EU numbering (*e.g.*, D to A), position 329 according to EU numbering (*e.g.*, P to A), position 234 according to EU numbering (*e.g.*, L to A), or position 235 according to EU numbering (*e.g.*, L to A), *e.g.*, as shown in Table 3. In certain embodiments, the anti-PD-1 antibody molecules comprises a human IgG1 mutated at one or more of the aforesaid positions, *e.g.*, as shown in Table 3; and a kappa light chain constant region, *e.g.*, as shown in Table 3.

In one embodiment, the anti-PD-1 antibody molecule is isolated or recombinant.

In one embodiment, the anti-PD-1 antibody molecule is a humanized antibody molecule.

In one embodiment, the anti-PD-1 antibody molecule has a risk score based on T cell epitope analysis of less than 700, 600, 500, 400 or less.

In one embodiment, the anti-PD-1 antibody molecue is a humanized antibody molecule and has a risk score based on T cell epitope analysis of 300 to 700, 400 to 650, 450 to 600, or a risk score as described herein.

The invention also features a nucleic acid molecule that comprise one or both nucleotide sequences that encode heavy and light chain variable regions, CDRs, hypervariable loops, framework regions of the anti-PD-1 antibody molecules, as described herein. In certain embodiments, the nucleotide sequence that encodes the anti-PD-1 antibody molecule is codon optimized. For example, the invention features a first and second nucleic acid encoding heavy

and light chain variable regions, respectively, of an anti-PD-1 antibody molecule chosen from one or more of, *e.g.*, any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E, as summarized in Table 1, or a sequence substantially identical thereto. For example, the nucleic acid can comprise a nucleotide sequence as set forth in Tables 1 and 2, or a sequence substantially identical thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, or which differs by no more than 3, 6, 15, 30, or 45 nucleotides from the sequences shown in Tables 1 and 2).

In other embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes a heavy chain variable domain and/or a heavy chain constant region comprising the amino acid sequence of BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1; or the nucleotide sequence in Table 1; or a sequence substantially identical (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical) to any of the aforesaid sequences.

In other embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes a light chain variable domain and/or a light chain constant region comprising the amino acid sequence of BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1; or the nucleotide sequence in Table 1; or a sequence substantially identical (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical) to any of the aforesaid sequences.

The aforesaid nucleotide sequences encoding the anti-PD-1 heavy and light chain variable domain and constant regions can be present in a separate nucleic acid molecule, or in the same nucleic acid molecule. In certain embodiments, the nucleic acid molecules comprise a nucleotide sequence encoding a leader sequence, *e.g.*, a leader sequence as shown in Table 4, or a sequence substantially identitical thereto.

In certain embodiments, the nucleic acid molecule comprises a nucleotide sequence encoding at least one, two, or three CDRs, or hypervariable loops, from a heavy chain variable region having an amino acid sequence as set forth in Table 1, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical

thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In another embodiment, the nucleic acid molecule comprises a nucleotide sequence encoding at least one, two, or three CDRs, or hypervariable loops, from a light chain variable region having an amino acid sequence as set forth in Table 1, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In yet another embodiment, the nucleic acid molecule comprises a nucleotide sequence encoding at least one, two, three, four, five, or six CDRs, or hypervariable loops, from heavy and light chain variable regions having an amino acid sequence as set forth in Table 1, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one embodiment, the nucleic acid molecule includes a nucleotide sequence encoding an anti-PD-1 antibody molecule that includes a substitution in the light chain CDR3 at position 102 of the light variable region, *e.g.*, a substitution of a cysteine to tyrosine, or a cysteine to serine residue, at position 102 of the light variable region according to Table 1 (*e.g.*, SEQ ID NO: 16 or 24 for murine or chimeric, unmodified; or any of SEQ ID NOs: 34, 42, 46, 54, 58, 62, 66, 70, 74, or 78 for a modified sequence).

In another embodiment, the nucleic acid molecule includes one or more heavy chain framework region (*e.g.*, any of VHFW1 (type a), VHFW1 (type b), VHFW2 (type a), VHFW2 (type b), VHFW2 (type c), VHFW3 (type a), VHFW3 (type b), or VHFW4, or any combination thereof, *e.g.*, a framework combination as described herein) for any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E, as summarized in Table 1 and 2, or a sequence substantially identical thereto. For example, the nucleic acid molecule can comprise a nucleotide sequence as set forth in Tables 1 and 2, or a sequence substantially identical thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or

more identical thereto, or which differs by no more than 3, 6, 15, 30, or 45 nucleotides from the sequences shown in Tables 1 and 2).

In another embodiment, the nucleic acid molecule includes one or more light chain framework region (*e.g.*, any of VLFW1 (type a), VLFW1 (type b), VLFW1 (type c), VLFW1 (type d), VLFW1 (type e), VLFW2 (type a), VLFW2 (type b), VLFW2 (type c), VLFW3 (type a), VLFW3 (type b), VLFW3 (type c), VLFW3 (type d), or VLFW4, or any combination thereof, *e.g.*, a framework combination as described herein) for any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E, as summarized in Table 1 and 2, or a sequence substantially identical thereto. For example, the nucleic acid molecule can comprise a nucleotide sequence as set forth in Tables 1 and 2, or a sequence substantially identical thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, or which differs by no more than 3, 6, 15, 30, or 45 nucleotides from the sequences shown in Tables 1 and 2).

In another embodiment, the nucleic acid molecule includes one or more heavy chain framework region and one or more light chain framework region as described herein. The heavy and light chain framework regions may be present in the same vector or separate vectors.

In another aspect, the application features host cells and vectors containing the nucleic acids described herein. The nucleic acids may be present in a single vector or separate vectors present in the same host cell or separate host cell. The host cell can be a eukaryotic cell, *e.g.*, a mammalian cell, an insect cell, a yeast cell, or a prokaryotic cell, *e.g.*, *E. coli*. For example, the mammalian cell can be a cultured cell or a cell line. Exemplary mammalian cells include lymphocytic cell lines (*e.g.*, NSO), Chinese hamster ovary cells (CHO), COS cells, oocyte cells, and cells from a transgenic animal, *e.g.*, mammary epithelial cell.

In one aspect, the invention features a method of providing an antibody molecule described herein. The method includes: providing a PD-1 antigen (*e.g.*, an antigen comprising at least a portion of a PD-1 epitope); obtaining an antibody molecule that specifically binds to the PD-1 polypeptide; and evaluating if the antibody molecule specifically binds to the PD-1

polypeptide, or evaluating efficacy of the antibody molecule in modulating, *e.g.*, inhibiting, the activity of the PD-1. The method can further include administering the antibody molecule to a subject, *e.g.*, a human or non-human animal.

In another aspect, the invention provides, compositions, *e.g.*, pharmaceutical compositions, which include a pharmaceutically acceptable carrier, excipient or stabilizer, and at least one of the anti-PD-1 antibody molecules described herein. In one embodiment, the composition, *e.g.*, the pharmaceutical composition, includes a combination of the antibody molecule and one or more agents, *e.g.*, a therapeutic agent or other antibody molecule, as described herein. In one embodiment, the antibody molecule is conjugated to a label or a therapeutic agent.

The anti-PD-1 antibody molecules disclosed herein can inhibit, reduce or neutralize one or more activities of PD-1, resulting in blockade or reduction of an immune checkpoint. In one embodiment, the antibody molecule results in one or more of: an increase in tumor infiltrating lymphocytes, an increase in T-cell receptor mediated proliferation, a decrease in immune evasion by cancerous cells, restoration of effector cell function (*e.g.*, one or more of T cell proliferation, IFN-γ secretion or cytolytic function), inhibition of regulatory T cell function, or an effect on the activity of multiple cell types, such as regulatory T cell, effector T cells and NK cells). Thus, such antibody molecules can be used to treat or prevent disorders where enhancing an immune response in a subject is desired.

Uses of the Anti-PD-1 Antibody Molecules

Accordingly, in another aspect, a method of modulating an immune response in a subject is provided. The method comprises administering to the subject an anti-PD-1 antibody molecule disclosed herein (*e.g.,* a therapeutically effective amount of an anti-PD-1 antibody molecule), alone or in combination with one or more agents or procedures, such that the immune response in the subject is modulated. In one embodiment, the antibody molecule enhances, stimulates or increases the immune response in the subject. The subject can be a mammal, *e.g.*, a primate, preferably a higher primate, *e.g.*, a human (*e.g.*, a patient having, or at risk of having, a disorder described herein). In one embodiment, the subject is in need of enhancing an immune response.

In one embodiment, the subject has, or is at risk of, having a disorder described herein, *e.g.*, a cancer or an infectious disorder as described herein. In certain embodiments, the subject is, or is at risk of being, immunocompromised. For example, the subject is undergoing or has undergone a chemotherapeutic treatment and/or radiation therapy. Alternatively, or in combination, the subject is, or is at risk of being, immunocompromised as a result of an infection.

In one aspect, a method of treating (*e.g.*, one or more of reducing, inhibiting, or delaying progression) a cancer or a tumor in a subject is provided. The method comprises administering to the subject an anti-PD-1 antibody molecule described herein, *e.g.*, a therapeutically effective amount of an anti-PD-1 antibody molecule, alone or in combination with one or more agents or procedures. In certain embodiments, the anti-PD-1 antibody molecule is administered in combination with a modulator of a costimulatory molecule (*e.g.*, an agonist of a costimulatory molecule) or a modulator of an inhibitory molecule (*e.g.*, an inhibitor of an immune checkpoint inhibitor), *e.g.*, as described herein.

In certain embodiments, the cancer treated with the anti-PD-1 antibody molecule, includes but is not limited to, a solid tumor, a hematological cancer (*e.g.*, leukemia, lymphoma, myeloma, *e.g.*, multiple myeloma), and a metastatic lesion. In one embodiment, the cancer is a solid tumor. Examples of solid tumors include malignancies, *e.g.*, sarcomas and carcinomas, *e.g.*, adenocarcinomas of the various organ systems, such as those affecting the lung, breast, ovarian, lymphoid, gastrointestinal (*e.g.*, colon), anal, genitals and genitourinary tract (*e.g.*, renal, urothelial, bladder cells, prostate), pharynx, CNS (*e.g.*, brain, neural or glial cells), head and neck, skin (*e.g.*, melanoma), and pancreas, as well as adenocarcinomas which include malignancies such as colon cancers, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell lung cancer, cancer of the small intestine and cancer of the esophagus. The cancer may be at an early, intermediate, late stage or metastatic cancer.

In one embodiment, the cancer is chosen from a lung cancer (*e.g.*, a non-small cell lung cancer (NSCLC) (*e.g.*, a NSCLC with squamous and/or non-squamous histology, or a NSCLC adenocarcinoma)), a melanoma (*e.g.*, an advanced melanoma), a renal cancer (*e.g.*, a renal cell carcinoma), a liver cancer, a myeloma (*e.g.*, a multiple myeloma), a prostate cancer, a breast cancer (*e.g.*, a breast cancer that does not express one, two or all of estrogen receptor, progesterone receptor, or Her2/neu, *e.g.*, a triple negative breast cancer), a colorectal cancer, a pancreatic cancer, a head and neck cancer (*e.g.*, head and neck squamous cell carcinoma

(HNSCC), anal cancer, gastro-esophageal cancer, thyroid cancer, cervical cancer, a lymphoproliferative disease (*e.g.*, a post-transplant lymphoproliferative disease) or a hematological cancer, T-cell lymphoma, B-cell lymphoma, a non-Hogdkin lymphoma, or a leukemia (*e.g.*, a myeloid leukemia or a lymphoid leukemia).

In another embodiment, the cancer is chosen form a carcinoma (*e.g.*, advanced or metastatic carcinoma), melanoma or a lung carcinoma, *e.g.*, a non-small cell lung carcinoma.

In one embodiment, the cancer is a lung cancer, *e.g.*, a non-small cell lung cancer or small cell lung cancer.

In one embodiment, the cancer is a melanoma, *e.g.*, an advanced melanoma. In one embodiment, the cancer is an advanced or unresectable melanoma that does not respond to other therapies. In other embodiments, the cancer is a melanoma with a BRAF mutation (*e.g.*, a BRAF V600 mutation). In yet other embodiments, the anti-PD-1 antibody molecule is administered after treatment with an anti-CTLA4 antibody (*e.g.*, ipilimumab) with or without a BRAF inhibitor (*e.g.*, vemurafenib or dabrafenib).

In another embodiment, the cancer is a hepatocarcinoma, *e.g.*, an advanced hepatocarcinoma, with or without a viral infection, *e.g.*, a chronic viral hepatitis.

In another embodiment, the cancer is a prostate cancer, *e.g.*, an advanced prostate cancer.

In yet another embodiment, the cancer is a myeloma, *e.g.*, multiple myeloma.

In yet another embodiment, the cancer is a renal cancer, *e.g.*, a renal cell carcinoma (RCC) (*e.g.*, a metastatic RCC or clear cell renal cell carcinoma (CCRCC)).

In one embodiment, the cancer microenvironment has an elevated level of PD-L1 expression. Alternatively, or in combination, the cancer microenvironment can have increased IFNγ and/or CD8 expression.

In some embodiments, the subject has, or is identified as having, a tumor that has one or more of high PD-L1 level or expression, or as being Tumor Infiltrating Lymphocyte (TIL)+ (*e.g.*, as having an increased number of TILs), or both. In certain embodiments, the subject has, or is identified as having, a tumor that has high PD-L1 level or expression and that is TIL+. In some embodiments, the methods described herein further include identifying a subject based on having a tumor that has one or more of high PD-L1 level or expression, or as being TIL+, or both. In certain embodiments, the methods described herein further include identifying a subject based on having a tumor that has high PD-L1 level or expression and as being TIL+. In some

embodiments, tumors that are TIL+ are positive for CD8 and IFNγ. In some embodiments, the subject has, or is identified as having, a high percentage of cells that are positive for one, two or more of PD-L1, CD8, and/or IFNγ. In certain embodiments, the subject has or is identified as having a high percentage of cells that are positive for all of PD-L1, CD8, and IFNγ.

In some embodiments, the methods described herein further include identifying a subject based on having a high percentage of cells that are positive for one, two or more of PD-L1, CD8, and/or IFNγ. In certain embodiments, the methods described herein further include identifying a subject based on having a high percentage of cells that are positive for all of PD-L1, CD8, and IFNγ. In some embodiments, the subject has, or is identified as having, one, two or more of PD-L1, CD8, and/or IFNγ, and one or more of a lung cancer, *e.g.*, squamous cell lung cancer or lung adenocarcinoma; a head and neck cancer; a squamous cell cervical cancer; a stomach cancer; an esophageal cancer; a thyroid cancer; a melanoma, and/or a nasopharyngeal cancer (NPC). In certain embodiments, the methods described herein further describe identifying a subject based on having one, two or more of PD-L1, CD8, and/or IFNγ, and one or more of a lung cancer, *e.g.*, squamous cell lung cancer or lung adenocarcinoma; a head and neck cancer; a squamous cell cervical cancer; a stomach cancer; a thyroid cancer; a melanoma, and or a nasopharyngeal cancer.

Methods and compositions disclosed herein are useful for treating metastatic lesions associated with the aforementioned cancers.

In a further aspect, the invention provides a method of treating an infectious disease in a subject, comprising administering to a subject a therapeutically effective amount of an anti-PD-1 antibody molecule described herein, alone or in combination with one or more agents or procedures. In one embodiment, the infection disease is chosen from hepatitis (*e.g.*, hepatis C infection), or sepsis.

Still further, the invention provides a method of enhancing an immune response to an antigen in a subject, comprising administering to the subject: (i) the antigen; and (ii) an anti-PD-1 antibody molecule, such that an immune response to the antigen in the subject is enhanced. The antigen can be, for example, a tumor antigen, a viral antigen, a bacterial antigen or an antigen from a pathogen.

The anti-PD-1 antibody molecule can be administered to the subject systemically (*e.g.*, orally, parenterally, subcutaneously, intravenously, rectally, intramuscularly, intraperitoneally,

intranasally, transdermally, or by inhalation or intracavitary installation), topically, or by application to mucous membranes, such as the nose, throat and bronchial tubes.

Dosages and therapeutic regimens of the anti-PD-1 antibody molecule can be determined by a skilled artisan. In certain embodiments, the anti-PD-1 antibody molecule is administered by injection (*e.g.*, subcutaneously or intravenously) at a dose of about 1 to 30 mg/kg, *e.g.*, about 5 to 25 mg/kg, about 10 to 20 mg/kg, about 1 to 5 mg/kg, or about 3 mg/kg. The dosing schedule can vary from *e.g.*, once a week to once every 2, 3, or 4 weeks. In one embodiment, the anti-PD-1 antibody molecule is administered at a dose from about 10 to 20 mg/kg every other week.

The antibody molecules described herein are preferred for use in the methods described herein, although other anti-PD-1 antibodies can be used instead, or in combination with an anti-PD-1 antibody molecule of the invention.

Combination therapies

The methods and compositions described herein can be used in combination with other agents or therapeutic modalities. In one embodiment, the methods described herein include administering to the subject an anti-PD-1 antibody molecule as described herein, in combination with an agent or therapeutic procedure or modality, in an amount effective to treat or prevent a disorder. The anti-PD-1 antibody molecule and the agent or therapeutic procedure or modality can be administered simultaneously or sequentially in any order. Any combination and sequence of the anti-PD-1 antibody molecules and other therapeutic agents, procedures or modalities (*e.g.*, as described herein) can be used. The antibody molecule and/or other therapeutic agents, procedures or modalities can be administered during periods of active disorder, or during a period of remission or less active disease. The antibody molecule can be administered before the other treatment, concurrently with the treatment, post-treatment, or during remission of the disorder.

In certain embodiments, the methods and compositions described herein are administered in combination with one or more of other antibody molecules, chemotherapy, other anti-cancer therapy (*e.g.*, targeted anti-cancer therapies, gene therapy, viral therapy, RNA therapy bone marrow transplantation, nanotherapy, or oncolytic drugs), cytotoxic agents, immune-based therapies (*e.g.*, cytokines or cell-based immune therapies), surgical procedures (*e.g.*, lumpectomy or mastectomy) or radiation procedures, or a combination of any of the foregoing. The

additional therapy may be in the form of adjuvant or neoadjuvant therapy. In some embodiments, the additional therapy is an enzymatic inhibitor (*e.g.*, a small molecule enzymatic inhibitor) or a metastatic inhibitor. Exemplary cytotoxic agents that can be administered in combination with include antimicrotubule agents, topoisomerase inhibitors, anti-metabolites, mitotic inhibitors, alkylating agents, anthracyclines, vinca alkaloids, intercalating agents, agents capable of interfering with a signal transduction pathway, agents that promote apoptosis, proteosome inhibitors, and radiation (*e.g.*, local or whole body irradiation (*e.g.*, gamma irradiation). In other embodiments, the additional therapy is surgery or radiation, or a combination thereof. In other embodiments, the additional therapy is a therapy targeting one or more of PI3K/AKT/mTOR pathway, an HSP90 inhibitor, or a tubulin inhibitor.

Alternatively, or in combination with the aforesaid combinations, the methods and compositions described herein can be administered in combination with one or more of: an immunomodulator (*e.g.*, an activator of a costimulatory molecule or an inhibitor of an inhibitory molecule, *e.g.,* an immune checkpoint molecule); a vaccine, *e.g.*, a therapeutic cancer vaccine; or other forms of cellular immunotherapy.

Exemplary non-limiting combinations and uses of the anti-PD-1 antibody molecules include the following.

In certain embodiments, the anti-PD-1 antibody molecule is administered in combination with a modulator of a costimulatory molecule or an inhibitory molecule, *e.g.*, a co-inhibitory ligand or receptor.

In one embodiment, the anti-PD-1 antibody molecule is administered in combination with a modulator, *e.g.*, agonist, of a costimulatory molecule. In one embodiment, the agonist of the costimulatory molecule is chosen from an agonist (*e.g.*, an agonistic antibody or antigen-binding fragment thereof, or a soluble fusion) of OX40, CD2, CD27, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD30, CD40, BAFFR, HVEM, CD7, LIGHT, NKG2C, SLAMF7, NKp80, CD160, B7-H3 or CD83 ligand.

In one embodiment, the anti-PD-1 antibody molecule is administered in combination with an inhibitor of an inhibitory (or immune checkpoint) molecule chosen from PD-L1, PD-L2, CTLA-4, TIM-3, LAG-3, CEACAM (*e.g.*, CEACAM-1, CEACAM-3, and/or CEACAM-5), VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 and/or TGFR beta. Inhibition of an inhibitory molecule can be performed by inhibition at the DNA, RNA or protein level. In embodiments, an

inhibitory nucleic acid (*e.g.*, a dsRNA, siRNA or shRNA), can be used to inhibit expression of an inhibitory molecule. In other embodiments, the inhibitor of an inhibitory signal is, a polypeptide *e.g.*, a soluble ligand, or an antibody or antigen-binding fragment thereof, that binds to the inhibitory molecule. In one embodiment, the inhibitor is a soluble ligand (*e.g.*, a CTLA-4-Ig), or an antibody or antibody fragment that binds to PD-L1, PD-L2 or CTLA-4. For example, the anti-PD-1 antibody molecule can be administered in combination with an anti-CTLA-4 antibody, *e.g.*, ipilimumab, for example, to treat a cancer (*e.g.*, a cancer chosen from: a melanoma, *e.g.*, a metastatic melanoma; a lung cancer, *e.g.*, a non-small cell lung carcinoma; or a prostate cancer). In one embodiment, the anti-PD-1 antibody molecule is administered after treatment with an anti-CTLA-4 antibody (*e.g.*, ipilimumab) with or without a BRAF inhibitor (*e.g.*, vemurafenib or dabrafenib).

In another embodiment, the anti-PD-1 antibody molecule is administered in combination with an anti-LAG-3 antibody or antigen-binding fragment thereof.

In another embodiment, the anti-PD-1 antibody molecule is administered in combination with an anti-TIM-3 antibody or antigen-binding fragment thereof.

In yet other embodiments, the anti-PD-1 antibody molecule is administered in combination with an anti-LAG-3 antibody and an anti-TIM-3 antibody (or antigen-binding fragments thereof).

In another embodiment, the anti-PD-1 antibody molecule is administered in combination with a CEACAM inhibitor (*e.g.*, CEACAM-1 and/or CEACAM-5 inhibitor), *e.g.*, an anti-CEACAM antibody molecule. In another embodiment, the anti-PD-1 antibody molecule is administered in combination with a CEACAM-1 inhibitor, *e.g.*, an anti- CEACAM-1 antibody molecule. In another embodiment, the anti-PD-1 antibody molecule is administered in combination with a CEACAM-5 inhibitor, *e.g.*, an anti- CEACAM-5 antibody molecule.

The combination of antibodies recited herein can be administered separately, *e.g.*, as separate antibodies or antigen-binding fragments thereof, or linked, *e.g.*, as a bispecific or trispecific antibody molecule. In one embodiment, a bispecific antibody that includes an anti-PD-1 antibody molecule and an anti-TIM-3, anti-CEACAM (*e.g.*, anti-CEACAM-1, CEACAM-3, and/or anti-CEACAM-5), or anti-LAG-3 antibody, or an antigen-binding fragment thereof, is administered. In certain embodiments, the combination of antibodies recited herein is used to

treat a cancer, *e.g.*, a cancer as described herein (*e.g.*, a solid tumor or a hematologic malignancy).

In other embodiments, the anti-PD-1 antibody molecule is administered in combination with a cytokine. The cytokine can be administered as a fusion molecule to the anti-PD-1 antibody molecule, or as separate compositions. In one embodiment, the anti-PD-1 antibody is administered in combination with one, two, three or more cytokines, *e.g.*, as a fusion molecule or as separate compositions. In one embodiment, the cytokine is an interleukin (IL) chosen from one, two, three or more of IL-1, IL-2, IL-12, IL-15 or IL-21. In one embodiment, a bispecific antibody molecule has a first binding specificity to a first target (*e.g.,* to PD-1), a second binding specificity to a second target (*e.g.,* LAG-3 or TIM-3), and is optionally linked to an interleukin (*e.g.,* IL-12) domain *e.g.,* full length IL-12 or a portion thereof. In certain embodiments, the combination of anti-PD-1 antibody molecule and the cytokine described herein is used to treat a cancer, *e.g.*, a cancer as described herein (*e.g.*, a solid tumor).

In certain embodiments, the anti-PD-1 antibody molecule is administered in combination with an antibody specific against an HLA C, *e.g.*, an antibody specific to Killer-cell Immunoglobulin-like Receptors (also referred to herein as an "anti-KIR antibody"). In certain embodiments, the combination of anti-PD-1 antibody molecule and anti-KIR antibody is used to treat a cancer, *e.g.*, a cancer as described herein (*e.g.*, a solid tumor, *e.g.*, an advanced solid tumor).

In one embodiment, the anti-PD-1 antibody molecule is administered in combination with a cellular immunotherapy (*e.g.*, Provenge® (*e.g.*, Sipuleucel-T)), and optionally in combination with cyclophosphamide. In certain embodiments, the combination of anti-PD-1 antibody molecule, Provenge® and/or cyclophosphamide is used to treat a cancer, *e.g.*, a cancer as described herein (*e.g.*, a prostate cancer, *e.g.*, an advanced prostate cancer).

In another embodiment, the anti-PD-1 antibody molecule is administered in combination with a vaccine, *e.g.*, a cancer vaccine, (*e.g.*, a dendritic cell renal carcinoma (DC-RCC) vaccine). In one embodiment, the vaccine is peptide-based, DNA-based, RNA-based, or antigen-based, or a combination thereof. In embodiments, the vaccine comprises one or more peptides, nucleic acids (*e.g.*, DNA or RNA), antigens, or a combination thereof. In certain embodiments, the combination of anti-PD-1 antibody molecule and the DC-RCC vaccine is used to treat a cancer,

e.g., a cancer as described herein (*e.g.*, a renal carcinoma, *e.g.*, metastatic renal cell carcinoma (RCC) or clear cell renal cell carcinoma (CCRCC)).

In another embodiment, the anti-PD-1 antibody molecule is administered in combination with an adjuvant.

In yet another embodiment, the anti-PD-1 antibody molecule is administered in combination with chemotherapy, and/or immunotherapy. For example, the anti-PD-1 antibody molecule can be used to treat a myeloma, alone or in combination with one or more of: chemotherapy or other anti-cancer agents (*e.g.*, thalidomide analogs, *e.g.*, lenalidomide), an anti-TIM-3 antibody, tumor antigen-pulsed dendritic cells, fusions (*e.g.*, electrofusions) of tumor cells and dendritic cells, or vaccination with immunoglobulin idiotype produced by malignant plasma cells. In one embodiment, the anti-PD-1 antibody molecule is used in combination with an anti-TIM-3 antibody to treat a myeloma, *e.g.*, a multiple myeloma.

In one embodiment, the anti-PD-1 antibody molecule is used in combination with chemotherapy to treat a lung cancer, *e.g.*, non-small cell lung cancer. In one embodiment, the anti-PD-1 antibody molecule is used with standard lung, *e.g.*, NSCLC, chemotherapy, *e.g.*, platinum doublet therapy, to treat lung cancer. In yet other embodiments, the anti-PD-1 antibody molecule is used in combination with an indoleamine-pyrrole 2,3-dioxygenase (IDO) inhibitor (*e.g.*, (4E)-4-[(3-chloro-4-fluoroanilino)-nitrosomethylidene]-1,2,5-oxadiazol-3-amine (also known as INCB24360), indoximod (1-methyl-D-tryptophan), α-cyclohexyl-5H-Imidazo[5,1-a]isoindole-5-ethanol (also known as NLG919), etc.) in a subject with advanced or metastatic cancer (*e.g.*, a patient with metastic and recurrent NSCL cancer).

In yet other embodiments, the anti-PD-1 antibody molecule is used in combination with one or more of: an immune-based strategy (*e.g.*, interleukin-2 or interferon-α), a targeting agent (*e.g.*, a VEGF inhibitor such as a monoclonal antibody to VEGF); a VEGF tyrosine kinase inhibitor such as sunitinib, sorafenib, axitinib and pazopanib; an RNAi inhibitor; or an inhibitor of a downstream mediator of VEGF signaling, *e.g.*, an inhibitor of the mammalian target of rapamycin (mTOR), *e.g.*, everolimus and temsirolimus. Any of such combinations can be used to treat a renal cancer, *e.g.*, renal cell carcinoma (RCC) (*e.g.*, clear cell renal cell carcinoma (CCRCC)) or metastatic RCC.

In some embodiments, the anti-PD-1 antibody molecule, *e.g.*, the anti-PD-1 antibody molecule described herein, is used in combination with a MEK inhibitor (*e.g.*, a MEK inhibitor

as described herein). In some embodiments, the combination of the anti-PD-1 antibody and the MEK inhibitor is used to treat a cancer (*e.g.*, a cancer described herein). In some embodiments, the cancer treated with the combination is chosen from a melanoma, a colorectal cancer, a non-small cell lung cancer, an ovarian cancer, a breast cancer, a prostate cancer, a pancreatic cancer, a hematological malignancy or a renal cell carcinoma. In certain embodiments, the cancer includes a BRAF mutation (*e.g.*, a BRAF V600E mutation), a BRAF wildtype, a KRAS wildtype or an activating KRAS mutation. The cancer may be at an early, intermediate or late stage.

In another embodiment, the anti-PD-1 antibody molecule is used in combination with one, two or all of oxaliplatin, leucovorin or 5-FU (*e.g.*, a FOLFOX co-treatment). Alternatively or in combination, combination further includes a VEGF inhibitor (*e.g.*, a VEGF inhibitor as disclosed herein). In some embodiments, the combination of the anti-PD-1 antibody, the FOLFOX co-treatment, and the VEGF inhibitor is used to treat a cancer (*e.g.*, a cancer described herein). In some embodiments, the cancer treated with the combination is chosen from a melanoma, a colorectal cancer, a non-small cell lung cancer, an ovarian cancer, a breast cancer, a prostate cancer, a pancreatic cancer, a hematological malignancy or a renal cell carcinoma. The cancer may be at an early, intermediate or late stage.

In other embodiments, the anti-PD-1 antibody molecule is administered with a tyrosine kinase inhibitor (*e.g.*, axitinib) to treat renal cell carcinoma and other solid tumors.

In other embodiments, the anti-PD-1 antibody molecule is administered with a 4-1BB receptor targeting agent (*e.g.*, an antibody that stimulates signaling through 4-1BB (CD-137), *e.g.*, PF-2566). In one embodiment, the anti-PD-1 antibody molecule is administered in combination with a tyrosine kinase inhibitor (*e.g.*, axitinib) and a 4-1BB receptor targeting agent.

The anti-PD-1 antibody molecule can be bound to a substance, *e.g.*, a cytotoxic agent or moiety (*e.g.*, a therapeutic drug; a compound emitting radiation; molecules of plant, fungal, or bacterial origin; or a biological protein (*e.g.*, a protein toxin) or particle (*e.g.*, a recombinant viral particle, *e.g.*, via a viral coat protein). For example, the antibody can be coupled to a radioactive isotope such as an α-, β-, or γ-emitter, or a β-and γ-emitter.

Any combination and sequence of the anti-PD-1 antibody molecules and other therapeutic agents, procedures or modalities (*e.g.*, as described herein) can be used. The antibody molecule and/or other therapeutic agents, procedures or modalities can be administered during periods of active disorder, or during a period of remission or less active disease. The

antibody molecule can be administered before the other treatment, concurrently with the treatment, post-treatment, or during remission of the disorder.

Additional Combination Therapies

The methods and compositions described herein (*e.g.*, PD-1 antibodies and methods of using them) can be used in combination with other agents or therapeutic modalities, *e.g.*, a second therapeutic agent chosen from one or more of the agents listed in Table 7. In one embodiment, the methods described herein include administering to the subject an anti-PD-1 antibody molecule as described herein (optionally in combination with one or more inhibitors of PD-L1, LAG-3, TIM-3, CEACAM (*e.g.*, CEACAM-1 and/or CEACAM-5), or CTLA-4)), further include administration of a second therapeutic agent chosen from one or more of the agents listed in Table 7, in an amount effective to treat or prevent a disorder, *e.g.*, a disorder as described herein, *e.g.*, a cancer. When administered in combination, the anti-PD-1 antibody molecule, the additional agent (*e.g.*, second or third agent), or all, can be administered in an amount or dose that is higher, lower or the same than the amount or dosage of each agent used individually, *e.g.*, as a monotherapy. In certain embodiments, the administered amount or dosage of the anti-PD-1 antibody, the additional agent (*e.g.*, second or third agent), or all, is lower (*e.g.*, at least 20%, at least 30%, at least 40%, or at least 50%) than the amount or dosage of each agent used individually, *e.g.*, as a monotherapy. In other embodiments, the amount or dosage of the anti-PD-1 antibody, the additional agent (*e.g.*, second or third agent), or all, that results in a desired effect (*e.g.*, treatment of cancer) is lower (*e.g.*, at least 20%, at least 30%, at least 40%, or at least 50% lower).

In other embodiments, the second therapeutic agent is chosen from one or more of the agents listed in Table 7. In one embodiment, the cancer is chosen from a lung cancer (*e.g.*, a non-small cell lung cancer (NSCLC) (*e.g.*, a NSCLC with squamous and/or non-squamous histology, or a NSCLC adenocarcinoma), or disclosed in a publication listed in Table 7. In some embodiments, the second therapeutic agent is chosen from one or more of: 1) a protein kinase C (PKC) inhibitor; 2) a heat shock protein 90 (HSP90) inhibitor; 3) an inhibitor of a phosphoinositide 3-kinase (PI3K) and/or target of rapamycin (mTOR); 4) an inhibitor of cytochrome P450 (*e.g.*, a CYP17 inhibitor or a 17alpha-Hydroxylase/C17-20 Lyase inhibitor); 5) an iron chelating agent; 6) an aromatase inhibitor; 7) an inhibitor of p53, *e.g.*, an inhibitor of a

p53/Mdm2 interaction; 8) an apoptosis inducer; 9) an angiogenesis inhibitor; 10) an aldosterone synthase inhibitor; 11) a smoothened (SMO) receptor inhibitor; 12) a prolactin receptor (PRLR) inhibitor; 13) a Wnt signaling inhibitor; 14) a CDK4/6 inhibitor; 15) a fibroblast growth factor receptor 2 (FGFR2)/fibroblast growth factor receptor 4 (FGFR4) inhibitor; 16) an inhibitor of macrophage colony-stimulating factor (M-CSF); 17) an inhibitor of one or more of c-KIT, histamine release, Flt3 (*e.g.*, FLK2/STK1) or PKC; 18) an inhibitor of one or more of VEGFR-2 (*e.g.*, FLK-1/KDR), PDGFRbeta, c-KIT or Raf kinase C; 19) a somatostatin agonist and/or a growth hormone release inhibitor; 20) an anaplastic lymphoma kinase (ALK) inhibitor; 21) an insulin-like growth factor 1 receptor (IGF-1R) inhibitor; 22) a P-Glycoprotein 1 inhibitor; 23) a vascular endothelial growth factor receptor (VEGFR) inhibitor; 24) a BCR-ABL kinase inhibitor; 25) an FGFR inhibitor; 26) an inhibitor of CYP11B2; 27) a HDM2 inhibitor, *e.g.*, an inhibitor of the HDM2-p53 interaction; 28) an inhibitor of a tyrosine kinase; 29) an inhibitor of c-MET; 30) an inhibitor of JAK; 31) an inhibitor of DAC; 32) an inhibitor of 11β-hydroxylase; 33) an inhibitor of IAP; 34) an inhibitor of PIM kinase; 35) an inhibitor of Porcupine; 36) an inhibitor of BRAF, *e.g.*, BRAF V600E or wild-type BRAF; 37) an inhibitor of HER3; 38) an inhibitor of MEK; or 39) an inhibitor of a lipid kinase, *e.g.*, as described herein and in Table 7.

In one embodiment, the second therapeutic agent is chosen from one or more of: Compound A8, Compound A17, Compound A23, Compound A24, Compound A27, Compound A29, Compound A33, and Compound A13.

In other embodiments, the second therapeutic agent is chosen from one or more of: Compound A5, Compound A8, Compound A17, Compound A23, Compound A24, Compound A29, and Compound A40.

In other embodiments, the second therapeutic agent is chosen from one or more of: Compound A9, Compound A16, Compound A17, Compound A21, Compound A22, Compound A25, Compound A28, Compound A48, and Compound 49.

In embodiments, the second therapeutic agent is administered at a therapeutic or lower-than therapeutic dose. In certain embodiments, the concentration of the second therapeutic agent that is required to achieve inhibition, *e.g.*, growth inhibition, is lower when the second therapeutic agent is administered in combination with the anti-PD-1 antibody molecule than when the second therapeutic agent is administered individually. In certain embodiments, the concentration of the anti-PD-1 antibody molecule that is required to achieve inhibition, *e.g.*,

growth inhibition, is lower when the anti-PD-1 antibody molecule is administered in combination with the second therapeutic agent than when the anti-PD-1 antibody molecule is administered individually. In certain embodiments, in a combination therapy, the concentration of the second therapeutic agent that is required to achieve inhibition, *e.g.*, growth inhibition, is lower than the therapeutic dose of the second therapeutic agent as a monotherapy, *e.g.*, 10-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, or 80-90% lower. In certain embodiments, in a combination therapy, the concentration of the anti-PD-1 antibody molecule that is required to achieve inhibition, *e.g.*, growth inhibition, is lower than the therapeutic dose of the anti-PD-1 antibody molecule as a monotherapy, *e.g.*, 10-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, or 80-90% lower.

Detection

In another aspect, the invention features methods for detecting the presence of PD-1 in a sample, *e.g.*, *in vitro* or *in vivo* (*e.g.*, a biological sample, *e.g.*, serum, semen or urine, or a tissue biopsy, *e.g.*, from a hyperproliferative or cancerous lesion). The subject method can be used to evaluate (*e.g.*, monitor treatment or progression of, diagnose and/or stage a disorder described herein, *e.g.*, a hyperproliferative or cancerous disorder, in a subject). The method includes: (i) contacting the sample with (and optionally, a reference, *e.g.*, a control sample), or administering to the subject, an antibody molecule as described herein, under conditions that allow interaction to occur, and (ii) detecting formation of a complex between the antibody molecule, and the sample (and optionally, the reference, *e.g.*, control, sample). Formation of the complex is indicative of the presence of PD-1, and can indicate the suitability or need for a treatment described herein. The method can involve an immunohistochemistry, immunocytochemistry, FACS, antibody molecule complexed magnetic beads, ELISA assays, PCR-techniques (*e.g.*, RT-PCR).

Typically, the antibody molecule used in the *in vivo* and *in vitro* diagnostic methods is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound binding agent. Suitable detectable substances include various biologically active enzymes, prosthetic groups, fluorescent materials, luminescent materials, paramagnetic (*e.g.*, nuclear magnetic resonance active) materials, and radioactive materials.

Additional embodiments provide a method of treating a cancer, comprising: identifying in a subject or a sample (*e.g.*, a subject's sample comprising cancer cells and optionally immune cells such as TILs) the presence of one, two or all of PD-L1, CD8, or IFN-γ, thereby providing a value for one, two or all of PD-L1, CD8, and IFN-γ. The method can further include comparing the PD-L1, CD8, and/or IFN-γ values to a reference value, *e.g.*, a control value. If the PD-L1, CD8, and/or IFN-γ values are greater than the reference value, *e.g.*, the control values, administering a therapeutically effective amount of an anti-PD-1 antibody (*e.g.*, an anti-PD-1 antibody described herein) to the subject, optionally in combination with one or more other agents, thereby treating the cancer. The cancer may be, *e.g.*, a cancer described herein, such as lung cancer (squamous), lung cancer (adenocarcinoma), head and neck cancer, cervical cancer (squamous), stomach cancer, thyroid cancer, melanoma, nasopharyngeal cancer, or breast cancer, *e.g.*, TN breast cancer, *e.g.*, IM-TN breast cancer. In some embodiments, the cancer is ER+ breast cancer or pancreatic cancer.

Also provided is a method of treating a cancer, comprising: testing a subject or a sample (*e.g.*, a subject's sample comprising cancer cells) for the presence of PD-L1, thereby identifying a PD-L1 value, comparing the PD-L1 value to a control value, and if the PD-L1 value is greater than the control value, administering a therapeutically effective amount of an anti-PD-1 antibody (*e.g.*, an anti-PD-1 antibody described herein) to the subject, optionally in combination with one or more other agents, thereby treating the cancer. The cancer may be, *e.g.*, a cancer as described herein, such as cancer is non-small cell lung (NSCLC) adenocarcinoma (ACA), NSCLC squamous cell carcinoma (SCC), or hepatocellular carcinoma (HCC).

In another aspect, the invention features diagnostic or therapeutic kits that include the antibody molecules described herein and instructions for use.

All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the amino acid sequences of the light and heavy chain variable regions of murine anti-PD-1 mAb BAP049. The upper and lower sequences were from two independent analyses. The light and heavy chain CDR sequences based on Kabat numbering are underlined. The light heavy chain CDR sequences based on Chothia numbering are shown in bold italics. The unpaired Cys residue at position 102 of the light chain sequence is boxed. Sequences are disclosed as SEQ ID NOs: 8, 228, 16 and 229, respectively, in order of appearance.

Figure 2A depicts the amino acid sequences of the light and heavy chain variable regions of murine anti-PD-1 mAb BAP049 aligned with the germline sequences. The upper and lower sequences are the germline (GL) and BAP049 (Mu mAb) sequences, respectively. The light and heavy chain CDR sequences based on Kabat numbering are underlined. The light heavy chain CDR sequences based on Chothia numbering are shown in bold italics. "-" means identical amino acid residue. Sequences disclosed as SEQ ID NOs: 230, 8, 231 and 16, respectively, in order of appearance.

Figure 2B depicts the sequence of murine κ J2 gene and the corresponding mutation in murine anti-PD-1 mAb BAP049. "-" means identical nucleotide residue. Sequences disclosed as SEQ ID NOs: 233, 232, 234 and 235, respectively, in order of appearance.

Figures 3A-3B depict the competition binding between fluorescently labeled murine anti-PD-1 mAb BAP049 (Mu mAb) and three chimeric versions of BAP049 (Chi mAb). Experiment was performed twice, and the results are shown in Figures 3A and 3B, respectively. The three chimeric BAP049 antibodies (Chi mAb (Cys), Chi mAb (Tyr) and Chi mAb (Ser)) have Cys, Tyr and Ser residue at position 102 of the light chain variable region, respectively. Chi mAb (Cys), Chi mAb (Tyr) and Chi mAb (Ser) are also known as BAP049-chi, BAP049-chi-Y, and BAP049-chi-S, respectively.

Figure 4 is a bar graph showing the results of FACS binding analysis for the sixteen humanized BAP049 clones (BAP049-hum01 to BAP049-hum16). The antibody concentrations are 200, 100, 50, 25 and 12.5 ng/ml from the leftmost bar to the rightmost bar for each tested mAb.

Figure 5 depicts the structural analysis of the humanized BAP049 clones (a, b, c, d and e represent various types of framework region sequences). The concentrations of the mAbs in the samples are also shown.

Figure 6A-6B depicts the binding affinity and specificity of humanized BAP049 mAbs measured in a competition binding assay using a constant concentration of Alexa 488-labeled murine mAb BAP049, serial dilutions of the test antibodies, and PD-1-expressing 300.19 cells. Experiment was performed twice, and the results are shown in Figures 6A and 6B, respectively.

Figure 7 depicts the ranking of humanized BAP049 clones based on FACS data, competition binding and structural analysis. The concentrations of the mAbs in the samples are also shown.

Figures 8A-8B depict blocking of ligand binding to PD-1 by selected humanized BAP049 clones. Blocking of PD-L1-Ig and PD-L2-Ig binding to PD-1 is shown in Figire 8A. Blocking of PD-L2-Ig binding to PD-1 is shown in Figire 8B. BAP049-hum01, BAP049-hum05, BAP049-hum08, BAP049-hum09, BAP049-hum10, and BAP049-hum11 were evaluated. Murine mAb BAP049 and chimeric mAb having Tyr at position 102 of the light chain variable region were also included in the analyses.

Figures 9A-9B depict the alignment of heavy chain variable domain sequences for the sixteen humanized BAP049 clones and BAP049 chimera (BAP049-chi). In Figure 9A, all of the sequences are shown (SEQ ID NOs: 22, 38, 38, 38, 38, 38, 38, 38, 38, 38, 50, 50, 50, 50, 82, 82 and 86, respectively, in order of appearance). In Figure 9B, only amino acid sequences that are different from mouse sequence are shown (SEQ ID NOs: 22, 38, 38, 38, 38, 38, 38, 38, 38, 38, 50, 50, 50, 50, 82, 82 and 86, respectively, in order of appearance).

Figures 10A-10B depict the alignment of light chain variable domain sequences for the sixteen humanized BAP049 clones and BAP049 chimera (BAP049-chi). In Figure 10A, all of the sequences are shown (SEQ ID NOs: 24, 66, 66, 66, 66, 70, 70, 70, 58, 62, 78, 74, 46, 46, 42, 54 and 54, respectively, in order of appearance). In Figure 10B, only amino acid sequences that are different from mouse sequence are shown (SEQ ID NOs: 24, 66, 66, 66, 66, 70, 70, 70, 58, 62, 78, 74, 46, 46, 42, 54 and 54, respectively, in order of appearance).

Figure 11 shows exemplary cancers having relatively high proportions of patients that are triple-positive for PD-L1/CD8/IFN-γ.

Figure 12 shows exemplary ER+ breast cancer and pancreatic cancer having relatively low proportions for patients that are triple positive for PD-L1/CD8/IFN-γ.

Figure 13 shows the proportion of exemplary breast cancer patients that are triple positive for PD-L1/CD8/IFN-γ.

Figure 14 shows the proportion of exemplary colon cancer patients that are triple positive for PD-L1/CD8/IFN-γ.

Figure 15 shows a graphical representation of flow cytometry of PD-L1 surface expression in EBC-1 cells *in vitro* with or without Compound A17 treatment. EBC-1 cells are non-small cell lung cancer cells with a cMET amplification.

Figure 16 shows a graphical representation of PD-L1 mRNA expression in Hs.746.T cells in a tumor xenograft model with or without Compound A17 treatment. Hs.746.T cells are gastric cancer cells with a c-MET amplification and a c-MET mutation.

Figure 17 shows a graphical representation of PD-L1 mRNA expression in H3122 cells *in vitro* with or without Compound A23. H3122 cells are non-small cell lung cancer (NSCLC) cells with an ALK translocation.

Figure 18 shows a graphical representation of PD-L1 mRNA expression in LOXIMV1 cells (BRAF mutant melanoma cells) in a tumor xenograft model with or without Compound A29 treatment.

Figure 19 shows a graphical representation of PD-L1 mRNA expression in HEYA8 cells (KRAS mutant ovarian cancer cells) in a tumor xenograft model with or without Compound A34 treatment.

Figure 20 shows a graphical representation of PD-L1 mRNA expression in UKE-1 cells (JAK2 V617F mutant myeloproliferative neoplasm cells) in a tumor xenograft model with or without Compound A18 treatment.

BRIEF DESCRIPTION OF THE TABLES

Table 1 is a summary of the amino acid and nucleotide sequences for the murine, chimeric and humanized anti-PD-1 antibody molecules. The antibody molecules include murine mAb BAP049, chimeric mAbs BAP049-chi and BAP049-chi-Y, and humanized mAbs BAP049-hum01 to BAP049-hum16 and BAP049-Clone-A to BAP049-Clone-E. The amino acid and nucleotide sequences of the heavy and light chain CDRs, the amino acid and nucleotide sequences of the heavy and light chain variable regions, and the amino acid and nucleotide sequences of the heavy and light chains are shown in this Table.

Table 2 depicts the amino acid and nucleotide sequences of the heavy and light chain framework regions for humanized mAbs BAP049-hum01 to BAP049-hum16 and BAP049-Clone-A to BAP049-Clone-E.

Table 3 depicts the constant region amino acid sequences of human IgG heavy chains and human kappa light chain.

Table 4 shows the amino acid sequences of the heavy and light chain leader sequences for humanized mAbs BAP049-Clone-A to BAP049-Clone-E.

Table 5 is a summary of yield, titre, monomer content and endotoxin levels for selected humanized BAP049 mAbs expressed in CHO cells.

Table 6 shows the charge isoforms as detected by Novex IEF analysis for selected humanized BAP049 mAbs expressed in CHO cells.

Table 7 is a summary of selected therapeutic agents that can be administered in combination with the anti-PD-1 antibody molecules and other immunomodulators (*e.g.*, one or more of: an activator of a costimulatory molecule and/or an inhibitor of an immune checkpoint molecule) described herein. **Table 7** provides from left to right the following: the Compound Designation of the second therapeutic agent, the Compound structure, and Patent publication(s) disclosing the Compound.

DETAILED DESCRIPTION

Programmed Death 1 (PD-1) is a CD28/CTLA-4 family member expressed on activated $CD4^+$ and $CD8^+$ T cells, T_{regs}, and B cells. It negatively regulates effector T cell signaling and function. PD-1 is induced on tumor-infiltrating T cells, resulting in functional exhaustion or dysfunction (Keir *et al.* (2008) *Annu. Rev. Immunol.* 26:677-704; Pardoll *et al.* (2012) *Nat Rev Cancer* 12(4):252-64).

PD-1 delivers a coinhibitory signal upon binding to either of its two ligands, Programmed Death-Ligand 1 (PD-L1) or Programmed Death-Ligand 2 (PD-L2). PD-L1 is expressed on T cells, natural killer (NK) cells, macrophages, dendritic cells (DCs), B cells, epithelial cells, vascular endothelial cells, as well as many types of tumors. High expression of PD-L1 on murine and human tumors has been linked to poor clinical outcomes in a variety of cancers (Keir *et al.* (2008) *Annu. Rev. Immunol.* 26:677-704; Pardoll *et al.* (2012) *Nat Rev Cancer* 12(4):252-64). PD-L2 is expressed on dendritic cells, macrophages, and some tumors.

Blockade of the PD-1 pathway has been pre-clinically and clinically validated for cancer immunotherapy. Both preclinical and clinical studies have demonstrated that anti-PD-1 blockade restores activity of effector T cells and results in robust anti-tumor response. For example, blockade of PD-1 pathway restores exhausted/dysfunctional effector T cell function (*e.g.*, proliferation, IFN-γ secretion, or cytolytic function) and inhibits T_{reg} cell function (Keir *et al.* (2008) *Annu. Rev. Immunol.* 26:677-704; Pardoll *et al.* (2012) *Nat Rev Cancer* 12(4):252-64).

Accordingly, the present invention provides, at least in part, antibody molecules (*e.g.*, humanized antibody molecules) that bind to Programmed Death 1 (PD-1) with high affinity and specificity. In one embodiment, humanized antibodies against PD-1 are disclosed, which show a surprisingly low immunogenicity. For example, humanized BAP049 antibodies were found to have a risk score of less than 650, 600, 550, or less than 500, according to the T cell epitope assays described herein. In other embodiments, selected combination of framework regions, *e.g.*, as shown in FIGs. 5 and 7, were shown to have distinct production efficiencies and binding properties.

Additional aspects of the invention include nucleic acid molecules encoding the antibody molecules, expression vectors, host cells and methods for making the antibody molecules. Immunoconjugates, multi- or bispecific molecules and pharmaceutical compositions comprising the antibody molecules are also provided. The anti-PD-1 antibody molecules disclosed herein can be used to treat, prevent and/or diagnose cancerous or malignant disorders (*e.g.*, solid and soft-tissue tumors; melanoma, *e.g.*, advanced melanoma; hepatocellular carcinoma; pancreatic cancer; renal cell carcinoma (RCC), *e.g.*, metastatic RCC or clear cell RCC; gliomas or glioblastomas; multiple myeloma; colorectal cancer; and lung cancer, *e.g.*, non-small cell carcinoma), as well as infectious diseases (*e.g.*, infectious disorders such as hepatitis, *e.g.*, hepatitis C (*e.g.*, chronic viral hepatitis); sepsis). Thus, methods for detecting PD-1, as well as methods for treating various disorders, including cancer and infectious diseases using the anti-PD-1 antibody molecules are disclosed herein.

The term "Programmed Death 1" or "PD-1" include isoforms, mammalian, *e.g.*, human PD-1, species homologs of human PD-1, and analogs comprising at least one common epitope with PD-1. The amino acid sequence of PD-1, *e.g.*, human PD-1, is known in the art, *e.g.*, Shinohara T *et al.* (1994) *Genomics* 23(3):704-6; Finger LR, *et al. Gene* (1997) 197(1-2):177-87.

Additional terms are defined below and throughout the application.

As used herein, the articles "a" and "an" refer to one or to more than one (*e.g.*, to at least one) of the grammatical object of the article.

The term "or" is used herein to mean, and is used interchangeably with, the term "and/or", unless context clearly indicates otherwise.

"About" and "approximately" shall generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Exemplary degrees of error are within 20 percent (%), typically, within 10%, and more typically, within 5% of a given value or range of values.

The compositions and methods of the present invention encompass polypeptides and nucleic acids having the sequences specified, or sequences substantially identical or similar thereto, *e.g.*, sequences at least 85%, 90%, 95% identical or higher to the sequence specified. In the context of an amino acid sequence, the term "substantially identical" is used herein to refer to a first amino acid that contains a sufficient or minimum number of amino acid residues that are i) identical to, or ii) conservative substitutions of aligned amino acid residues in a second amino acid sequence such that the first and second amino acid sequences can have a common structural domain and/or common functional activity. For example, amino acid sequences that contain a common structural domain having at least about 85%, 90%. 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence, *e.g.*, a sequence provided herein.

In the context of nucleotide sequence, the term "substantially identical" is used herein to refer to a first nucleic acid sequence that contains a sufficient or minimum number of nucleotides that are identical to aligned nucleotides in a second nucleic acid sequence such that the first and second nucleotide sequences encode a polypeptide having common functional activity, or encode a common structural polypeptide domain or a common functional polypeptide activity. For example, nucleotide sequences having at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence, *e.g.*, a sequence provided herein.

The term “functional variant” refers to polypeptides that have a substantially identical amino acid sequence to the naturally-occurring sequence, or are encoded by a substantially identical nucleotide sequence, and are capable of having one or more activities of the naturally-occurring sequence.

Calculations of homology or sequence identity between sequences (the terms are used interchangeably herein) are performed as follows.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology").

The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch ((1970) *J. Mol. Biol.* 48:444-453) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used unless otherwise specified) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of E. Meyers and W. Miller ((1989) *CABIOS*, 4:11-17) which has been

incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences described herein can be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid (SEQ ID NO: 1) molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25:3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

As used herein, the term "hybridizes under low stringency, medium stringency, high stringency, or very high stringency conditions" describes conditions for hybridization and washing. Guidance for performing hybridization reactions can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6, which is incorporated by reference. Aqueous and nonaqueous methods are described in that reference and either can be used. Specific hybridization conditions referred to herein are as follows: 1) low stringency hybridization conditions in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by two washes in 0.2X SSC, 0.1% SDS at least at 50°C (the temperature of the washes can be increased to 55°C for low stringency conditions); 2) medium stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C; 3) high stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C; and preferably 4) very high stringency hybridization conditions are 0.5M sodium phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Very high stringency conditions (4) are the preferred conditions and the ones that should be used unless otherwise specified.

It is understood that the molecules of the present invention may have additional conservative or non-essential amino acid substitutions, which do not have a substantial effect on their functions.

The term "amino acid" is intended to embrace all molecules, whether natural or synthetic, which include both an amino functionality and an acid functionality and capable of being included in a polymer of naturally-occurring amino acids. Exemplary amino acids include naturally-occurring amino acids; analogs, derivatives and congeners thereof; amino acid analogs having variant side chains; and all stereoisomers of any of any of the foregoing. As used herein the term "amino acid" includes both the D- or L- optical isomers and peptidomimetics.

A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine).

The terms "polypeptide", "peptide" and "protein" (if single chain) are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation, such as conjugation with a labeling component. The polypeptide can be isolated from natural sources, can be a produced by recombinant techniques from a eukaryotic or prokaryotic host, or can be a product of synthetic procedures.

The terms "nucleic acid," "nucleic acid sequence," "nucleotide sequence," or "polynucleotide sequence," and "polynucleotide" are used interchangeably. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. The polynucleotide may be either single-stranded or double-stranded, and if single-stranded may be the coding strand or non-coding (antisense) strand. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. The

sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. The nucleic acid may be a recombinant polynucleotide, or a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which either does not occur in nature or is linked to another polynucleotide in a nonnatural arrangement.

The term "isolated," as used herein, refers to material that is removed from its original or native environment (*e.g.*, the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated by human intervention from some or all of the co-existing materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of the environment in which it is found in nature.

Various aspects of the invention are described in further detail below. Additional definitions are set out throughout the specification.

Antibody Molecules

In one embodiment, the antibody molecule binds to a mammalian, *e.g.*, human, PD-1. For example, the antibody molecule binds specifically to an epitope, *e.g.*, linear or conformational epitope, (*e.g.*, an epitope as described herein) on PD-1.

As used herein, the term "antibody molecule" refers to a protein, *e.g.*, an immunoglobulin chain or fragment thereof, comprising at least one immunoglobulin variable domain sequence. The term "antibody molecule" includes, for example, a monoclonal antibody (including a full length antibody which has an immunoglobulin Fc region). In an embodiment, an antibody molecule comprises a full length antibody, or a full length immunoglobulin chain. In an embodiment, an antibody molecule comprises an antigen binding or functional fragment of a full length antibody, or a full length immunoglobulin chain.

In an embodiment, an antibody molecule is a monospecific antibody molecule and binds a single epitope. *E.g.*, a monospecific antibody molecule having a plurality of immunoglobulin variable domain sequences, each of which binds the same epitope.

In an embodiment an antibody molecule is a multispecific antibody molecule, *e.g.*, it comprises a plurality of immunoglobulin variable domains sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope. In an embodiment the first and second epitopes are on the same antigen, *e.g.*, the same protein (or subunit of a multimeric protein). In an embodiment the first and second epitopes overlap. In an embodiment the first and second epitopes do not overlap. In an embodiment the first and second epitopes are on different antigens, *e.g.*, the different proteins (or different subunits of a multimeric protein). In an embodiment a multispecific antibody molecule comprises a third, fourth or fifth immunoglobulin variable domain. In an embodiment, a multispecific antibody molecule is a bispecific antibody molecule, a trispecific antibody molecule, or tetraspecific antibody molecule,

In an embodiment a multispecific antibody molecule is a bispecific antibody molecule. A bispecific antibody has specificity for no more than two antigens. A bispecific antibody molecule is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope. In an embodiment the first and second epitopes are on the same antigen, *e.g.*, the same protein (or subunit of a multimeric protein). In an embodiment the first and second epitopes overlap. In an embodiment the first and second epitopes do not overlap. In an embodiment the first and second epitopes are on different antigens, *e.g.*, the different proteins (or different subunits of a multimeric protein). In an embodiment a bispecific antibody molecule comprises a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a first epitope and a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a half antibody having binding specificity for a first epitope and a half antibody having binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a half antibody, or fragment thereof, having binding specificity for a first epitope and a half antibody, or fragment thereof, having binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a scFv, or fragment thereof, have binding specificity for a first epitope and a scFv, or fragment thereof, have binding specificity for

a second epitope. In an embodiment the first epitope is located on PD-1 and the second epitope is located on a TIM-3, LAG-3, CEACAM (*e.g.*, CEACAM-1 and/or CEACAM-5), PD-L1, or PD-L2.

In an embodiment, an antibody molecule comprises a diabody, and a single-chain molecule, as well as an antigen-binding fragment of an antibody (*e.g.*, Fab, $F(ab')_2$, and Fv). For example, an antibody molecule can include a heavy (H) chain variable domain sequence (abbreviated herein as VH), and a light (L) chain variable domain sequence (abbreviated herein as VL). In an embodiment an antibody molecule comprises or consists of a heavy chain and a light chain (referred to herein as a half antibody. In another example, an antibody molecule includes two heavy (H) chain variable domain sequences and two light (L) chain variable domain sequence, thereby forming two antigen binding sites, such as Fab, Fab', $F(ab')_2$, Fc, Fd, Fd', Fv, single chain antibodies (scFv for example), single variable domain antibodies, diabodies (Dab) (bivalent and bispecific), and chimeric (*e.g.*, humanized) antibodies, which may be produced by the modification of whole antibodies or those synthesized de novo using recombinant DNA technologies. These functional antibody fragments retain the ability to selectively bind with their respective antigen or receptor. Antibodies and antibody fragments can be from any class of antibodies including, but not limited to, IgG, IgA, IgM, IgD, and IgE, and from any subclass (*e.g.*, IgG1, IgG2, IgG3, and IgG4) of antibodies. The a preparation of antibody molecules can be monoclonal or polyclonal. An antibodymolecule can also be a human, humanized, CDR-grafted, or *in vitro* generated antibody. The antibody can have a heavy chain constant region chosen from, *e.g.*, IgG1, IgG2, IgG3, or IgG4. The antibody can also have a light chain chosen from, *e.g.*, kappa or lambda. The term "immunoglobulin" (Ig) is used interchangeably with the term "antibody" herein.

Examples of antigen-binding fragments of an antibody molecule include: (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 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 VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a diabody (dAb) fragment, which consists of a VH domain; (vi) a camelid or camelized variable domain; (vii) a single chain Fv (scFv), *see e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883); (viii) a single domain antibody. These antibody

fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

The term "antibody" includes intact molecules as well as functional fragments thereof. Constant regions of the antibodies can be altered, *e.g.*, mutated, to modify the properties of the antibody (*e.g.*, to increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, effector cell function, or complement function).

Antibody molecules can also be single domain antibodies. Single domain antibodies can include antibodies whose complementary determining regions are part of a single domain polypeptide. Examples include, but are not limited to, heavy chain antibodies, antibodies naturally devoid of light chains, single domain antibodies derived from conventional 4-chain antibodies, engineered antibodies and single domain scaffolds other than those derived from antibodies. Single domain antibodies may be any of the art, or any future single domain antibodies. Single domain antibodies may be derived from any species including, but not limited to mouse, human, camel, llama, fish, shark, goat, rabbit, and bovine. According to another aspect of the invention, a single domain antibody is a naturally occurring single domain antibody known as heavy chain antibody devoid of light chains. Such single domain antibodies are disclosed in WO 9404678, for example. For clarity reasons, this variable domain derived from a heavy chain antibody naturally devoid of light chain is known herein as a VHH or nanobody to distinguish it from the conventional VH of four chain immunoglobulins. Such a VHH molecule can be derived from antibodies raised in *Camelidae* species, for example in camel, llama, dromedary, alpaca and guanaco. Other species besides *Camelidae* may produce heavy chain antibodies naturally devoid of light chain; such VHHs are within the scope of the invention.

The VH and VL regions can be subdivided into regions of hypervariability, termed "complementarity determining regions" (CDR), interspersed with regions that are more conserved, termed "framework regions" (FR or FW).

The extent of the framework region and CDRs has been precisely defined by a number of methods (*see*, Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Chothia, C. *et al.* (1987) *J. Mol. Biol.* 196:901-917; and the AbM definition used by Oxford Molecular's AbM antibody modeling software. *See*, generally, *e.g.*, *Protein Sequence and*

Structure Analysis of Antibody Variable Domains. In: Antibody Engineering Lab Manual (Ed.: Duebel, S. and Kontermann, R., Springer-Verlag, Heidelberg).

The terms "complementarity determining region," and "CDR," as used herein refer to the sequences of amino acids within antibody variable regions which confer antigen specificity and binding affinity. In general, there are three CDRs in each heavy chain variable region (HCDR1, HCDR2, HCDR3) and three CDRs in each light chain variable region (LCDR1, LCDR2, LCDR3).

The precise amino acid sequence boundaries of a given CDR can be determined using any of a number of well-known schemes, including those described by Kabat *et al.* (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD ("Kabat" numbering scheme), Al-Lazikani *et al.*, (1997) *JMB* 273,927-948 ("Chothia" numbering scheme). As used herein, the CDRs defined according the "Chothia" number scheme are also sometimes referred to as "hypervariable loops."

For example, under Kabat, the CDR amino acid residues in the heavy chain variable domain (VH) are numbered 31-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3); and the CDR amino acid residues in the light chain variable domain (VL) are numbered 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3). Under Chothia the CDR amino acids in the VH are numbered 26-32 (HCDR1), 52-56 (HCDR2), and 95-102 (HCDR3); and the amino acid residues in VL are numbered 26-32 (LCDR1), 50-52 (LCDR2), and 91-96 (LCDR3). By combining the CDR definitions of both Kabat and Chothia, the CDRs consist of amino acid residues 26-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3) in human VH and amino acid residues 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3) in human VL.

Generally, unless specifically indicated, the anti-PD-1 antibody molecules can include any combination of one or more Kabat CDRs and/or Chothia hypervariable loops, *e.g.,* described in Table 1. In one embodiment, the following definitions are used for the anti-PD-1 antibody molecules described in Table 1: HCDR1 according to the combined CDR definitions of both Kabat and Chothia, and HCCDRs 2-3 and LCCDRs 1-3 according the CDR definition of Kabat. Under all definitions, each VH and VL typically includes three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

As used herein, an "immunoglobulin variable domain sequence" refers to an amino acid sequence which can form the structure of an immunoglobulin variable domain. For example, the sequence may include all or part of the amino acid sequence of a naturally-occurring variable domain. For example, the sequence may or may not include one, two, or more N- or C-terminal amino acids, or may include other alterations that are compatible with formation of the protein structure.

The term "antigen-binding site" refers to the part of an antibody molecule that comprises determinants that form an interface that binds to the PD-1 polypeptide, or an epitope thereof. With respect to proteins (or protein mimetics), the antigen-binding site typically includes one or more loops (of at least four amino acids or amino acid mimics) that form an interface that binds to the PD-1 polypeptide. Typically, the antigen-binding site of an antibody molecule includes at least one or two CDRs and/or hypervariable loops, or more typically at least three, four, five or six CDRs and/or hypervariable loops.

The terms "compete" or "cross-compete" are used interchangeably herein to refer to the ability of an antibody molecule to interfere with binding of an anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule provided herein, to a target, *e.g.*, human PD-1. The interference with binding can be direct or indirect (*e.g.*, through an allosteric modulation of the antibody molecule or the target). The extent to which an antibody molecule is able to interfere with the binding of another antibody molecule to the target, and therefore whether it can be said to compete, can be determined using a competition binding assay, for example, a FACS assay, an ELISA or BIACORE assay. In some embodiments, a competition binding assay is a quantitative competition assay. In some embodiments, a first anti-PD-1 antibody molecule is said to compete for binding to the target with a second anti-PD-1 antibody molecule when the binding of the first antibody molecule to the target is reduced by 10% or more, *e.g.*, 20% or more, 30% or more, 40% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, 99% or more in a competition binding assay (*e.g.*, a competition assay described herein).

The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

A monoclonal antibody can be made by hybridoma technology or by methods that do not use hybridoma technology (*e.g.*, recombinant methods).

An "effectively human" protein is a protein that does not evoke a neutralizing antibody response, *e.g.*, the human anti-murine antibody (HAMA) response. HAMA can be problematic in a number of circumstances, *e.g.*, if the antibody molecule is administered repeatedly, *e.g.*, in treatment of a chronic or recurrent disease condition. A HAMA response can make repeated antibody administration potentially ineffective because of an increased antibody clearance from the serum (*see, e.g.,* Saleh *et al.*, *Cancer Immunol. Immunother.*, 32:180-190 (1990)) and also because of potential allergic reactions (*see, e.g.,* LoBuglio *et al.*, *Hybridoma*, 5:5117-5123 (1986)).

The antibody molecule can be a polyclonal or a monoclonal antibody. In other embodiments, the antibody can be recombinantly produced, *e.g.,* produced by phage display or by combinatorial methods.

Phage display and combinatorial methods for generating antibodies are known in the art (as described in, *e.g.,* Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* International Publication No. WO 92/18619; Dower *et al.* International Publication No. WO 91/17271; Winter *et al.* International Publication WO 92/20791; Markland *et al.* International Publication No. WO 92/15679; Breitling *et al.* International Publication WO 93/01288; McCafferty *et al.* International Publication No. WO 92/01047; Garrard *et al.* International Publication No. WO 92/09690; Ladner *et al.* International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum Antibod Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J Mol Biol* 226:889-896; Clackson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *PNAS* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc Acid Res* 19:4133-4137; and Barbas *et al.* (1991) *PNAS* 88:7978-7982, the contents of all of which are incorporated by reference herein).

In one embodiment, the antibody is a fully human antibody (*e.g.*, an antibody made in a mouse which has been genetically engineered to produce an antibody from a human immunoglobulin sequence), or a non-human antibody, *e.g.,* a rodent (mouse or rat), goat, primate (*e.g.*, monkey), camel antibody. Preferably, the non-human antibody is a rodent (mouse or rat antibody). Methods of producing rodent antibodies are known in the art.

Human monoclonal antibodies can be generated using transgenic mice carrying the human immunoglobulin genes rather than the mouse system. Splenocytes from these transgenic mice immunized with the antigen of interest are used to produce hybridomas that secrete human mAbs with specific affinities for epitopes from a human protein (see, *e.g.*, Wood *et al.* International Application WO 91/00906, Kucherlapati *et al.* PCT publication WO 91/10741; Lonberg *et al.* International Application WO 92/03918; Kay *et al.* International Application 92/03917; Lonberg, N. *et al.* 1994 *Nature* 368:856-859; Green, L.L. *et al.* 1994 *Nature Genet.* 7:13-21; Morrison, S.L. *et al.* 1994 *Proc. Natl. Acad. Sci. USA* 81:6851-6855; Bruggeman *et al.* 1993 *Year Immunol* 7:33-40; Tuaillon *et al.* 1993 *PNAS* 90:3720-3724; Bruggeman *et al.* 1991 *Eur J Immunol* 21:1323-1326).

An antibody can be one in which the variable region, or a portion thereof, *e.g.*, the CDRs, are generated in a non-human organism, *e.g.*, a rat or mouse. Chimeric, CDR-grafted, and humanized antibodies are within the invention. Antibodies generated in a non-human organism, *e.g.*, a rat or mouse, and then modified, *e.g.*, in the variable framework or constant region, to decrease antigenicity in a human are within the invention.

Chimeric antibodies can be produced by recombinant DNA techniques known in the art (see Robinson *et al.*, International Patent Publication PCT/US86/02269; Akira, *et al.*, European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.*, European Patent Application 173,494; Neuberger *et al.*, International Application WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.*, European Patent Application 125,023; Better *et al.* (1988 *Science* 240:1041-1043); Liu *et al.* (1987) *PNAS* 84:3439-3443; Liu *et al.*, 1987, *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *PNAS* 84:214-218; Nishimura *et al.*, 1987, *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.*, 1988, *J. Natl Cancer Inst.* 80:1553-1559).

A humanized or CDR-grafted antibody will have at least one or two but generally all three recipient CDRs (of heavy and or light immuoglobulin chains) replaced with a donor CDR. The antibody may be replaced with at least a portion of a non-human CDR or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized antibody to PD-1. Preferably, the donor will be a rodent antibody, *e.g.*, a rat or mouse antibody, and the recipient will be a human framework or a human consensus framework. Typically, the immunoglobulin providing the CDRs is called the

"donor" and the immunoglobulin providing the framework is called the "acceptor." In one embodiment, the donor immunoglobulin is a non-human (*e.g.,* rodent). The acceptor framework is a naturally-occurring (*e.g.,* a human) framework or a consensus framework, or a sequence about 85% or higher, preferably 90%, 95%, 99% or higher identical thereto.

As used herein, the term "consensus sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related sequences (See *e.g.*, Winnaker, From Genes to Clones (Verlagsgesellschaft, Weinheim, Germany 1987). In a family of proteins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence. A "consensus framework" refers to the framework region in the consensus immunoglobulin sequence.

An antibody can be humanized by methods known in the art (*see e.g.,* Morrison, S. L., 1985, *Science* 229:1202-1207, by Oi *et al.,* 1986, *BioTechniques* 4:214, and by Queen *et al.* US 5,585,089, US 5,693,761 and US 5,693,762, the contents of all of which are hereby incorporated by reference).

Humanized or CDR-grafted antibodies can be produced by CDR-grafting or CDR substitution, wherein one, two, or all CDRs of an immunoglobulin chain can be replaced. *See e.g.*, U.S. Patent 5,225,539; Jones *et al.* 1986 *Nature* 321:552-525; Verhoeyan *et al.* 1988 *Science* 239:1534; Beidler *et al.* 1988 *J. Immunol.* 141:4053-4060; Winter US 5,225,539, the contents of all of which are hereby expressly incorporated by reference. Winter describes a CDR-grafting method which may be used to prepare the humanized antibodies of the present invention (UK Patent Application GB 2188638A, filed on March 26, 1987; Winter US 5,225,539), the contents of which is expressly incorporated by reference.

Also within the scope of the invention are humanized antibodies in which specific amino acids have been substituted, deleted or added. Criteria for selecting amino acids from the donor are described in US 5,585,089, *e.g.*, columns 12-16 of US 5,585,089, *e.g.,* columns 12-16 of US 5,585,089, the contents of which are hereby incorporated by reference. Other techniques for humanizing antibodies are described in Padlan *et al.* EP 519596 A1, published on December 23, 1992.

The antibody molecule can be a single chain antibody. A single-chain antibody (scFV) may be engineered (see, for example, Colcher, D. *et al.* (1999) *Ann N Y Acad Sci* 880:263-80;

and Reiter, Y. (1996) *Clin Cancer Res* 2:245-52). The single chain antibody can be dimerized or multimerized to generate multivalent antibodies having specificities for different epitopes of the same target protein.

In yet other embodiments, the antibody molecule has a heavy chain constant region chosen from, *e.g.*, the heavy chain constant regions of IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE; particularly, chosen from, *e.g.*, the (*e.g.*, human) heavy chain constant regions of IgG1, IgG2, IgG3, and IgG4. In another embodiment, the antibody molecule has a light chain constant region chosen from, *e.*g., the (*e.g.*, human) light chain constant regions of kappa or lambda. The constant region can be altered, *e.g.*, mutated, to modify the properties of the antibody (*e.g.*, to increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, effector cell function, and/or complement function). In one embodiment the antibody has: effector function; and can fix complement. In other embodiments the antibody does not; recruit effector cells; or fix complement. In another embodiment, the antibody has reduced or no ability to bind an Fc receptor. For example, it is a isotype or subtype, fragment or other mutant, which does not support binding to an Fc receptor, *e.g.*, it has a mutagenized or deleted Fc receptor binding region.

Methods for altering an antibody constant region are known in the art. Antibodies with altered function, *e.g.* altered affinity for an effector ligand, such as FcR on a cell, or the C1 component of complement can be produced by replacing at least one amino acid residue in the constant portion of the antibody with a different residue (*see e.g.,* EP 388,151 A1, U.S. Pat. No. 5,624,821 and U.S. Pat. No. 5,648,260, the contents of all of which are hereby incorporated by reference). Similar type of alterations could be described which if applied to the murine, or other species immunoglobulin would reduce or eliminate these functions.

An antibody molecule can be derivatized or linked to another functional molecule (*e.g.*, another peptide or protein). As used herein, a "derivatized" antibody molecule is one that has been modified. Methods of derivatization include but are not limited to the addition of a fluorescent moiety, a radionucleotide, a toxin, an enzyme or an affinity ligand such as biotin. Accordingly, the antibody molecules of the invention are intended to include derivatized and otherwise modified forms of the antibodies described herein, including immunoadhesion molecules. For example, an antibody molecule can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities,

such as another antibody (*e.g.*, a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

One type of derivatized antibody molecule is produced by crosslinking two or more antibodies (of the same type or of different types, *e.g.*, to create bispecific antibodies). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (*e.g.*, m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (*e.g.*, disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, Ill.

Useful detectable agents with which an antibody molecule of the invention may be derivatized (or labeled) to include fluorescent compounds, various enzymes, prosthetic groups, luminescent materials, bioluminescent materials, fluorescent emitting metal atoms, *e.g.*, europium (Eu), and other anthanides, and radioactive materials (described below). Exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin and the like. An antibody may also be derivatized with detectable enzymes, such as alkaline phosphatase, horseradish peroxidase, β-galactosidase, acetylcholinesterase, glucose oxidase and the like. When an antibody is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction product. For example, when the detectable agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody molecule may also be derivatized with a prosthetic group (*e.g.*, streptavidin/biotin and avidin/biotin). For example, an antibody may be derivatized with biotin, and detected through indirect measurement of avidin or streptavidin binding. Examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of bioluminescent materials include luciferase, luciferin, and aequorin.

Labeled antibody molecule can be used, for example, diagnostically and/or experimentally in a number of contexts, including (i) to isolate a predetermined antigen by standard techniques, such as affinity chromatography or immunoprecipitation; (ii) to detect a

predetermined antigen (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the protein; (iii) to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to determine the efficacy of a given treatment regimen.

An antibody molecules may be conjugated to another molecular entity, typically a label or a therapeutic (*e.g.*, a cytotoxic or cytostatic) agent or moiety. Radioactive isotopes can be used in diagnostic or therapeutic applications. Radioactive isotopes that can be coupled to the anti-PD-1 antibodies include, but are not limited to α-, β-, or γ-emitters, or β-and γ-emitters. Such radioactive isotopes include, but are not limited to iodine (^{131}I or ^{125}I), yttrium (^{90}Y), lutetium (^{177}Lu), actinium (^{225}Ac), praseodymium, astatine (^{211}At), rhenium (^{186}Re), bismuth (^{212}Bi or ^{213}Bi), indium (^{111}In), technetium (^{99} mTc), phosphorus (^{32}P), rhodium (^{188}Rh), sulfur (^{35}S) , carbon (^{14}C), tritium (^{3}H), chromium (^{51}Cr), chlorine (^{36}Cl), cobalt (^{57}Co or ^{58}Co), iron (^{59}Fe), selenium (^{75}Se), or gallium (^{67}Ga). Radioisotopes useful as therapeutic agents include yttrium (^{90}Y), lutetium (^{177}Lu), actinium (^{225}Ac), praseodymium, astatine (^{211}At), rhenium (^{186}Re), bismuth (^{212} Bi or ^{213}Bi), and rhodium (^{188}Rh). Radioisotopes useful as labels, *e.g.*, for use in diagnostics, include iodine (^{131}I or ^{125}I), indium (^{111}In), technetium (^{99}mTc), phosphorus (^{32}P), carbon (^{14}C), and tritium (^{3} H), or one or more of the therapeutic isotopes listed above.

The invention provides radiolabeled antibody molecules and methods of labeling the same. In one embodiment, a method of labeling an antibody molecule is disclosed. The method includes contacting an antibody molecule, with a chelating agent, to thereby produce a conjugated antibody. The conjugated antibody is radiolabeled with a radioisotope, *e.g.*, ^{111}Indium, ^{90}Yttrium and ^{177}Lutetium, to thereby produce a labeled antibody molecule.

As is discussed above, the antibody molecule can be conjugated to a therapeutic agent. Therapeutically active radioisotopes have already been mentioned. Examples of other therapeutic agents include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, maytansinoids, *e.g.,* maytansinol (*see* U.S. Pat. No. 5,208,020), CC-1065 (see U.S. Pat. Nos. 5,475,092, 5,585,499, 5,846, 545) and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.,* mechlorethamine, thioepa

chlorambucil, CC-1065, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclinies (*e.g.,* daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.,* dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine, vinblastine, taxol and maytansinoids).

In one aspect, the invention features a method of providing a target binding molecule that specifically binds to a PD-1 receptor. For example, the target binding molecule is an antibody molecule. The method includes: providing a target protein that comprises at least a portion of non-human protein, the portion being homologous to (at least 70, 75, 80, 85, 87, 90, 92, 94, 95, 96, 97, 98% identical to) a corresponding portion of a human target protein, but differing by at least one amino acid (*e.g.*, at least one, two, three, four, five, six, seven, eight, or nine amino acids); obtaining an antibody molecule that specifically binds to the antigen; and evaluating efficacy of the binding agent in modulating activity of the target protein. The method can further include administering the binding agent (*e.g.*, antibody molecule) or a derivative (*e.g.*, a humanized antibody molecule) to a human subject.

In certain embodiments, the antibody molecule is a multi-specific (*e.g.*, a bispecific or a trispecific) antibody molecule. Protocols for generating bispecific or heterodimeric antibody molecules are known in the art; including but not limited to, for example, the "knob in a hole" approach described in, *e.g.*, US 5731168; the electrostatic steering Fc pairing as described in, *e.g.*, WO 09/089004, WO 06/106905 and WO 2010/129304; Strand Exchange Engineered Domains (SEED) heterodimer formation as described in, *e.g.*, WO 07/110205; Fab arm exchange as described in, *e.g.*, WO 08/119353, WO 2011/131746, and WO 2013/060867; double antibody conjugate, *e.g.*, by antibody cross-linking to generate a bi-specific structure using a heterobifunctional reagent having an amine-reactive group and a sulfhydryl reactive group as described in, *e.g.*, US 4433059; bispecific antibody determinants generated by recombining half antibodies (heavy-light chain pairs or Fabs) from different antibodies through cycle of reduction and oxidation of disulfide bonds between the two heavy chains, as described in, *e.g.*, US 4444878; trifunctional antibodies, *e.g.*, three Fab' fragments cross-linked through sulfhdryl reactive groups, as described in, *e.g.*, US5273743; biosynthetic binding proteins, *e.g.*, pair of scFvs cross-linked through C-terminal tails preferably through disulfide or amine-reactive

chemical cross-linking, as described in, *e.g.*, US5534254; bifunctional antibodies, *e.g.*, Fab fragments with different binding specificities dimerized through leucine zippers (*e.g.*, c-fos and c-jun) that have replaced the constant domain, as described in, *e.g.*, US5582996; bispecific and oligospecific mono-and oligovalent receptors, *e.g.*, VH-CH1 regions of two antibodies (two Fab fragments) linked through a polypeptide spacer between the CH1 region of one antibody and the VH region of the other antibody typically with associated light chains, as described in, *e.g.*, US5591828; bispecific DNA-antibody conjugates, *e.g.*, crosslinking of antibodies or Fab fragments through a double stranded piece of DNA, as described in, *e.g.*, US5635602; bispecific fusion proteins, *e.g.*, an expression construct containing two scFvs with a hydrophilic helical peptide linker between them and a full constant region, as described in, *e.g.*, US5637481; multivalent and multispecific binding proteins, *e.g.*, dimer of polypeptides having first domain with binding region of Ig heavy chain variable region, and second domain with binding region of Ig light chain variable region, generally termed diabodies (higher order structures are also disclosed creating bispecifc, trispecific, or tetraspecific molecules, as described in, *e.g.*, US5837242; minibody constructs with linked VL and VH chains further connected with peptide spacers to an antibody hinge region and CH3 region, which can be dimerized to form bispecific/multivalent molecules, as described in, *e.g.*, US5837821; VH and VL domains linked with a short peptide linker (*e.g.*, 5 or 10 amino acids) or no linker at all in either orientation, which can form dimers to form bispecific diabodies; trimers and tetramers, as described in, *e.g.*, US5844094; String of VH domains (or VL domains in family members) connected by peptide linkages with crosslinkable groups at the C-terminus futher associated with VL domains to form a series of FVs (or scFvs), as described in, *e.g.*, US5864019; and single chain binding polypeptides with both a VH and a VL domain linked through a peptide linker are combined into multivalent structures through non-covalent or chemical crosslinking to form, *e.g.*, homobivalent, heterobivalent, trivalent, and tetravalent structures using both scFV or diabody type format, as described in, *e.g.*, US5869620. Additional exemplary multispecific and bispecific molecules and methods of making the same are found, for example, in US5910573, US5932448, US5959083, US5989830, US6005079, US6239259, US6294353, US6333396, US6476198, US6511663, US6670453, US6743896, US6809185, US6833441, US7129330, US7183076, US7521056, US7527787, US7534866, US7612181, US2002004587A1, US2002076406A1, US2002103345A1, US2003207346A1, US2003211078A1,

US2004219643A1, US2004220388A1, US2004242847A1, US2005003403A1, US2005004352A1, US2005069552A1, US2005079170A1, US2005100543A1, US2005136049A1, US2005136051A1, US2005163782A1, US2005266425A1, US2006083747A1, US2006120960A1, US2006204493A1, US2006263367A1, US2007004909A1, US2007087381A1, US2007128150A1, US2007141049A1, US2007154901A1, US2007274985A1, US2008050370A1, US2008069820A1, US2008152645A1, US2008171855A1, US2008241884A1, US2008254512A1, US2008260738A1, US2009130106A1, US2009148905A1, US2009155275A1, US2009162359A1, US2009162360A1, US2009175851A1, US2009175867A1, US2009232811A1, US2009234105A1, US2009263392A1, US2009274649A1, EP346087A2, WO0006605A2, WO02072635A2, WO04081051A1, WO06020258A2, WO2007044887A2, WO2007095338A2, WO2007137760A2, WO2008119353A1, WO2009021754A2, WO2009068630A1, WO9103493A1, WO9323537A1, WO9409131A1, WO9412625A2, WO9509917A1, WO9637621A2, WO9964460A1. The contents of the above-referenced applications are incorporated herein by reference in their entireties.

In other embodiments, the anti-PD-1 antibody molecule (*e.g.*, a monospecific, bispecific, or multispecific antibody molecule) is covalently linked, *e.g.*, fused, to another partner *e.g.*, a protein *e.g.*, one, two or more cytokines, *e.g.*, as a fusion molecule for example a fusion protein. In other embodiments, the fusion molecule comprises one or more proteins, *e.g.*, one, two or more cytokines. In one embodiment, the cytokine is an interleukin (IL) chosen from one, two, three or more of IL-1, IL-2, IL-12, IL-15 or IL-21. In one embodiment, a bispecific antibody molecule has a first binding specificity to a first target (*e.g.*, to PD-1), a second binding specificity to a second target (*e.g.*, LAG-3 or TIM-3), and is optionally linked to an interleukin (*e.g.*, IL-12) domain *e.g.*, full length IL-12 or a portion thereof.

A "fusion protein" and a "fusion polypeptide" refer to a polypeptide having at least two portions covalently linked together, where each of the portions is a polypeptide having a different property. The property may be a biological property, such as activity *in vitro* or *in vivo*. The property can also be simple chemical or physical property, such as binding to a target molecule, catalysis of a reaction, etc. The two portions can be linked directly by a single peptide bond or through a peptide linker, but are in reading frame with each other.

This invention provides an isolated nucleic acid molecule encoding the above antibody molecule, vectors and host cells thereof. The nucleic acid molecule includes but is not limited to RNA, genomic DNA and cDNA.

Exemplary Anti-PD-1 Antibody Molecules

In one embodiment, the anti-PD-1 antibody molecule includes:

(a) a heavy chain variable region (VH) comprising a VHCDR1 amino acid sequence of SEQ ID NO: 4, a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a light chain variable region (VL) comprising a VLCDR1 amino acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 33;

(b) a VH comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 32;

(c) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 224, a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 33; or

(d) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 224; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 32.

In certain embodiments, the anti-PD-1 antibody molecule comprises:

(i) a heavy chain variable region (VH) comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1, SEQ ID NO: 4 or SEQ ID NO: 224; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and

(ii) a light chain variable region (VL) comprising a VLCDR1 amino acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 32.

In other embodiments, the anti-PD-1 antibody molecule comprises:

(i) a heavy chain variable region (VH) comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1, SEQ ID NO: 4 or SEQ ID NO: 224; a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and

(ii) a light chain variable region (VL) comprising a VLCDR1 amino acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 33.

In embodiments of the aforesaid antibody molecules, the VHCDR1 comprises the amino acid sequence of SEQ ID NO: 1. In other embodiments, the VHCDR1 comprises the amino acid sequence of SEQ ID NO: 4. In yet other embodiments, the VHCDR1 amino acid sequence of SEQ ID NO: 224.

In embodiments, the aforesaid antibody molecules have a heavy chain variable region comprising at least one framework (FW) region comprising the amino acid sequence of any of SEQ ID NOs: 147, 151, 153, 157, 160, 162, 166, or 169, or an amino acid sequence at least 90% identical thereto, or having no more than two amino acid substitutions, insertions or deletions compared to the amino acid sequence of any of SEQ ID NOs: 147, 151, 153, 157, 160, 162, 166, or 169.

In other embodiments, the aforesaid antibody molecules have a heavy chain variable region comprising at least one framework region comprising the amino acid sequence of any of SEQ ID NOs: 147, 151, 153, 157, 160, 162, 166, or 169.

In yet other embodiments, the aforesaid antibody molecules have a heavy chain variable region comprising at least two, three, or four framework regions comprising the amino acid sequences of any of SEQ ID NOs: 147, 151, 153, 157, 160, 162, 166, or 169.

In other embodiments, the aforesaid antibody molecules comprise a VHFW1 amino acid sequence of SEQ ID NO: 147 or 151, a VHFW2 amino acid sequence of SEQ ID NO: 153, 157, or 160, and a VHFW3 amino acid sequence of SEQ ID NO: 162 or 166, and, optionally, further comprising a VHFW4 amino acid sequence of SEQ ID NO: 169.

In other embodiments, the aforesaid antibody molecules have a light chain variable region comprising at least one framework region comprising the amino acid sequence of any of SEQ ID NOs: 174, 177, 181, 183, 185, 187, 191, 194, 196, 200, 202, 205, or 208, or an amino acid sequence at least 90% identical thereto, or having no more than two amino acid

substitutions, insertions or deletions compared to the amino acid sequence of any of 174, 177, 181, 183, 185, 187, 191, 194, 196, 200, 202, 205, or 208.

In other embodiments, the aforesaid antibody molecules have a light chain variable region comprising at least one framework region comprising the amino acid sequence of any of SEQ ID NOs: 174, 177, 181, 183, 185, 187, 191, 194, 196, 200, 202, 205, or 208.

In other embodiments, the aforesaid antibody molecules have a light chain variable region comprising at least two, three, or four framework regions comprising the amino acid sequences of any of SEQ ID NOs: 174, 177, 181, 183, 185, 187, 191, 194, 196, 200, 202, 205, or 208.

In other embodiments, the aforesaid antibody molecules comprise a VLFW1 amino acid sequence of SEQ ID NO: 174, 177, 181, 183, or 185, a VLFW2 amino acid sequence of SEQ ID NO: 187, 191, or 194, and a VLFW3 amino acid sequence of SEQ ID NO: 196, 200, 202, or 205, and, optionally, further comprising a VLFW4 amino acid sequence of SEQ ID NO: 208.

In other embodiments, the aforesaid antibodies comprise a heavy chain variable domain comprising an amino acid sequence at least 85% identical to any of SEQ ID NOs: 38, 50, 82, or 86.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38, 50, 82, or 86.

In other embodiments, the aforesaid antibody molecules comprise a light chain variable domain comprising an amino acid sequence at least 85% identical to any of SEQ ID NOs: 42, 46, 54, 58, 62, 66, 70, 74, or 78.

In other embodiments, the aforesaid antibody molecules comprise a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 42, 46, 54, 58, 62, 66, 70, 74, or 78.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 40.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 91.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 50.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 52 or SEQ ID NO: 102.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 82.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 84.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 86.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 88.

In other embodiments, the aforesaid antibody molecules comprise a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 42.

In other embodiments, the aforesaid antibody molecules comprise a light chain comprising the amino acid sequence of SEQ ID NO: 44.

In other embodiments, the aforesaid antibody molecules comprise a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 46.

In other embodiments, the aforesaid antibody molecules comprise a light chain comprising the amino acid sequence of SEQ ID NO: 48.

In other embodiments, the aforesaid antibody molecules comprise a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 54.

In other embodiments, the aforesaid antibody molecules comprise a light chain comprising the amino acid sequence of SEQ ID NO: 56.

In other embodiments, the aforesaid antibody molecules comprise a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 58.

In other embodiments, the aforesaid antibody molecules comprise a light chain comprising the amino acid sequence of SEQ ID NO: 60.

In other embodiments, the aforesaid antibody molecules comprise a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 62.

In other embodiments, the aforesaid antibodies comprise a light chain comprising the amino acid sequence of SEQ ID NO: 64.

In other embodiments, the aforesaid antibody molecules comprise a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 66.

In other embodiments, the aforesaid antibody molecules comprise a light chain comprising the amino acid sequence of SEQ ID NO: 68.

In other embodiments, the aforesaid antibody molecules comprise a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 70.

In other embodiments, the aforesaid antibody molecules comprise a light chain comprising the amino acid sequence of SEQ ID NO: 72.

In other embodiments, the aforesaid antibody molecules comprise a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 74.

In other embodiments, the aforesaid antibody molecules comprise a light chain comprising the amino acid sequence of SEQ ID NO: 76.

In other embodiments, the aforesaid antibody molecules comprise a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 78.

In other embodiments, the aforesaid antibody molecules comprise a light chain comprising the amino acid sequence of SEQ ID NO: 80.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 42.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 66.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 70.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 50 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 70.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 46.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 50 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 46.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 50 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 54.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 54.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 58.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 62.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 50 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 66.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 74.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 78.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 82 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 70.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 82 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 66.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 86 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 66.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 91 and a light chain comprising the amino acid sequence of SEQ ID NO: 44.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 91 and a light chain comprising the amino acid sequence of SEQ ID NO: 56.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 91 and a light chain comprising the amino acid sequence of SEQ ID NO: 68.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 91 and a light chain comprising the amino acid sequence of SEQ ID NO: 72.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 102 and a light chain comprising the amino acid sequence of SEQ ID NO: 72.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 44.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 48.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 52 and a light chain comprising the amino acid sequence of SEQ ID NO: 48.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 52 and a light chain comprising the amino acid sequence of SEQ ID NO: 56.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 56.

In other embodiments, the aforesaid antibodies comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 60.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 64.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 52 and a light chain comprising the amino acid sequence of SEQ ID NO: 68.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 68.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 52 and a light chain comprising the amino acid sequence of SEQ ID NO: 72.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 72.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 76.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 80.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 84 and a light chain comprising the amino acid sequence of SEQ ID NO: 72.

In other embodiments, the aforesaid antibodies comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 84 and a light chain comprising the amino acid sequence of SEQ ID NO: 68.

In other embodiments, the aforesaid antibody molecules comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 88 and a light chain comprising the amino acid sequence of SEQ ID NO: 68.

In other embodiments, the aforesaid antibody molecules are chosen from a Fab, F(ab')2, Fv, or a single chain Fv fragment (scFv).

In other embodiments, the aforesaid antibody molecules comprise a heavy chain constant region selected from IgG1, IgG2, IgG3, and IgG4.

In other embodiments, the aforesaid antibody molecules comprise a light chain constant region chosen from the light chain constant regions of kappa or lambda.

In other embodiments, the aforesaid antibody molecules comprise a human IgG4 heavy chain constant region with a mutation at position 228 according to EU numbering or position 108 of SEQ ID NO: 212 or 214 and a kappa light chain constant region.

In other embodiments, the aforesaid antibody molecules comprise a human IgG4 heavy chain constant region with a Serine to Proline mutation at position 228 according to EU numbering or position 108 of SEQ ID NO: 212 or 214 and a kappa light chain constant region.

In other embodiments, the aforesaid antibody molecules comprise a human IgG1 heavy chain constant region with an Asparagine to Alanine mutation at position 297 according to EU numbering or position 180 of SEQ ID NO: 216 and a kappa light chain constant region.

In other embodiments, the aforesaid antibody molecules comprise a human IgG1 heavy chain constant region with an Aspartate to Alanine mutation at position 265 according to EU numbering or position 148 of SEQ ID NO: 217, and Proline to Alanine mutation at position 329 according to EU numbering or position 212 of SEQ ID NO: 217 and a kappa light chain constant region.

In other embodiments, the aforesaid antibody molecules comprise a human IgG1 heavy chain constant region with a Leucine to Alanine mutation at position 234 according to EU numbering or position 117 of SEQ ID NO: 218, and Leucine to Alanine mutation at position 235 according to EU numbering or position 118 of SEQ ID NO: 218 and a kappa light chain constant region.

In other embodiments, the aforesaid antibody molecules are capable of binding to human PD-1 with a dissociation constant (K_D) of less than about 0.2 nM.

In some embodiments, the aforesaid antibody molecules bind to human PD-1 with a K_D of less than about 0.2 nM, 0.15 nM, 0.1 nM, 0.05 nM, or 0.02 nM, *e.g.,* about 0.13 nM to 0.03 nM, *e.g.,* about 0.077 nM to 0.088 nM, *e.g.,* about 0.083 nM, *e.g.,* as measured by a Biacore method.

In other embodiments, the aforesaid antibody molecules bind to cynomolgus PD-1 with a K_D of less than about 0.2 nM, 0.15 nM, 0.1 nM, 0.05 nM, or 0.02 nM, *e.g.,* about 0.11 nM to 0.08 nM, *e.g.,* about 0.093 nM, *e.g.,* as measured by a Biacore method.

In certain embodiments, the aforesaid antibody molecules bind to both human PD-1 and cynomolgus PD-1 with similar K_D, *e.g.,* in the nM range, *e.g.,* as measured by a Biacore method. In some embodiments, the aforesaid antibody molecules bind to a human PD-1-Ig fusion protein with a K_D of less than about 0.1 nM, 0.075 nM, 0.05 nM, 0.025 nM, or 0.01 nM, *e.g.,* about 0.04 nM, *e.g.,* as measured by ELISA.

In some embodiments, the aforesaid antibody molecules bind to Jurkat cells that express human PD-1 (*e.g.,* human PD-1-transfected Jurkat cells) with a K_D of less than about 0.1 nM, 0.075 nM, 0.05 nM, 0.025 nM, or 0.01 nM, *e.g.,* about 0.06 nM, *e.g.,* as measured by FACS analysis.

In some embodiments, the aforesaid antibody molecules bind to cynomolgus T cells with a K_D of less than about 1nM, 0.75 nM, 0.5 nM, 0.25 nM, or 0.1 nM, *e.g.,* about 0.4 nM, *e.g.,* as measured by FACS analysis.

In some embodiments, the aforesaid antibody molecules bind to cells that express cynomolgus PD-1 (*e.g.,* cells transfected with cynomolgus PD-1) with a K_D of less than about 1nM, 0.75 nM, 0.5 nM, 0.25 nM, or 0.01 nM, *e.g.,* about 0.6 nM, *e.g.,* as measured by FACS analysis.

In certain embodiments, the aforesaid antibody molecules are not cross-reactive with mouse or rat PD-1. In other embodiments, the aforesaid antibodies are cross-reactive with rhesus PD-1. For example, the cross-reactivity can be measured by a Biacore method or a binding assay using cells that expresses PD-1 (*e.g.,* human PD-1-expressing 300.19 cells). In other embodiments, the aforesaid antibody molecules bind an extracellular Ig-like domain of PD-1.

In other embodiments, the aforesaid antibody molecules are capable of reducing binding of PD-1 to PD-L1, PD-L2, or both, or a cell that expresses PD-L1, PD-L2, or both. In some embodiments, the aforesaid antibody molecules reduce (*e.g.*, block) PD-L1 binding to a cell that expresses PD-1 (*e.g.*, human PD-1-expressing 300.19 cells) with an IC50 of less than about 1.5 nM, 1 nM, 0.8 nM, 0.6 nM, 0.4 nM, 0.2 nM, or 0.1 nM, *e.g.*, between about 0.79 nM and about 1.09 nM, *e.g.*, about 0.94 nM, or about 0.78 nM or less, *e.g.*, about 0.3 nM. In some embodiments, the aforesaid antibodies reduce (*e.g.*, block) PD-L2 binding to a cell that expresses PD-1 (*e.g.*, human PD-1-expressing 300.19 cells) with an IC50 of less than about 2 nM, 1.5 nM, 1 nM, 0.5 nM, or 0.2 nM, *e.g.*, between about 1.05 nM and about 1.55 nM, or about 1.3 nM or less, *e.g.*, about 0.9 nM.

In other embodiments, the aforesaid antibody molecules are capable of enhancing an antigen-specific T cell response.

In embodiments, the antibody molecule is a monospecific antibody molecule or a bispecific antibody molecule. In embodiments, the antibody molecule has a first binding specificity for PD-1 and a second binding specifity for TIM-3, LAG-3, CEACAM (*e.g.*, CEACAM-1, CEACAM-3, and/or CEACAM-5), PD-L1 or PD-L2. In embodiments, the antibody molecule comprises an antigen binding fragment of an antibody, *e.g.*, a half antibody or antigen binding fragment of a half antibody.

In some embodiments, the aforesaid antibody molecules increase the expression of IL-2 from cells activated by Staphylococcal enterotoxin B (SEB) (*e.g.*, at 25 μg/mL) by at least about 2, 3, 4, 5-fold, *e.g.*, about 2 to 3-fold, *e.g.*, about 2 to 2.6-fold, *e.g.*, about 2.3-fold, compared to the expression of IL-2 when an isotype control (*e.g.*, IgG4) is used, *e.g.*, as measured in a SEB T cell activation assay or a human whole blood *ex vivo* assay.

In some embodiments, the aforesaid antibody molecules increase the expression of IFN-γ from T cells stimulated by anti-CD3 (*e.g.*, at 0.1 μg/mL) by at least about 2, 3, 4, 5-fold, *e.g.*, about 1.2 to 3.4-fold, *e.g.*, about 2.3-fold, compared to the expression of IFN-γ when an isotype control (*e.g.*, IgG4) is used, *e.g.*, as measured in an IFN-γ activity assay.

In some embodiments, the aforesaid antibody molecules increase the expression of IFN-γ from T cells activated by SEB (*e.g.*, at 3 pg/mL) by at least about 2, 3, 4, 5-fold, *e.g.*, about 0.5 to 4.5-fold, *e.g.*, about 2.5-fold, compared to the expression of IFN-γ when an isotype control (*e.g.*, IgG4) is used, *e.g.*, as measured in an IFN-γ activity assay.

In some embodiments, the aforesaid antibody molecules increase the expression of IFN-γ from T cells activated with an CMV peptide by at least about 2, 3, 4, 5-fold, *e.g.,* about 2 to 3.6-fold, *e.g.,* about 2.8-fold, compared to the expression of IFN-γ when an isotype control (*e.g.,* IgG4) is used, *e.g.,* as measured in an IFN-γ activity assay.

In some embodiments, the aforesaid antibody molecules increase the proliferation of $CD8^+$ T cells activated with an CMV peptide by at least about 1, 2, 3, 4, 5-fold, *e.g.,* about 1.5-fold, compared to the proliferation of $CD8^+$ T cells when an isotype control (*e.g.,* IgG4) is used, *e.g.,* as measured by the percentage of CD8+ T cells that passed through at least n (*e.g.,* n = 2 or 4) cell divisions.

In certain embodiments, the aforesaid antibody molecules has a Cmax between about 100 μg/mL and about 500 μg/mL, between about 150 μg/mL and about 450 μg/mL, between about 250 μg/mL and about 350 μg/mL, or between about 200 μg/mL and about 400 μg/mL, *e.g.,* about 292.5 μg/mL, *e.g.,* as measured in monkey.

In certain embodiments, the aforesaid antibody molecules has a $T_{1/2}$ between about 250 hours and about 650 hours, between about 300 hours and about 600 hours, between about 350 hours and about 550 hours, or between about 400 hours and about 500 hours, *e.g.,* about 465.5 hours, *e.g.,* as measured in monkey.

In some embodiments, the aforesaid antibody molecules bind to PD-1 with a Kd slower than 5×10^{-4}, 1×10^{-4}, 5×10^{-5}, or 1×10^{-5} s^{-1}, *e.g.,* about 2.13×10^{-4} s^{-1}, *e.g.*, as measured by a Biacore method. In some embodiments, the aforesaid antibody molecules bind to PD-1 with a Ka faster than 1×10^{4}, 5×10^{4}, 1×10^{5}, or 5×10^{5} $M^{-1}s^{-1}$, *e.g.,* about 2.78×10^{5} $M^{-1}s^{-1}$, *e.g.*, as measured by a Biacore method.

In some embodiments, the aforesaid anti-PD-1 antibody molecules bind to one or more residues within the C strand, CC' loop, C' strand and FG loop of PD-1. The domain structure of PD-1 is described, *e.g.*, in Cheng et al., "Structure and Interactions of the Human Programmed Cell Death 1 Receptor" *J. Biol. Chem.* 2013, 288:11771-11785. As described in Cheng *et. al.*, the C strand comprises residues F43-M50, the CC' loop comprises S51-N54, the C' strand comprises residues Q55-F62, and the FG loop comprises residues L108-I114 (amino acid numbering according to Chang *et al. supra*). Accordingly, in some embodiments, an anti-PD-1 antibody as described herein binds to at least one residue in one or more of the ranges F43-M50, S51-N54, Q55-F62, and L108-I114 of PD-1. In some embodiments, an anti-PD-1 antibody as

described herein binds to at least one residue in two, three, or all four of the ranges F43-M50, S51-N54, Q55-F62, and L108-I114 of PD-1. In some embodiments, the anti-PD-1 antibody binds to a residue in PD-1 that is also part of a binding site for one or both of PD-L1 and PD-L2.

In another aspect, the invention provides an isolated nucleic acid molecule encoding any of the aforesaid antibody molecules, vectors and host cells thereof.

An isolated nucleic acid encoding the antibody heavy chain variable region or light chain variable region, or both, of any the aforesaid antibody molecules is also provided.

In one embodiment, the isolated nucleic acid encodes heavy chain CDRs 1-3, wherein said nucleic acid comprises a nucleotide sequence of SEQ ID NO: 108-112, 223, 122-126, 133-137, or 144-146.

In another embodiment, the isolated nucleic acid encodes light chain CDRs 1-3, wherein said nucleic acid comprises a nucleotide sequence of SEQ ID NO: 113-120, 127-132, or 138-143.

In other embodiments, the aforesaid nucleic acid further comprises a nucleotide sequence encoding a heavy chain variable domain, wherein said nucleotide sequence is at least 85% identical to any of SEQ ID NO: 39, 51, 83, 87, 90, 95, or 101.

In other embodiments, the aforesaid nucleic acid further comprises a nucleotide sequence encoding a heavy chain variable domain, wherein said nucleotide sequence comprises any of SEQ ID NO: 39, 51, 83, 87, 90, 95, or 101.

In other embodiments, the aforesaid nucleic acid further comprises a nucleotide sequence encoding a heavy chain, wherein said nucleotide sequence is at least 85% identical to any of SEQ ID NO: 41, 53, 85, 89, 92, 96, or 103.

In other embodiments, the aforesaid nucleic acid further comprises a nucleotide sequence encoding a heavy chain, wherein said nucleotide sequence comprises any of SEQ ID NO: 41, 53, 85, 89, 92, 96, or 103.

In other embodiments, the aforesaid nucleic acid further comprises a nucleotide sequence encoding a light chain variable domain, wherein said nucleotide sequence is at least 85% identical to any of SEQ ID NO: 45, 49, 57, 61, 65, 69, 73, 77, 81, 94, 98, 100, 105, or 107.

In other embodiments, the aforesaid nucleic acid further comprises a nucleotide sequence encoding a light chain variable domain, wherein said nucleotide sequence comprises any of SEQ ID NO: 45, 49, 57, 61, 65, 69, 73, 77, 81, 94, 98, 100, 105, or 107.

In other embodiments, the aforesaid nucleic acid further comprises a nucleotide sequence encoding a light chain, wherein said nucleotide sequence is at least 85% identical to any of SEQ ID NO: 45, 49, 57, 61, 65, 69, 73, 77, 81, 94, 98, 100, 105 or 107.

In other embodiments, the aforesaid nucleic acid further comprises a nucleotide sequence encoding a light chain, wherein said nucleotide sequence comprises any of SEQ ID NO: 45, 49, 57, 61, 65, 69, 73, 77, 81, 94, 98, 100, 105 or 107.

In certain embodiments, one or more expression vectors and host cells comprising the aforesaid nucleic acids are provided.

A method of producing an antibody molecule or fragment thereof, comprising culturing the host cell as described herein under conditions suitable for gene expression is also provided.

Pharmaceutical Compositions and Kits

In another aspect, the present invention provides compositions, *e.g.*, pharmaceutically acceptable compositions, which include an antibody molecule described herein, formulated together with a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, isotonic and absorption delaying agents, and the like that are physiologically compatible. The carrier can be suitable for intravenous, intramuscular, subcutaneous, parenteral, rectal, spinal or epidermal administration (*e.g.* by injection or infusion).

The compositions of this invention may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions. The preferred mode of administration is parenteral (*e.g.*, intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal,

intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

Therapeutic compositions typically should be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high antibody concentration. Sterile injectable solutions can be prepared by incorporating the active compound (*i.e.*, antibody or antibody portion) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

The antibody molecules can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is intravenous injection or infusion. For example, the antibody molecules can be administered by intravenous infusion at a rate of more than 20 mg/min, *e.g.*, 20-40 mg/min, and typically greater than or equal to 40 mg/min to reach a dose of about 35 to 440 mg/m^2, typically about 70 to 310 mg/m^2, and more typically, about 110 to 130 mg/m^2. In embodiments, the antibody molecules can be administered by intravenous infusion at a rate of less than 10mg/min; preferably less than or equal to 5 mg/min to reach a dose of about 1 to 100 mg/m ^{2}, preferably about 5 to 50 mg/m^2, about 7 to 25 mg/m^2 and more preferably, about 10 mg/m^2. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants,

transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. *See, e.g., Sustained and Controlled Release Drug Delivery Systems*, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

In certain embodiments, an antibody molecule can be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. Therapeutic compositions can also be administered with medical devices known in the art.

Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody molecule is 0.1-30 mg/kg, more preferably 1-25 mg/kg. Dosages and therapeutic regimens of the anti-PD-1 antibody molecule can be determined by a skilled artisan. In certain embodiments, the anti-PD-1 antibody molecule is administered by injection (*e.g.*,

subcutaneously or intravenously) at a dose of about 1 to 40 mg/kg, *e.g.*, 1 to 30 mg/kg, *e.g.*, about 5 to 25 mg/kg, about 10 to 20 mg/kg, about 1 to 5 mg/kg, 1 to 10 mg/kg, 5 to 15 mg/kg, 10 to 20 mg/kg, 15 to 25 mg/kg, or about 3 mg/kg. The dosing schedule can vary from *e.g.*, once a week to once every 2, 3, or 4 weeks. In one embodiment, the anti-PD-1 antibody molecule is administered at a dose from about 10 to 20 mg/kg every other week. The antibody molecule can be administered by intravenous infusion at a rate of more than 20 mg/min, *e.g.*, 20-40 mg/min, and typically greater than or equal to 40 mg/min to reach a dose of about 35 to 440 mg/m^2, typically about 70 to 310 mg/m^2, and more typically, about 110 to 130 mg/m^2. In embodiments, the infusion rate of about 110 to 130 mg/m^2 achieves a level of about 3 mg/kg. In other embodiments, the antibody molecule can be administered by intravenous infusion at a rate of less than 10 mg/min, *e.g.*, less than or equal to 5 mg/min to reach a dose of about 1 to 100 mg/m^2, *e.g.*, about 5 to 50 mg/m^2, about 7 to 25 mg/m^2, or, about 10 mg/m^2. In some embodiments, the antibody is infused over a period of about 30 min. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody or antibody portion of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the modified antibody or antibody fragment may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the modified antibody or antibody fragment is outweighed by the therapeutically beneficial effects. A "therapeutically effective dosage" preferably inhibits a measurable parameter, *e.g.,* tumor growth rate by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to inhibit a measurable parameter, *e.g.,* cancer, can be evaluated in an animal model

system predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit, such inhibition in vitro by assays known to the skilled practitioner.

A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Also within the scope of the invention is a kit comprising an antibody molecule described herein. The kit can include one or more other elements including: instructions for use; other reagents, *e.g.,* a label, a therapeutic agent, or an agent useful for chelating, or otherwise coupling, an antibody to a label or therapeutic agent, or a radioprotective composition; devices or other materials for preparing the antibody for administration; pharmaceutically acceptable carriers; and devices or other materials for administration to a subject.

Uses of Anti-PD-1 Antibody Molecules

The anti-PD-1 antibody molecules disclosed herein have *in vitro and in vivo* diagnostic, as well as therapeutic and prophylactic utilities. For example, these molecules can be administered to cells in culture, *in vitro* or *ex vivo*, or to a subject, *e.g.*, a human subject, to treat, prevent, and/or diagnose a variety of disorders, such as cancers and infectious disorders.

Accordingly, in one aspect, the invention provides a method of modifying an immune response in a subject comprising administering to the subject the antibody molecule described herein, such that the immune response in the subject is modified. In one embodiment, the immune response is enhanced, stimulated or up-regulated. In one embodiment, the antibody molecules enhance an immune response in a subject by blockade of PD-1.

As used herein, the term "subject" is intended to include human and non-human animals. In one embodiment, the subject is a human subject, *e.g.*, a human patient having a disorder or condition characterized by abnormal PD-1 functioning. The term "non-human animals" includes mammals and non-mammals, such as non-human primates. In one embodiment, the subject is a human. In one embodiment, the subject is a human patient in need of enhancement of an immune response. In one embodiment, the subject is immunocompromised, *e.g.*, the subject is undergoing, or has undergone a chemotherapeutic or radiation therapy. Alternatively, or in

combination, the subject is, or is at risk of being, immunocompromised as a result of an infection. The methods and compositions described herein are suitable for treating human patients having a disorder that can be treated by augmenting the T-cell mediated immune response. For example, the methods and compositions described herein can enhance a number of immune activities. In one embodiment, the subject has increased number or activity of tumour-infiltrating T lymphocytes (TILs). In another embodiment, the subject has increased expression or activity of interferon-gamma (IFN-γ). In yet another embodiment, the subject has decreased PD-L1 expression or activity.

Therapeutic Uses

Cancer

Blockade of PD-1 can enhance an immune response to cancerous cells in a subject. The ligand for PD-1, PD-L1, is not expressed in normal human cells, but is abundant in a variety of human cancers (Dong *et al.* (2002) *Nat Med* 8:787-9). The interaction between PD-1 and PD-L1 can result in a decrease in tumor infiltrating lymphocytes, a decrease in T-cell receptor mediated proliferation, and/or immune evasion by the cancerous cells (Dong *et al.* (2003) *J Mol Med* 81:281-7; Blank *et al.* (2005) *Cancer Immunol. Immunother.* 54:307-314; Konishi *et al.* (2004) *Clin. Cancer Res.* 10:5094-100). Immune suppression can be reversed by inhibiting the local interaction of PD-1 to PD-L1; the effect is additive when the interaction of PD-1 to PD-L2 is blocked as well (Iwai *et al.* (2002) *PNAS* 99:12293-7; Brown *et al.* (2003) *J. Immunol.* 170:1257-66). Thus, inhibition of PD-1 can result in augmenting an immune response.

In one aspect, the invention relates to treatment of a subject *in vivo* using an anti-PD-1 antibody molecule such that growth of cancerous tumors is inhibited or reduced. An anti-PD-1 antibody may be used alone to inhibit the growth of cancerous tumors. Alternatively, an anti-PD-1 antibody may be used in combination with one or more of: a standard of care treatment (*e.g.*, for cancers or infectious disorders), another antibody or antigen-binding fragment thereof, an immunomodulator (*e.g.*, an activator of a costimulatory molecule or an inhibitor of an inhibitory molecule); a vaccine, *e.g.*, a therapeutic cancer vaccine; or other forms of cellular immunotherapy, as described below.

Accordingly, in one embodiment, the invention provides a method of inhibiting growth of tumor cells in a subject, comprising administering to the subject a therapeutically effective amount of an anti-PD-1 antibody molecule described herein.

In one embodiment, the methods are suitable for the treatment of cancer *in vivo*. To achieve antigen-specific enhancement of immunity, the anti-PD-1 antibody molecule can be administered together with an antigen of interest. When antibodies to PD-1 are administered in combination with one or more agents, the combination can be administered in either order or simultaneously.

Types of cancer; theranostic methods

In another aspect, a method of treating a subject, *e.g.*, reducing or ameliorating, a hyperproliferative condition or disorder (*e.g.*, a cancer), *e.g.*, solid tumor, a hematological cancer, soft tissue tumor, or a metastatic lesion, in a subject is provided. The method includes administering to the subject one or more anti-PD-1 antibody molecules described herein, alone or in combination with other agents or therapeutic modalities.

As used herein, the term "cancer" is meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. Examples of cancerous disorders include, but are not limited to, solid tumors, hematological cancers, soft tissue tumors, and metastatic lesions. Examples of solid tumors include malignancies, *e.g.*, sarcomas, and carcinomas (including adenocarcinomas and squamous cell carcinomas), of the various organ systems, such as those affecting liver, lung, breast, lymphoid, gastrointestinal (*e.g.*, colon), genitourinary tract (*e.g.*, renal, urothelial cells), prostate and pharynx. Adenocarcinomas include malignancies such as most colon cancers, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus. Squamous cell carcinomas include malignancies, *e.g.*, in the lung, esophagus, skin, head and neck region, oral cavity, anus, and cervix. In one embodiment, the cancer is a melanoma, *e.g.*, an advanced stage melanoma. Metastatic lesions of the aforementioned cancers can also be treated or prevented using the methods and compositions of the invention.

Exemplary cancers whose growth can be inhibited using the antibodies molecules disclosed herein include cancers typically responsive to immunotherapy. Non-limiting examples

of preferred cancers for treatment include melanoma (*e.g.,* metastatic malignant melanoma), renal cancer (*e.g.,* clear cell carcinoma), prostate cancer (*e.g.,* hormone refractory prostate adenocarcinoma), breast cancer, colon cancer and lung cancer (*e.g.,* non-small cell lung cancer). Additionally, refractory or recurrent malignancies can be treated using the antibody molecules described herein.

Examples of other cancers that can be treated include bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, anal cancer, gastro-esophageal, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Merkel cell cancer, Hodgkin lymphoma, non-Hodgkin lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, multiple myeloma, myelodisplastic syndromes, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos (*e.g.,* mesothelioma), and combinations of said cancers.

Treatment of metastatic cancers, *e.g.*, metastatic cancers that express PD-L1 (Iwai *et al.* (2005) *Int. Immunol.* 17:133-144) can be effected using the antibody molecules described herein. In one embodiment, the cancer expresses an elevated level of PD-L1, IFNγ and /or CD8.

While not wishing to be bound by theory, in some embodiments, a patient is more likely to respond to treatment with an immunomodulator (optionally in combination with one or more agents as described herein) if the patient has a cancer that highly expresses PD-L1, and/or the cancer is infiltrated by anti-tumor immune cells, *e.g.,* TILs. The anti-tumor immunce cells may be positive for CD8, PD-L1, and/or IFN-γ; thus levels of CD8, PD-L1, and/or IFN-γ can serve as a readout for levels of TILs in the microenvironment. In certain embodiments, the cancer microenvironment is referred to as triple-positive for PD-L1/CD8/IFN-γ.

Accordingly, in certain aspects, this application provides methods of determining whether a tumor sample is positive for one or more of PD-L1, CD8, and IFN-γ, and if the tumor sample is positive for one or more, *e.g.*, two, or all three, of the markers, then administering to the patient a therapeutically effective amount of an anti-PD-1 antibody molecule, optionally in combination with one or more other immunnomodulators or anti-cancer agents.

In the following indications, a large fraction of patients are triple-positive for PD-L1/CD8/IFN-γ: Lung cancer (squamous); lung cancer (adenocarcinoma); head and neck cancer; stomach cancer; NSCLC; HNSCC; gastric cancers (*e.g.*, MSIhi and/or EBV+); CRC (*e.g.*, MSIhi); nasopharyngeal cancer (NPC); cervical cancer (*e.g.*, squamous); thyroid cancer *e.g.*, papillary thyroid; melanoma; TN breast cancer; and DLBCL (Diffuse Large B-Cell Lymphoma). In breast cancer generally and in colon cancer generally, a moderate fraction of patients is triple-positive for PD-L1/CD8/IFN-γ. In the following indications, a small fraction of patients are triple-positive for PD-L1/CD8/IFN-γ: ER+ breast cancer, and pancreatic cancer. These findings are discussed further in Example 4. Regardless of whether a large or small fraction of patients is triple-positive for these markers, screening the patients for these markers allows one to identify a fraction of patients that has an especially high likelihood of responding favorably to therapy with a PD-1 antibody (*e.g.*, a blocking PD-1 antibody), optionally in combination with one or more other immunomodulators (*e.g.*, an anti-TIM-3 antibody molecule, an anti-LAG-3 antibody molecule, or an anti-PD-L1 antibody molecule) and/or anti-cancer agents, *e.g.*, those listed in Table 7 and disclosed in the publications listed in Table 7.

In some embodiments, the cancer sample is classified as triple-positive for PD-L1/CD8/IFN-γ. This measurement can roughly be broken down into two thresholds: whether an individual cell is classified as positive, and whether the sample as a whole is classified as positive. First, one can measure, within an individual cell, the level of PD-L1, CD8, and/or IFN-γ. In some embodiments, a cell that is positive for one or more of these markers is a cell that has a higher level of the marker compared to a control cell or a reference value. For example, in some embodiments, a high level of PD-L1 in a given cell is a level higher than the level of PD-L1 in a corresponding non-cancerous tissue in the patient. As another example, in some embodiments, a high level of CD8 or IFN-γ in a given cell is a level of that protein typically seen in a TIL. Second, one can also measure the percentage of cells in the sample that are positive for PD-L1, CD8, and/or IFN-γ. (It is not necessary for a single cell to express all three markers.) In

some embodiments, a triple positive sample is one that has a high percentage of cells, *e.g.*, higher than a reference value or higher than a control sample, that are positive for these markers.

In other embodiments, one can measure the levels of PD-L1, CD8, and/or IFN-γ overall in the sample. In this case, a high level of CD8 or IFN-γ in the sample can be the level of that protein typically seen in a tumor infiltrated with TIL. Similarly, a high level of PD-L1 can be the level of that protein typically seen in a tumor sample, *e.g.*, a tumor microenvironment.

The identification of subsets of patients that are triple-positive for PD-L1/CD8/IFN-γ, as shown in Example 4 herein, reveals certain sub-populations of patients that are likely to be responsive to PD-1 antibody therapy. For instance, many IM-TN (immunomodulatory, triple negative) breast cancer patients are triple-positive for PD-L1/CD8/IFN-γ. IM-TN breast cancer is described in, *e.g.*, Brian D. Lehmann *et al.*, "Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies", *J Clin Invest.* Jul 1, 2011; 121(7): 2750–2767. Triple-negative breast cancers are those that do not express estrogen receptor (ER), progesterone receptor (PR) and Her2/neu. These cancers are difficult to treat because they are typically not responsive to agents that target ER, PR, and Her2/neu. Triple-negative breast cancers can be further subdivided into different classes, one of which is immunomodulatory. As described in Lehmann *et al.*, IM-TN breast cancer is enriched for factors involved in immune cell processes, for example, one or more of immune cell signaling (*e.g.*, TH1/TH2 pathway, NK cell pathway, B cell receptor signaling pathway, DC pathway, and T cell receptor signaling), cytokine signaling (*e.g.*, cytokine pathway, IL-12 pathway, and IL-7 pathway), antigen processing and presentation, signaling through core immune signal transduction pathways (*e.g.*, NFKB, TNF, and JAK/STAT signaling), genes involved in T-cell function, immune transcription, interferon (IFN) response and antigen processing. Accordingly, in some embodiments, the cancer treated is a cancer that is, or is determined to be, positive for one or more marker of IM-TN breast cancer, *e.g.*, a factor that promotes one or more of immune cell signaling (*e.g.*, TH1/TH2 pathway, NK cell pathway, B cell receptor signaling pathway, DC pathway, and T cell receptor signaling), cytokine signaling (*e.g.*, cytokine pathway, IL-12 pathway, and IL-7 pathway), antigen processing and presentation, signaling through core immune signal transduction pathways (*e.g.*, NFKB, TNF, and JAK/STAT signaling), genes involved in T-cell function, immune transcription, interferon (IFN) response and antigen processing.

As another example, it is shown herein that a subset of colon cancer patients having high MSI (microsatellite instability) is also triple-positive for PD-L1/CD8/IFN-γ. Accordingly, in some embodiments, a PD-1 antibody, *e.g.*, a PD-1 antibody as described herein, (optionally in combination with one or more immunomodulators such as a LAG-3 antibody, TIM-3 antibody, or PD-L1 antibody, and one or more anti-cancer agents, *e.g.*, an anti-cancer agent described in Table 7 or in a publication in Table 7) is administered to a patient who has, or who is identified as having, colon cancer with high MSI, thereby treating the cancer. In some embodiments, a cell with high MSI is a cell having MSI at a level higher than a reference value or a control cell, *e.g.*, a non-cancerous cell of the same tissue type as the cancer.

As another example, it is shown herein that a subset of gastric cancer patients having high MSI, and/or which is EBV+, is also triple-positive for PD-L1/CD8/IFN-γ. Accordingly, in some embodiments, a PD-1 antibody, *e.g.*, a PD-1 antibody as described herein, (optionally in combination with one or more immunomodulators such as a LAG-3 antibody, TIM-3 antibody, or PD-L1 antibody, and one or more anti-cancer agents, *e.g.,* an anti-cancer agent described in Table 7 or in a publication in Table 7) is administered to a patient who has, or who is identified as having, gastric cancer with high MSI and/or EBV+, thereby treating the cancer. In some embodiments, a cell with high MSI is a cell having MSI at a level higher than a reference value or a control cell, *e.g.,* a non-cancerous cell of the same tissue type as the cancer.

Additionally disclosed herein are methods of assaying a cancer for PD-L1, and then treating the cancer with a PD-1 antibody. As described in Example 5 herein, a cancer sample can be assayed for PD-L1 protein levels or mRNA levels. A sample having levels of PD-L1 (protein or mRNA) higher than a reference value or a control cell (*e.g.*, a non-cancerous cell) can be classified as PD-L1 positive. Accordingly, in some embodiments, a PD-1 antibody, *e.g.*, a PD-1 antibody as described herein, (optionally in combination with one or more anti-cancer agents) is administered to a patient who has, or who is identified as having, a cancer that is PD-L1 positive. The cancer may be, *e.g.,* non-small cell lung (NSCLC) adenocarcinoma (ACA), NSCLC squamous cell carcinoma (SCC), or hepatocellular carcinoma (HCC).

In some embodiments, the methods herein involve using a PD-1 antibody, *e.g.,* a PD-1 antibody as described herein, *e.g.*, as a monotherapy, for treating a cancer that is (or is identified as being) positive for PD-L1. In some embodiments, the cancer is colorectal cancer (*e.g.,* MSI-high), gastric cancer (*e.g.,* MSI-high and/or EBV+), NPC, cervical cancer, breast cancer (*e.g.,*

TN breast cancer), and ovarian cancer. In some embodiments, the cancer is NSCLC, melanoma, or HNSCC. In some embodiments, the PD-1 antibody is administered at a dose of, *e.g.,* 1, 3, 10, or 20 mg/kg.

Based on, *e.g*, Example 4 herein, it was found that certain gastric cancers that are triple-positive for PD-L1/CD8/IFN-γ are also positive for PIK3CA. Accordingly, in some embodiments, a cancer can be treated with an anti-PD-1 antibody molecule (optionally in combination with one or more immunomodulators, *e.g.,* an anti-LAG-3 antibody molecule, an anti-TIM-3 antibody molecule, or an anti-PD-L1 antibody molecule) and an agent that inhibits PIK3CA. Exemplary agents in this category are described in Stein RC (September 2001). "Prospects for phosphoinositide 3-kinase inhibition as a cancer treatment". *Endocrine-related Cancer* 8 (3): 237–48 and Marone R, Cmiljanovic V, Giese B, Wymann MP (January 2008). "Targeting phosphoinositide 3-kinase: moving towards therapy". *Biochimica et Biophysica Acta* 1784 (1): 159–85.

Based on, *e.g*, Example 4 herein, CRC, *e.g.,* a patient that has (or is identified as having) MSI-high CRC may be treated with a PD-1 antibody, optionally in combination with a therapeutic that targets one or more of LAG-3, RNF43, and BRAF. For instance, these cancers may be treated with a PD-1 antibody, optionally in combination with one or more therapeutics that target one or more of LAG-3, PD-1, RNF43, and BRAF. In embodiments, the one or more therapeutics include an immunomodulators such as an anti-LAG-3 antibody molecule, and an anti-cancer agent described in Table 7 or a publication listed in Table 7. LAG-3 inhibitors, *e.g.,* antibodies, are described herein. RNF43 can be inhibited, *e.g.,* with an antibody, small molecule (*e.g.,* 2-(2',3-dimethyl-[2,4'-bipyridin]-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide (Compound A28)), siRNA, or a Rspo ligand or derivative thereof. BRAF inhibitors (*e.g.*, vemurafenib or dabrafenib) are described herein.

Based on, *e.g*, Example 4 herein, a patient that has (or is identified as having) a squamous cell lung cancer may be treated with a PD-1 antibody molecule in combination with a therapeutic that targets LAG-3, *e.g.,* a LAG-3 antibody molecule, and optionally with one or more anti-cancer agents, *e.g.*, an anti-cancer agent described in Table 7 or in a publication in Table 7.

In some embodiments, a subject that has (or is identified as having) a squamous cell lung cancer may be treated with a PD-1 antibody, optionally in combination with a therapeutic that targets TIM-3, *e.g.,* a TIM-3 antibody. TIM-3 inhibitors, *e.g.*, antibodies, are described herein.

Based on, *e.g*, Example 4 herein, a patient that has (or is identified as having) a thyroid cancer may be treated with a PD-1 antibody molecule, optionally in combination with a therapeutic that targets BRAF, and optionally in combination with one or more immunomodulators, *e.g.,* an anti-LAG-3 antibody molecule, an anti-TIM-3 antibody molecule, and an anti-PD-L1 antibody molecule. BRAF inhibitors (*e.g.*, vemurafenib or dabrafenib) are described herein, *e.g.,* in Table 7 and the publications listed in Table 7.

In some embodiments, the therapies here can be used to treat a patient that has (or is identified as having) a cancer associated with an infection, *e.g.,* a viral or bacterial infection. Exemplary cancers include cervical cancer, anal cancer, HPV-associated head and neck squamous cell cancer, HPV-associated esophageal papillomas, HHV6-associated lymphomas, EBV-associated lymphomas (including Burkitt lymphoma), Gastric MALT lymphoma, other infection-associated MALT lymphomas, HCC, and Kaposi's sarcoma.

In other embodiments, the cancer is a hematological malignancy or cancer including but is not limited to a leukemia or a lymphoma. For example, the anti-PD-1 antibody molecule can be used to treat cancers and malignancies including, but not limited to, *e.g.*, acute leukemias including but not limited to, *e.g.,* B-cell acute lymphoid leukemia ("BALL"), T-cell acute lymphoid leukemia ("TALL"), acute lymphoid leukemia (ALL); one or more chronic leukemias including but not limited to, *e.g.*, chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL); additional hematologic cancers or hematologic conditions including, but not limited to, *e.g.*, B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, Follicular lymphoma, Hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, and "preleukemia" which are a diverse collection of hematological conditions united by ineffective production (or dysplasia) of myeloid blood cells, and the like.

In one embodiment, the cancer is chosen from a lung cancer (*e.g.*, a non-small cell lung cancer (NSCLC) (*e.g.*, a NSCLC with squamous and/or non-squamous histology, or a NSCLC adenocarcinoma)), a melanoma (*e.g.*, an advanced melanoma), a renal cancer (*e.g.*, a renal cell carcinoma, *e.g.,* clear cell renal cell carcinoma), a liver cancer, a myeloma (*e.g.*, a multiple

myeloma), a prostate cancer, a breast cancer (*e.g.*, a breast cancer that does not express one, two or all of estrogen receptor, progesterone receptor, or Her2/neu, *e.g.*, a triple negative breast cancer), a colorectal cancer, a pancreatic cancer, a head and neck cancer (*e.g.*, head and neck squamous cell carcinoma (HNSCC), anal cancer, gastro-esophageal cancer, thyroid cancer, cervical cancer, a lymphoproliferative disease (*e.g.*, a post-transplant lymphoproliferative disease) or a hematological cancer, T-cell lymphoma, a non-Hogdkin's lymphoma, or a leukemia (*e.g.*, a myeloid leukemia).

In another embodiment, the cancer is chosen form a carcinoma (*e.g.*, advanced or metastatic carcinoma), melanoma or a lung carcinoma, *e.g.*, a non-small cell lung carcinoma.

In one embodiment, the cancer is a lung cancer, *e.g.*, a non-small cell lung cancer.

In another embodiment, the cancer is a hepatocarcinoma, *e.g.*, an advanced hepatocarcinoma, with or without a viral infection, *e.g.*, a chronic viral hepatitis.

In another embodiment, the cancer is a prostate cancer, *e.g.*, an advanced prostate cancer.

In yet another embodiment, the cancer is a myeloma, *e.g.*, multiple myeloma.

In yet another embodiment, the cancer is a renal cancer, *e.g.*, a renal cell carcinoma (RCC) (*e.g.*, a metastatic RCC or clear cell renal cell carcinoma).

In one embodiment, the cancer is a melanoma, *e.g.*, an advanced melanoma. In one embodiment, the cancer is an advanced or unresectable melanoma that does not respond to other therapies. In other embodiments, the cancer is a melanoma with a BRAF mutation (*e.g.*, a BRAF V600 mutation). In yet other embodiments, the anti-PD-1 antibody molecule is administered after treatment with an anti-CTLA-4 antibody (*e.g.*, ipilimumab) with or without a BRAF inhibitor (*e.g.*, vemurafenib or dabrafenib).

Methods and compositions disclosed herein are useful for treating metastatic lesions associated with the aforementioned cancers.

Combination of Anti-PD-1 antibodies with cancer vaccines

Antibody molecules to PD-1 can be combined with an immunogenic agent, such as cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), cells, and cells transfected with genes encoding immune stimulating cytokines (He *et al.* (2004) *J. Immunol.* 173:4919-28). Non-limiting examples of tumor vaccines that can be used include peptides of melanoma antigens, such as peptides of gp100, MAGE

antigens, Trp-2, MART1 and/or tyrosinase, tumor cells transfected to express the cytokine GM-CSF, DNA-based vaccines, RNA-based vaccines, and viral transduction-based vaccines. The cancer vaccine may be prophylactic or therapeutic.

PD-1 blockade can be combined with a vaccination protocol. Many experimental strategies for vaccination against tumors have been devised (*see* Rosenberg, S., 2000, Development of Cancer Vaccines, ASCO Educational Book Spring: 60-62; Logothetis, C., 2000, ASCO Educational Book Spring: 300-302; Khayat, D. 2000, ASCO Educational Book Spring: 414-428; Foon, K. 2000, ASCO Educational Book Spring: 730-738; *see* also Restifo, N. and Sznol, M., *Cancer Vaccines*, Ch. 61, pp. 3023-3043 in DeVita, V. *et al.* (eds.), 1997, *Cancer*: Principles and Practice of Oncology. Fifth Edition). In one of these strategies, a vaccine is prepared using autologous or allogeneic tumor cells. These cellular vaccines have been shown to be most effective when the tumor cells are transduced to express GM-CSF. GM-CSF has been shown to be a potent activator of antigen presentation for tumor vaccination (Dranoff *et al.* (1993) *Proc. Natl. Acad. Sci.* U.S.A. 90: 3539-43).

PD-1 blockade can be used in conjunction with a collection of recombinant proteins and/or peptides expressed in a tumor in order to generate an immune response to these proteins. These proteins are normally viewed by the immune system as self antigens and are therefore tolerant to them. The tumor antigen may also include the protein telomerase, which is required for the synthesis of telomeres of chromosomes and which is expressed in more than 85% of human cancers and in only a limited number of somatic tissues (Kim, N *et al.* (1994) *Science* 266: 2011-2013). (These somatic tissues may be protected from immune attack by various means). Tumor antigen may also be "neo-antigens" expressed in cancer cells because of somatic mutations that alter protein sequence or create fusion proteins between two unrelated sequences (ie. bcr-abl in the Philadelphia chromosome), or idiotype from B cell tumors.

Other tumor vaccines may include the proteins from viruses implicated in human cancers such a Human Papilloma Viruses (HPV), Hepatitis Viruses (HBV and HCV), Kaposi's Herpes Sarcoma Virus (KHSV), and Epstein–Barr virus (EBV). Another form of tumor specific antigen which may be used in conjunction with PD-1 blockade is purified heat shock proteins (HSP) isolated from the tumor tissue itself. These heat shock proteins contain fragments of proteins from the tumor cells and these HSPs are highly efficient at delivery to antigen presenting cells

for eliciting tumor immunity (Suot, R & Srivastava, P (1995) *Science* 269:1585-1588; Tamura, Y. *et al.* (1997) *Science* 278:117-120).

Dendritic cells (DC) are potent antigen presenting cells that can be used to prime antigen-specific responses. DC's can be produced *ex vivo* and loaded with various protein and peptide antigens as well as tumor cell extracts (Nestle, F. *et al.* (1998) *Nature Medicine* 4: 328-332). DCs may also be transduced by genetic means to express these tumor antigens as well. DCs have also been fused directly to tumor cells for the purposes of immunization (Kugler, A. *et al.* (2000) *Nature Medicine* 6:332-336). As a method of vaccination, DC immunization may be effectively combined with PD-1 blockade to activate more potent anti-tumor responses.

In embodiments, the combination further includes an inhibitor or activator of an immune checkpoint modulator (*e.g.*, a LAG-3 inhibitor (*e.g.*, an anti-LAG-3 antibody molecule), a PD-L1 inhibitor (*e.g.*, an anti-PD-L1 antibody molecule), a TIM-3 modulator (*e.g.*, a TIM-3 activator or inhibitor, *e.g.*, an anti-TIM-3 antibody molecule), or a CTLA-4 inhibitor (*e.g.*, an anti-CTLA-4 antibody), or any combination thereof.

PD-1 blockade may also be combined with a standard cancer treatment. PD-1 blockade may be effectively combined with chemotherapeutic regimes. In these instances, it may be possible to reduce the dose of chemotherapeutic reagent administered (Mokyr, M. *et al.* (1998) *Cancer Research* 58: 5301-5304). In certain embodiments, the methods and compositions described herein are administered in combination with one or more of other antibody molecules, chemotherapy, other anti-cancer therapy (*e.g.*, targeted anti-cancer therapies, or oncolytic drugs), cytotoxic agents, immune-based therapies (*e.g.*, cytokines), surgical and/or radiation procedures. Exemplary cytotoxic agents that can be administered in combination with include antimicrotubule agents, topoisomerase inhibitors, anti-metabolites, mitotic inhibitors, alkylating agents, anthracyclines, vinca alkaloids, intercalating agents, agents capable of interfering with a signal transduction pathway, agents that promote apoptosis, proteosome inhibitors, and radiation (*e.g.*, local or whole body irradiation).

Alternatively, or in combination with the aforesaid combinations, the methods and compositions described herein can be administered in combination with one or more of: an immunomodulator (*e.g.*, an activator of a costimulatory molecule or an inhibitor of an inhibitory molecule); a vaccine, *e.g.*, a therapeutic cancer vaccine; or other forms of cellular immunotherapy.

Exemplary non-limiting combinations and uses of the anti-PD-1 antibody molecules include the following.

In certain embodiments, the anti-PD-1 antibody molecule is administered in combination with a modulator of a costimulatory molecule or an inhibitory molecule, *e.g.*, a co-inhibitory ligand or receptor.

In one embodiment, the anti-PD-1 antibody molecule is administered in combination with a modulator, *e.g.*, agonist, of a costimulatory molecule. In one embodiment, the agonist of the costimulatory molecule is chosen from an agonist (*e.g.*, an agonistic antibody or antigen-binding fragment thereof, or soluble fusion) of OX40, CD2, CD27, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD30, CD40, BAFFR, HVEM, CD7, LIGHT, NKG2C, SLAMF7, NKp80, CD160, B7-H3 or CD83 ligand.

In another embodiment, the anti-PD-1 antibody molecule is used in combination with a costimulatory molecule, *e.g.*, an agonist associated with a positive signal that includes a costimulatory domain of CD28, CD27, ICOS and GITR.

Exemplary GITR agonists include, *e.g.*, GITR fusion proteins and anti-GITR antibodies (*e.g.*, bivalent anti-GITR antibodies), such as, a GITR fusion protein described in U.S. Patent No.: 6,111,090, European Patent No.: 090505B1, U.S Patent No.: 8,586,023, PCT Publication Nos.: WO 2010/003118 and 2011/090754, or an anti-GITR antibody described, *e.g.,* in U.S. Patent No.: 7,025,962, European Patent No.: 1947183B1, U.S. Patent No.: 7,812,135, U.S. Patent No.: 8,388,967, U.S. Patent No.: 8,591,886, European Patent No.: EP 1866339, PCT Publication No.: WO 2011/028683, PCT Publication No.:WO 2013/039954, PCT Publication No.: WO2005/007190, PCT Publication No.: WO 2007/133822, PCT Publication No.: WO2005/055808, PCT Publication No.: WO 99/40196, PCT Publication No.: WO 2001/03720, PCT Publication No.: WO99/20758, PCT Publication No.: WO2006/083289, PCT Publication No.: WO 2005/115451, U.S. Patent No.: 7,618,632, and PCT Publication No.: WO 2011/051726.

In one embodiment, the anti-PD-1 antibody molecule is administered in combination with an inhibitor of an inhibitory molecule of an immune checkpoint molecule. It will be understood by those of ordinary skill in the art, that the term "immune checkpoints" means a group of molecules on the cell surface of CD4 and CD8 T cells. These molecules can effectively serve as "brakes" to down-modulate or inhibit an anti-tumor immune response. Immune checkpoint molecules include, but are not limited to, Programmed Death 1 (PD-1), Cytotoxic T-Lymphocyte

Antigen 4 (CTLA-4), B7H1, B7H4, OX-40, CD137, CD40, LAG-3 and TIM-3, which directly inhibit immune cells. Immunotherapeutic agents which can act as immune checkpoint inhibitors useful in the methods of the present invention, include, but are not limited to, inhibitors of PD-L1, PD-L2, CTLA4, TIM-3, LAG-3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CEACAM (*e.g.*, CEACAM-1 and/or CEACAM-5), and/or TGFR beta. Inhibition of an inhibitory molecule can be performed by inhibition at the DNA, RNA or protein level. In embodiments, an inhibitory nucleic acid (*e.g.*, a dsRNA, siRNA or shRNA), can be used to inhibit expression of an inhibitory molecule. In other embodiments, the inhibitor of an inhibitory signal is, a polypeptide *e.g.*, a soluble ligand, or an antibody or antigen-binding fragment thereof, that binds to the inhibitory molecule.

In one embodiment, the inhibitor is a soluble ligand (*e.g.*, a CTLA-4-Ig or a TIM-3-Ig), or an antibody or antibody fragment that binds to PD-L1, PD-L2 or CTLA4. For example, the anti-PD-1 antibody molecule can be administered in combination with an anti-CTLA-4 antibody, *e.g.*, ipilimumab, for example, to treat a cancer (*e.g.*, a cancer chosen from: a melanoma, *e.g.*, a metastatic melanoma; a lung cancer, *e.g.*, a non-small cell lung carcinoma; or a prostate cancer). Exemplary anti-CTLA4 antibodies include Tremelimumab (IgG2 monoclonal antibody available from Pfizer, formerly known as ticilimumab, CP-675,206); and Ipilimumab (CTLA-4 antibody, also known as MDX-010, CAS No. 477202-00-9). In one embodiment, the anti-PD-1 antibody molecule is administered after treatment, *e.g.*, after treatment of a melanoma, with an anti-CTLA4 antibody (*e.g.*, ipilimumab) with or without a BRAF inhibitor (*e.g.*, vemurafenib or dabrafenib). Exemplary doses that can be use include a dose of anti-PD-1 antibody molecule of about 1 to 10 mg/kg, *e.g.*, 3 mg/kg, and a dose of an anti-CTLA-4 antibody, *e.g.*, ipilimumab, of about 3 mg/kg.

Immune inhibitory molecules, *e.g.*, PD-1 and LAG-3, can regulate, *e.g.*, synergistically regulate, T-cell function to promote tumoral immune escape. In another embodiment, the anti-PD-1 antibody molecule is administered in combination with an anti-LAG-3 antibody or an antigen-binding fragment thereof. In another embodiment, the anti-PD-1 antibody molecule is administered in combination with an anti-TIM-3 antibody or antigen-binding fragment thereof. In yet other embodiments, the anti-PD-1 antibody molecule is administered in combination with an anti-LAG-3 antibody and an anti-TIM-3 antibody, or antigen-binding fragments thereof. The combination of antibodies recited herein can be administered separately, *e.g.*, as separate

antibodies, or linked, *e.g.*, as a bispecific or trispecific antibody molecule. In another embodiment, the anti-PD-1 antibody molecule is administered in combination with a CEACAM inhibitor (*e.g.*, CEACAM-1, CEACAM-3, and/or CEACAM-5 inhibitor), *e.g.*, an anti-CEACAM antibody molecule. In another embodiment, the anti-PD-1 antibody molecule is administered in combination with a CEACAM-1 inhibitor, *e.g.*, an anti- CEACAM-1 antibody molecule. In another embodiment, the anti-PD-1 antibody molecule is administered in combination with a CEACAM-5 inhibitor, *e.g.*, an anti- CEACAM-5 antibody molecule. In one embodiment, a bispecific antibody that includes an anti-PD-1 antibody molecule and an anti-TIM-3 or anti-LAG-3 antibody, or antigen-binding fragment thereof, is administered. In certain embodiments, the combination of antibodies recited herein is used to treat a cancer, *e.g.*, a cancer as described herein (*e.g.*, a solid tumor). The efficacy of the aforesaid combinations can be tested in animal models known in the art. For example, the animal models to test the synergistic effect of anti-PD-1 and anti-LAG-3 are described, *e.g.,* in Woo *et al.* (2012) *Cancer Res.* 72(4):917-27).

In one embodiment, the inhibitor of CEACAM (*e.g.*, CEACAM-1 and/or CEACAM-5) is an anti-CEACAM antibody molecule. Without wishing to be bound by theory, CEACAM-1 has been described as a ligand and partner of TIM-3 (see *e.g.*, WO 2014/022332). Synergistic in vivo effect of the combination of anti-TIM-3 and anti-CEACAM-1 antibodies have been detected in xenograft cancer models (*see e.g.*, WO 2014/022332). Tumors are believed to use CEACAM-1 or CEACAM-5 to inhibit the immune system, as described in, *e.g.*, Markel *et al. J Immunol.* 2002 Mar 15;168(6):2803-10; Markel et al. *J Immunol.* 2006 Nov 1;177(9):6062-71; Markel et al. *Immunology.* 2009 Feb;126(2):186-200; Markel et al. *Cancer Immunol Immunother.* 2010 Feb;59(2):215-30; Ortenberg et al. *Mol Cancer Ther.* 2012 Jun;11(6):1300-10; Stern et al. *J Immunol.* 2005 Jun 1;174(11):6692-701; Zheng et al. *PLoS One.* 2010 Sep 2;5(9). pii: e12529. Thus, CEACAM inhibitors can be used with the other immunomodulators described herein (*e.g.*, anti-PD-1 or anti-TIM-3 inhibitors) to enhance an immune response against a cancer, *e.g.*, melanoma, lung cancer (*e.g.*, NSCLC), bladder, colon or ovarian cancer, or other cancers as described herein. In one embodiment, the inhibitor of CEACAM is an anti-CEACAM-1 antibody as described in WO 2010/125571, WO 2013/82366 and WO 2014/022332, *e.g.,* a monoclonal antibody 34B1, 26H7, and 5F4 or a recombinant form thereof, as described in, *e.g.*, US 2004/0047858, US 7,132,255 and WO 99/52552. In other embodiments, the anti-CEACAM antibody is an anti-CEACAM-1 and/or anti-CEACAM-5

antibody molecule as described in, *e.g.*, WO 2010/125571, WO 2013/054331 and US 2014/0271618.

In some embodiments, the PD-1 and LAG-3 immune inhibitory molecules (*e.g.*, antibody molecules) are administered in combination with each other, *e.g.*, to treat cancer. In some embodiments, the patient is a patient who progressed (*e.g.*, experienced tumor growth) during therapy with a PD-1 inhibitor (*e.g.*, an antibody molecule as described herein) and/or a PD-L1 inhibitor (*e.g.*, antibody molecule). In some embodiments, therapy with the PD-1 antibody molecule and/or PD-L1 antibody molecule is continued, and a LAG-3 immune inhibitory molecule (*e.g.*, antibody) is added to the therapy.

In some embodiments, the PD-1 and TIM-3 immune inhibitory molecules (*e.g.*, antibody molecules) are administered in combination with each other, *e.g.*, to treat cancer. In some embodiments, the patient is a patient who progressed (*e.g.*, experienced tumor growth) during therapy with a PD-1 inhibitor (*e.g.*, an antibody molecule as described herein) and/or a PD-L1 inhibitor (*e.g.*, antibody molecule). In some embodiments, therapy with the PD-1 antibody molecule and/or PD-L1 antibody molecule is continued, and a TIM-3 immune inhibitory molecule (*e.g.*, antibody) is added to the therapy.

In other embodiments, the anti-PD-1 antibody molecule is administered in combination with a cytokine, *e.g.*, interleukin-21, interleukin-2, interleukin-12, or interleukin-15. In certain embodiments, the combination of anti-PD-1 antibody molecule and cytokine described herein is used to treat a cancer, *e.g.*, a cancer as described herein (*e.g.*, a solid tumor or melanoma).

Exemplary immunomodulators that can be used in combination with anti-PD-1 antibody molecules include, but are not limited to, *e.g.*, afutuzumab (available from Roche®); pegfilgrastim (Neulasta®); lenalidomide (CC-5013, Revlimid®); thalidomide (Thalomid®), actimid (CC4047); and cytokines, *e.g.*, IL-21 or IRX-2 (mixture of human cytokines including interleukin 1, interleukin 2, and interferon γ, CAS 951209-71-5, available from IRX Therapeutics).

In yet other embodiments, the anti-PD-1 antibody molecule is used in combination with an indoleamine-pyrrole 2,3-dioxygenase (IDO) inhibitor (*e.g.*, INCB24360) in a subject with advanced or metastatic cancer (*e.g.*, a patient with metastic and recurrent NSCL cancer).

In other embodiments, the anti-PD-1 antibody molecules are administered to a subject in conjunction with (*e.g.*, before, simultaneously or following) one or more of: bone marrow

transplantation, T cell ablative therapy using chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, and/or antibodies such as OKT3 or CAMPATH. In one embodiment, the anti-PD-1 antibody molecules are administered following B-cell ablative therapy such as agents that react with CD20, *e.g.*, Rituxan. For example, in one embodiment, subjects may undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain embodiments, following the transplant, subjects receive the anti-PD-1 antibody molecules. In an additional embodiment, the anti-PD-1 antibody molecules are administered before or following surgery.

Another example of a combination is an anti-PD-1 antibody in combination with decarbazine for the treatment of melanoma. Without being bound by theory, the combined use of PD-1 blockade and chemotherapy is believed to be facilitated by cell death, that is a consequence of the cytotoxic action of most chemotherapeutic compounds, which can result in increased levels of tumor antigen in the antigen presentation pathway. Other combination therapies that may result in synergy with PD-1 blockade through cell death are radiation, surgery, and hormone deprivation. Each of these protocols creates a source of tumor antigen in the host. Angiogenesis inhibitors may also be combined with PD-1 blockade. Inhibition of angiogenesis leads to tumor cell death which may feed tumor antigen into host antigen presentation pathways.

PD-1 blocking antibodies can also be used in combination with bispecific antibodies. Bispecific antibodies can be used to target two separate antigens. For example anti-Fc receptor/anti tumor antigen (*e.g.*, Her-2/neu) bispecific antibodies have been used to target macrophages to sites of tumor. This targeting may more effectively activate tumor specific responses. The T cell arm of these responses would by augmented by the use of PD-1 blockade. Alternatively, antigen may be delivered directly to DCs by the use of bispecific antibodies which bind to tumor antigen and a dendritic cell specific cell surface marker.

Tumors evade host immune surveillance by a large variety of mechanisms. Many of these mechanisms may be overcome by the inactivation of proteins which are expressed by the tumors and which are immunosuppressive. These include among others TGF-beta (Kehrl, J. *et al.* (1986) *J. Exp. Med.* 163: 1037-1050), IL-10 (Howard, M. & O'Garra, A. (1992) *Immunology Today* 13: 198-200), and Fas ligand (Hahne, M. *et al.* (1996) *Science* 274: 1363-1365). Antibodies or antigen-binding fragments thereof to each of these entities may be used in

combination with anti-PD-1 to counteract the effects of the immunosuppressive agent and favor tumor immune responses by the host.

Other antibodies which may be used to activate host immune responsiveness can be used in combination with anti-PD-1. These include molecules on the surface of dendritic cells which activate DC function and antigen presentation. Anti-CD40 antibodies are able to substitute effectively for T cell helper activity (Ridge, J. *et al.* (1998) *Nature* 393: 474-478) and can be used in conjunction with PD-1 antibodies (Ito, N. *et al.* (2000) *Immunobiology* 201 (5) 527-40). Antibodies to T cell costimulatory molecules such as CTLA-4 (*e.g.*, U.S. Pat. No. 5,811,097), OX-40 (Weinberg, A. *et al.* (2000) *Immunol* 164: 2160-2169), 4-1BB (Melero, I. *et al.* (1997) *Nature Medicine* 3: 682-685 (1997), and ICOS (Hutloff, A. *et al.* (1999) *Nature* 397: 262-266) may also provide for increased levels of T cell activation.

Additional exemplary standard of care treatments are described in the section entitled "Combination Therapies" below.

In all of the methods described herein, PD-1 blockade can be combined with other forms of immunotherapy such as cytokine treatment (*e.g.*, interferons, GM-CSF, G-CSF, IL-2, IL-21), or bispecific antibody therapy, which provides for enhanced presentation of tumor antigens (*see e.g.*, Holliger (1993) *Proc. Natl. Acad. Sci.* USA 90:6444-6448; Poljak (1994) *Structure* 2:1121-1123).

Methods of administering the antibody molecules are known in the art and are described below. Suitable dosages of the molecules used will depend on the age and weight of the subject and the particular drug used. Dosages and therapeutic regimens of the anti-PD-1 antibody molecule can be determined by a skilled artisan. In certain embodiments, the anti-PD-1 antibody molecule is administered by injection (*e.g.*, subcutaneously or intravenously) at a dose of about 1 to 30 mg/kg, *e.g.*, about 5 to 25 mg/kg, about 10 to 20 mg/kg, about 1 to 5 mg/kg, or about 3 mg/kg. In some embodiments, the anti-PD-1 antibody molecule is administered at a dose of about 1 mg/kg, about 3 mg/kg, or 10 mg/kg, about 20 mg/kg, about 30 mg/kg, or about 40 mg/kg. In some embodiments, the anti-PD-1 antibody molecule is administered at a dose of about 1-3 mg/kg, or about 3-10 mg/kg. In some embodiments, the anti-PD-1 antibody molecule is administered at a dose of about 0.5-2, 2-4, 2-5, 5-15, or 5-20 mg/kg. The dosing schedule can vary from *e.g.*, once a week to once every 2, 3, or 4 weeks. In one embodiment, the anti-PD-1 antibody molecule is administered at a dose from about 10 to 20 mg/kg every other week.

The antibody molecules can be used in unconjugated forms or conjugated to a second agent, *e.g.*, a cytotoxic drug, radioisotope, or a protein, *e.g.*, a protein toxin or a viral protein. This method includes: administering the antibody molecule, alone or conjugated to a cytotoxic drug, to a subject requiring such treatment. The antibody molecules can be used to deliver a variety of therapeutic agents, *e.g.*, a cytotoxic moiety, *e.g.*, a therapeutic drug, a radioisotope, molecules of plant, fungal, or bacterial origin, or biological proteins (*e.g.*, protein toxins) or particles (*e.g.*, a recombinant viral particles, *e.g.*; via a viral coat protein), or mixtures thereof.

Additional Combination Therapies

The anti-PD-1 antibody molecule can be used in combination with other therapies. For example, the combination therapy can include a composition of the present invention co-formulated with, and/or co-administered with, one or more additional therapeutic agents, *e.g.*, one or more anti-cancer agents, cytotoxic or cytostatic agents, hormone treatment, vaccines, and/or other immunotherapies. In other embodiments, the antibody molecules are administered in combination with other therapeutic treatment modalities, including surgery, radiation, cryosurgery, and/or thermotherapy. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

By "in combination with," it is not intended to imply that the therapy or the therapeutic agents must be administered at the same time and/or formulated for delivery together, although these methods of delivery are within the scope described herein. The anti-PD-1 antibody molecules can be administered concurrently with, prior to, or subsequent to, one or more other additional therapies or therapeutic agents. The anti-PD-1 antibody molecule and the other agent or therapeutic protocol can be administered in any order. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent. In will further be appreciated that the additional therapeutic agent utilized in this combination may be administered together in a single composition or administered separately in different compositions. In general, it is expected that additional therapeutic agents utilized in combination be utilized at levels that do not exceed the levels at which they are utilized individually. In some embodiments, the levels utilized in combination will be lower than those utilized individually.

In certain embodiments, the anti-PD-1 molecules described herein are administered in combination with one or more other inhibitors of PD-1, PD-L1 and/or PD-L2 known in the art. The antagonist may be an antibody, an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. In some embodiments, the other anti-PD-1 antibody is chosen from MDX-1106, Merck 3475 or CT-011. In some embodiments, the PD-1 inhibitor is an immunoadhesin (*e.g.*, an immunoadhesin comprising an extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region (*e.g.*, an Fc region of an immunoglobulin sequence). In some embodiments, the PD-1 inhibitor is AMP-224. In some embodiments, the PD-L1 inhibitor is anti-PD-L1 antibody. In some embodiments, the anti-PD-L1 binding antagonist is chosen from YW243.55.S70, MPDL3280A, MEDI-4736, MSB-0010718C, or MDX-1105. MDX-1105, also known as BMS-936559, is an anti-PD-L1 antibody described in WO2007/005874. Antibody YW243.55.S70 (heavy and light chain variable region sequences shown in SEQ ID Nos. 20 and 21, respectively) is an anti-PD-L1 described in WO 2010/077634.

MDX-1106, also known as MDX-1106-04, ONO-4538 or BMS-936558, is an anti-PD-1 antibody described in WO2006/121168. Merck 3745, also known as MK-3475 or SCH-900475, is an anti-PD-1 antibody described in WO2009/114335. Pidilizumab (CT-011; Cure Tech) is a humanized IgG1k monoclonal antibody that binds to PD-1. Pidilizumab and other humanized anti-PD-1 monoclonal antibodies are disclosed in WO2009/101611. In other embodiments, the anti-PD-1 antibody is pembrolizumab. Pembrolizumab (Trade name Keytruda formerly lambrolizumab also known as MK-3475) disclosed, *e.g.*, in Hamid, O. *et al.* (2013) *New England Journal of Medicine* 369 (2): 134–44. AMP-224 (B7-DCIg; Amplimmune; *e.g.*, disclosed in WO2010/027827 and WO2011/066342), is a PD-L2 Fc fusion soluble receptor that blocks the interaction between PD-1 and B7-H1. Other anti-PD-1 antibodies include AMP 514 (Amplimmune), among others, *e.g.*, anti-PD-1 antibodies disclosed in US 8,609,089, US 2010028330, and/or US 20120114649.

In some embodiments, the other anti-PD-1 antibody is MDX-1106. Alternative names for MDX- 1106 include MDX-1106-04, ONO-4538, BMS-936558 or Nivolumab. In some embodiments, the anti-PD-1 antibody is Nivolumab (CAS Registry Number: 946414-94-4). Nivolumab (also referred to as BMS-936558 or MDX1106; Bristol-Myers Squibb) is a fully human IgG4 monoclonal antibody which specifically blocks PD-1. Nivolumab (clone 5C4) and other human monoclonal antibodies that specifically bind to PD-1 are disclosed in US 8,008,449

and WO2006/121168. Lambrolizumab (also referred to as pembrolizumab or MK03475; Merck) is a humanized IgG4 monoclonal antibody that binds to PD-1. Pembrolizumab and other humanized anti-PD-1 antibodies are disclosed in US 8,354,509 and WO2009/114335. MDPL3280A (Genentech / Roche) is a human Fc optimized IgG1 monoclonal antibody that binds to PD-L1. MDPL3280A and other human monoclonal antibodies to PD-L1 are disclosed in U.S. Patent No.: 7,943,743 and U.S Publication No.: 20120039906. Other anti-PD-L1 binding agents include YW243.55.S70 (heavy and light chain variable regions are shown in SEQ ID NOs 20 and 21 in WO2010/077634) and MDX-1105 (also referred to as BMS-936559, and, *e.g.*, anti-PD-L1 binding agents disclosed in WO2007/005874).

Cancer Therapies

Exemplary combinations of anti-PD-1 antibody molecules (alone or in combination with other stimulatory agents) and standard of care for cancer, include at least the following. In certain embodiments, the anti-PD-1 antibody molecule, *e.g.*, the anti-PD-1 antibody molecule described herein, is used in combination with a standard of cancer care chemotherapeutic agent including, but not limited to, anastrozole (Arimidex®), bicalutamide (Casodex®), bleomycin sulfate (Blenoxane®), busulfan (Myleran®), busulfan injection (Busulfex®), capecitabine (Xeloda®), N4-pentoxycarbonyl-5-deoxy-5-fluorocytidine, carboplatin (Paraplatin®), carmustine (BiCNU®), chlorambucil (Leukeran®), cisplatin (Platinol®), cladribine (Leustatin®), cyclophosphamide (Cytoxan® or Neosar®), cytarabine, cytosine arabinoside (Cytosar-U®), cytarabine liposome injection (DepoCyt®), dacarbazine (DTIC-Dome®), dactinomycin (Actinomycin D, Cosmegan), daunorubicin hydrochloride (Cerubidine®), daunorubicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®), fludarabine phosphate (Fludara®), 5-fluorouracil (Adrucil®, Efudex®), flutamide (Eulexin®), tezacitibine, Gemcitabine (difluorodeoxycitidine), hydroxyurea (Hydrea®), Idarubicin (Idamycin®), ifosfamide (IFEX®), irinotecan (Camptosar®), L-asparaginase (ELSPAR®), leucovorin calcium, melphalan (Alkeran®), 6-mercaptopurine (Purinethol®), methotrexate (Folex®), mitoxantrone (Novantrone®), mylotarg, paclitaxel (Taxol®), phoenix (Yttrium90/MX-DTPA),

pentostatin, polifeprosan 20 with carmustine implant (Gliadel®), tamoxifen citrate (Nolvadex®), teniposide (Vumon®), 6-thioguanine, thiotepa, tirapazamine (Tirazone®), topotecan hydrochloride for injection (Hycamptin®), vinblastine (Velban®), vincristine (Oncovin®), vinorelbine (Navelbine®), Ibrutinib, idelalisib, and brentuximab vedotin.

Exemplary alkylating agents include, without limitation, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes): uracil mustard (Aminouracil Mustard®, Chlorethaminacil®, Demethyldopan®, Desmethyldopan®, Haemanthamine®, Nordopan®, Uracil nitrogen mustard®, Uracillost®, Uracilmostaza®, Uramustin®, Uramustine®), chlormethine (Mustargen®), cyclophosphamide (Cytoxan®, Neosar®, Clafen®, Endoxan®, Procytox®, Revimmune™), ifosfamide (Mitoxana®), melphalan (Alkeran®), Chlorambucil (Leukeran®), pipobroman (Amedel®, Vercyte®), triethylenemelamine (Hemel®, Hexalen®, Hexastat®), triethylenethiophosphoramine, Temozolomide (Temodar®), thiotepa (Thioplex®), busulfan (Busilvex®, Myleran®), carmustine (BiCNU®), lomustine (CeeNU®), streptozocin (Zanosar®), and Dacarbazine (DTIC-Dome®). Additional exemplary alkylating agents include, without limitation, Oxaliplatin (Eloxatin®); Temozolomide (Temodar® and Temodal®); Dactinomycin (also known as actinomycin-D, Cosmegen®); Melphalan (also known as L-PAM, L-sarcolysin, and phenylalanine mustard, Alkeran®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Carmustine (BiCNU®); Bendamustine (Treanda®); Busulfan (Busulfex® and Myleran®); Carboplatin (Paraplatin®); Lomustine (also known as CCNU, CeeNU®); Cisplatin (also known as CDDP, Platinol® and Platinol®-AQ); Chlorambucil (Leukeran®); Cyclophosphamide (Cytoxan® and Neosar®); Dacarbazine (also known as DTIC, DIC and imidazole carboxamide, DTIC-Dome®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Ifosfamide (Ifex®); Prednumustine; Procarbazine (Matulane®); Mechlorethamine (also known as nitrogen mustard, mustine and mechloroethamine hydrochloride, Mustargen®); Streptozocin (Zanosar®); Thiotepa (also known as thiophosphoamide, TESPA and TSPA, Thioplex®); Cyclophosphamide (Endoxan®, Cytoxan®, Neosar®, Procytox®, Revimmune®); and Bendamustine HCl (Treanda®).

Exemplary anthracyclines include, *e.g.*, doxorubicin (Adriamycin® and Rubex®); bleomycin (lenoxane®); daunorubicin (dauorubicin hydrochloride, daunomycin, and rubidomycin hydrochloride, Cerubidine®); daunorubicin liposomal (daunorubicin citrate liposome, DaunoXome®); mitoxantrone (DHAD, Novantrone®); epirubicin (Ellence™);

idarubicin (Idamycin®, Idamycin PFS®); mitomycin C (Mutamycin®); geldanamycin; herbimycin; ravidomycin; and desacetylravidomycin.

Exemplary vinca alkaloids that can be used in combination with the anti-PD-1 antibody molecules, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), include, but ate not limited to, vinorelbine tartrate (Navelbine®), Vincristine (Oncovin®), and Vindesine (Eldisine®)); vinblastine (also known as vinblastine sulfate, vincaleukoblastine and VLB, Alkaban-AQ® and Velban®); and vinorelbine (Navelbine®).

Exemplary proteosome inhibitors that can be used in combination with the anti-PD-1 antibody molecules, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), include, but ate not limited to, bortezomib (Velcade®); carfilzomib (PX-171-007, (*S*)-4-Methyl-*N*-((*S*)-1-(((*S*)-4-methyl-1-((*R*)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-2-((*S*)-2-(2-morpholinoacetamido)-4-phenylbutanamido)-pentanamide); marizomib (NPI-0052); ixazomib citrate (MLN-9708); delanzomib (CEP-18770); and *O*-Methyl-*N*-[(2-methyl-5-thiazolyl)carbonyl]-L-seryl-*O*-methyl-*N*-[(1*S*)-2-[(2*R*)-2-methyl-2-oxiranyl]-2-oxo-1-(phenylmethyl)ethyl]- L-serinamide (ONX-0912).

In some embodiments, the anti-PD-1 antibody molecule, *e.g.*, the anti-PD-1 antibody molecule described herein, is used, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), in combination with a tyrosine kinase inhibitor (*e.g.*, a receptor tyrosine kinase (RTK) inhibitor). Exemplary tyrosine kinase inhibitor include, but are not limited to, an epidermal growth factor (EGF) pathway inhibitor (*e.g.*, an epidermal growth factor receptor (EGFR) inhibitor), a vascular endothelial growth factor (VEGF) pathway inhibitor (*e.g.*, a vascular endothelial growth factor receptor (VEGFR) inhibitor (*e.g.*, a VEGFR-1 inhibitor, a VEGFR-2 inhibitor, a VEGFR-3 inhibitor)), a platelet derived growth factor (PDGF) pathway inhibitor (*e.g.*, a platelet derived growth factor receptor (PDGFR) inhibitor (*e.g.*, a PDGFR-ß inhibitor)), a RAF-1 inhibitor, a KIT inhibitor and a RET inhibitor. In some embodiments, the anti-cancer agent used in combination with the hedgehog inhibitor is selected from the group consisting of: axitinib (AG013736), bosutinib (SKI-606), cediranib (RECENTIN™, AZD2171), dasatinib (SPRYCEL®, BMS-354825), erlotinib (TARCEVA®), gefitinib (IRESSA®), imatinib (Gleevec®, CGP57148B, STI-571),

lapatinib (TYKERB®, TYVERB®), lestaurtinib (CEP-701), neratinib (HKI-272), nilotinib (TASIGNA®), semaxanib (semaxinib, SU5416), sunitinib (SUTENT®, SU11248), toceranib (PALLADIA®), vandetanib (ZACTIMA®, ZD6474), vatalanib (PTK787, PTK/ZK), trastuzumab (HERCEPTIN®), bevacizumab (AVASTIN®), rituximab (RITUXAN®), cetuximab (ERBITUX®), panitumumab (VECTIBIX®), ranibizumab (Lucentis®), nilotinib (TASIGNA®), sorafenib (NEXAVAR®), alemtuzumab (CAMPATH®), gemtuzumab ozogamicin (MYLOTARG®), ENMD-2076, PCI-32765, AC220, dovitinib lactate (TKI258, CHIR-258), BIBW 2992 (TOVOK™), SGX523, PF-04217903, PF-02341066, PF-299804, BMS-777607, ABT-869, MP470, BIBF 1120 (VARGATEF®), AP24534, JNJ-26483327, MGCD265, DCC-2036, BMS-690154, CEP-11981, tivozanib (AV-951), OSI-930, MM-121, XL-184, XL-647, XL228, AEE788, AG-490, AST-6, BMS-599626, CUDC-101, PD153035, pelitinib (EKB-569), vandetanib (zactima), WZ3146, WZ4002, WZ8040, ABT-869 (linifanib), AEE788, AP24534 (ponatinib), AV-951(tivozanib), axitinib, BAY 73-4506 (regorafenib), brivanib alaninate (BMS-582664), brivanib (BMS-540215), cediranib (AZD2171), CHIR-258 (dovitinib), CP 673451, CYC116, E7080, Ki8751, masitinib (AB1010), MGCD-265, motesanib diphosphate (AMG-706), MP-470, OSI-930, Pazopanib Hydrochloride, PD173074, Sorafenib Tosylate(Bay 43-9006), SU 5402, TSU-68(SU6668), vatalanib, XL880 (GSK1363089, EXEL-2880). Selected tyrosine kinase inhibitors are chosen from sunitinib, erlotinib, gefitinib, or sorafenib.

In certain embodiments, the anti-PD-1 antibody molecule, *e.g.*, the anti-PD-1 antibody molecule described herein, is used, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), in combination with a Vascular Endothelial Growth Factor (VEGF) receptor inhibitors, including but not limited to, Bevacizumab (Avastin®), axitinib (Inlyta®); Brivanib alaninate (BMS-582664, (*S*)-((*R*)-1-(4-(4-Fluoro-2-methyl-1*H*-indol-5-yloxy)-5-methylpyrrolo[2,1-*f*][1,2,4]triazin-6-yloxy)propan-2-yl)2-aminopropanoate); Sorafenib (Nexavar®); Pazopanib (Votrient®); Sunitinib malate (Sutent®); Cediranib (AZD2171, CAS 288383-20-1); Vargatef (BIBF1120, CAS 928326-83-4); Foretinib (GSK1363089); Telatinib (BAY57-9352, CAS 332012-40-5); Apatinib (YN968D1, CAS 811803-05-1); Imatinib (Gleevec®); Ponatinib (AP24534, CAS 943319-70-8); Tivozanib (AV951, CAS 475108-18-0); Regorafenib (BAY73-4506, CAS 755037-03-7); Vatalanib dihydrochloride (PTK787, CAS 212141-51-0); Brivanib (BMS-540215, CAS 649735-

46-6); Vandetanib (Caprelsa® or AZD6474); Motesanib diphosphate (AMG706, CAS 857876-30-3, N-(2,3-dihydro-3,3-dimethyl-1H-indol-6-yl)-2-[(4-pyridinylmethyl)amino]-3-pyridinecarboxamide, described in PCT Publication No. WO 02/066470); Dovitinib dilactic acid (TKI258, CAS 852433-84-2); Linfanib (ABT869, CAS 796967-16-3); Cabozantinib (XL184, CAS 849217-68-1); Lestaurtinib (CAS 111358-88-4); N-[5-[[[5-(1,1-Dimethylethyl)-2-oxazolyl]methyl]thio]-2-thiazolyl]-4-piperidinecarboxamide (BMS38703, CAS 345627-80-7); (3R,4R)-4-Amino-1-((4-((3-methoxyphenyl)amino)pyrrolo[2,1-f][1,2,4]triazin-5-yl)methyl)piperidin-3-ol (BMS690514); *N*-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[[(3aα,5β,6aα)-octahydro-2-methylcyclopenta[*c*]pyrrol-5-yl]methoxy]- 4-quinazolinamine (XL647, CAS 781613-23-8); 4-Methyl-3-[[1-methyl-6-(3-pyridinyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl]amino]-*N*-[3-(trifluoromethyl)phenyl]-benzamide (BHG712, CAS 940310-85-0); and Aflibercept (Eylea®).

Exemplary anti-VEGF antibodies include, but are not limited to, a monoclonal antibody that binds to the same epitope as the monoclonal anti-VEGF antibody A4.6.1 produced by hybridoma ATCC HB 10709; a recombinant humanized anti-VEGF monoclonal antibody generated according to Presta *et al.* (1997) *Cancer Res.* 57:4593-4599. In one embodiment, the anti-VEGF antibody is Bevacizumab (BV), also known as rhuMAb VEGF or AVASTIN®. It comprises mutated human IgGl framework regions and antigen-binding complementarity-determining regions from the murine anti-hVEGF monoclonal antibody A.4.6.1 that blocks binding of human VEGF to its receptors. Bevacizumab and other humanized anti-VEGF antibodies are further described in U.S. Pat. No. 6,884,879 issued Feb. 26, 2005. Additional antibodies include the G6 or B20 series antibodies (*e.g.*, G6-31, B20-4.1), as described in PCT Publication No. WO2005/012359, PCT Publication No. WO2005/044853, the contents of these patent applications are expressly incorporated herein by reference. For additional antibodies see U.S. Pat. Nos. 7,060,269, 6,582,959, 6,703,020, 6,054,297, W098/45332, WO 96/30046, WO94/10202, EP 0666868B1, U.S. Patent Application Publication Nos. 2006009360, 20050186208, 20030206899, 20030190317, 20030203409, and 20050112126; and Popkov *et al, Journal of Immunological Methods* 288: 149-164 (2004). Other antibodies include those that bind to a functional epitope on human VEGF comprising of residues F17, Ml 8, D19, Y21, Y25, Q89, 191 , Kl 01, El 03, and C104 or, alternatively, comprising residues F17, Y21, Q22, Y25, D63, 183 and Q89.

In some embodiments, the anti-PD-1 antibody molecule, *e.g.*, the anti-PD-1 antibody molecule described herein, is used, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), in combination with a PI3K inhibitor. In one embodiment, the PI3K inhibitor is an inhibitor of delta and gamma isoforms of PI3K. Exemplary PI3K inhibitors that can be used in combination are described in, *e.g.*, WO 2010/036380, WO 2010/006086, WO 09/114870, WO 05/113556, GSK 2126458, GDC-0980, GDC-0941, Sanofi XL147, XL756, XL147, PF-46915032, BKM 120, CAL-101, CAL 263, SF1126, PX-886, and a dual PI3K inhibitor (*e.g.*, Novartis BEZ235).

In some embodiments, the anti-PD-1 antibody molecules described herein is used, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), in combination with a mTOR inhibitor, *e.g.*, one or more mTOR inhibitors chosen from one or more of rapamycin, temsirolimus (TORISEL®), AZD8055, BEZ235, BGT226, XL765, PF-4691502, GDC0980, SF1126, OSI-027, GSK1059615, KU-0063794, WYE-354, Palomid 529 (P529), PF-04691502, or PKI-587. ridaforolimus (formally known as deferolimus, (1*R*,2*R*,4*S*)-4-[(2*R*)-2 [(1*R*,9*S*,12*S*,15*R*,16*E*,18*R*,19*R*,21*R*, 23*S*,24*E*,26*E*,28*Z*,30*S*,32*S*,35*R*)-1,18-dihydroxy-19,30-dimethoxy-15,17,21,23, 29,35-hexamethyl-2,3,10,14,20-pentaoxo-11,36-dioxa-4-azatricyclo[30.3.1.$0^{4,9}$] hexatriaconta-16,24,26,28-tetraen-12-yl]propyl]-2-methoxycyclohexyl dimethylphosphinate, also known as AP23573 and MK8669, and described in PCT Publication No. WO 03/064383); everolimus (Afinitor® or RAD001); rapamycin (AY22989, Sirolimus®); simapimod (CAS 164301-51-3); emsirolimus, (5-{2,4-Bis[(3*S*)-3-methylmorpholin-4-yl]pyrido[2,3-*d*]pyrimidin-7-yl}-2-methoxyphenyl)methanol (AZD8055); 2-Amino-8-[*trans*-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-pyridinyl)-4-methyl-pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (PF04691502, CAS 1013101-36-4); and N^2-[1,4-dioxo-4-[[4-(4-oxo-8-phenyl-4*H*-1-benzopyran-2-yl)morpholinium-4-yl]methoxy]butyl]-L-arginylglycyl-L-α-aspartylL-serine- (SEQ ID NO: 237), inner salt (SF1126, CAS 936487-67-1), and XL765.

In some embodiments, the anti-PD-1 antibody molecule, *e.g.*, the anti-PD-1 antibody molecule described herein, is used, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), in combination with a BRAF inhibitor, *e.g.*, GSK2118436, RG7204, PLX4032, GDC-0879, PLX4720, and sorafenib tosylate (Bay 43-9006).

In some embodiments, the anti-PD-1 antibody molecule, *e.g.*, the anti-PD-1 antibody molecule described herein, is used, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), in combination with a MEK inhibitor. In some embodiments, the combination of the anti-PD-1 antibody and the MEK inhibitor is used to treat a cancer (*e.g.*, a cancer described herein). In some embodiments, the cancer treated with the combination is chosen from a melanoma, a colorectal cancer, a non-small cell lung cancer, an ovarian cancer, a breast cancer, a prostate cancer, a pancreatic cancer, a hematological malignancy or a renal cell carcinoma. In certain embodiments, the cancer includes a BRAF mutation (*e.g.*, a BRAF V600E mutation), a BRAF wildtype, a KRAS wildtype or an activating KRAS mutation. The cancer may be at an early, intermediate or late stage. Any MEK inhibitor can be used in combination including, but not limited to, ARRY-142886, G02442104 (also known as GSK1120212), RDEA436, RDEA119/BAY 869766, AS703026, G00039805 (also known as AZD-6244 or selumetinib), BIX 02188, BIX 02189, CI-1040 (PD-184352), PD0325901, PD98059, U0126, GDC-0973 (Methanone, [3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl][3- hydroxy-3-(25)-2-piperidinyl- 1 -azetidinyl]-), G-38963, G02443714 (also known as AS703206), or a pharmaceutically acceptable salt or solvate thereof..Additional examples of MEK inhibitors are disclosed in WO 2013/019906, WO 03/077914, WO 2005/121142, WO 2007/04415, WO 2008/024725 and WO 2009/085983, the contents of which are incorporated herein by reference.

In some embodiments, the anti-PD-1 antibody molecule, *e.g.*, the anti-PD-1 antibody molecule described herein, is used, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), in combination with a JAK2 inhibitor, *e.g.,* CEP-701, INCB18424, CP-690550 (tasocitinib).

In some embodiments, the pharmaceutical composition described herein is used, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), in combination with paclitaxel or a paclitaxel agent, *e.g.,* TAXOL®, protein-bound paclitaxel (*e.g.*, ABRAXANE®). Exemplary paclitaxel agents include, but are not limited to, nanoparticle albumin-bound paclitaxel (ABRAXANE, marketed by Abraxis Bioscience), docosahexaenoic acid bound-paclitaxel (DHA-paclitaxel, Taxoprexin, marketed by Protarga), polyglutamate bound-paclitaxel (PG-paclitaxel, paclitaxel poliglumex, CT-2103, XYOTAX, marketed by Cell Therapeutic), the tumor-activated prodrug (TAP), ANG105

(Angiopep-2 bound to three molecules of paclitaxel, marketed by ImmunoGen), paclitaxel-EC-1 (paclitaxel bound to the erbB2-recognizing peptide EC-1; *see* Li *et al., Biopolymers* (2007) 87:225-230), and glucose-conjugated paclitaxel (*e.g.,* 2'-paclitaxel methyl 2-glucopyranosyl succinate, *see* Liu *et al., Bioorganic & Medicinal Chemistry Letters* (2007) 17:617-620).

Radiation therapy can be administered through one of several methods, or a combination of methods, including without limitation external-beam therapy, internal radiation therapy, implant radiation, stereotactic radiosurgery, systemic radiation therapy, radiotherapy and permanent or temporary interstitial brachytherapy. The term "brachytherapy," refers to radiation therapy delivered by a spatially confined radioactive material inserted into the body at or near a tumor or other proliferative tissue disease site. The term is intended without limitation to include exposure to radioactive isotopes (*e.g.*, At-211, I-131, I-125, Y-90, Re-186, Re-188, Sm-153, Bi-212, P-32, and radioactive isotopes of Lu). Suitable radiation sources for use as a cell conditioner of the present invention include both solids and liquids. By way of non-limiting example, the radiation source can be a radionuclide, such as I-125, I-131, Yb-169, Ir-192 as a solid source, I-125 as a solid source, or other radionuclides that emit photons, beta particles, gamma radiation, or other therapeutic rays. The radioactive material can also be a fluid made from any solution of radionuclide(s), *e.g.,* a solution of I-125 or I-131, or a radioactive fluid can be produced using a slurry of a suitable fluid containing small particles of solid radionuclides, such as Au-198, Y-90. Moreover, the radionuclide(s) can be embodied in a gel or radioactive micro spheres.

Anti-PD-1 antibody molecules, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), can be administered in combination with one or more of the existing modalities for treating cancers, including, but not limited to: surgery; radiation therapy (*e.g.,* external-beam therapy which involves three dimensional, conformal radiation therapy where the field of radiation is designed, local radiation (*e.g.,* radition directed to a preselected target or organ), or focused radiation). Focused radiation can be selected from the group consisting of stereotactic radiosurgery, fractionated stereotactic radiosurgery, and intensity-modulated radiation therapy. The focused radiation can have a radiation source selected from the group consisting of a particle beam (proton), cobalt-60 (photon), and a linear accelerator (x-ray), *e.g.,* as decribed in WO 2012/177624.

In certain embodiments, the anti-PD-1 antibody molecule, alone or in combination with another immunomodulator (*e.g.*, an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), is administered in combination with an antibody against a Killer-cell Immunoglobulin-like Receptors (also referred to herein as an "anti-KIR antibody"), a pan-KIR antibody, an anti-NKG2D antibody, and an anti-MICA antibody. In certain embodiments, the combination of anti-PD-1 antibody molecule and anti-KIR antibody, pan-KIR antibody, or an anti-NKG2D antibody described herein is used to treat a cancer, *e.g.*, a cancer as described herein (*e.g.*, a solid tumor, *e.g.*, an advanced solid tumor).

In one embodiment, the anti-PD-1 antibody molecule, alone or in combination with another immunomodulator (*e.g.*, an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), is administered in combination with a cellular immunotherapy (*e.g.*, Provenge (*e.g.*, Sipuleucel)), and optionally in combination with cyclophosphamide. In certain embodiments, the combination of anti-PD-1 antibody molecule, Provenge and/or cyclophosphamide is used to treat a cancer, *e.g.*, a cancer as described herein (*e.g.*, a prostate cancer, *e.g.*, an advanced prostate cancer).

In another embodiment, the anti-PD-1 antibody molecule, alone or in combination with another immunomodulator (*e.g.*, an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), is administered in combination with a vaccine, *e.g.*, a dendritic cell renal carcinoma (DC-RCC) vaccine. In certain embodiments, the combination of anti-PD-1 antibody molecule and the DC-RCC vaccine is used to treat a cancer, *e.g.*, a cancer as described herein (*e.g.*, a renal carcinoma, *e.g.*, metastatic renal cell carcinoma (RCC) or clear cell renal cell carcinoma (CCRCC)).

In yet another embodiment, the anti-PD-1 antibody molecule, alone or in combination with another immunomodulator (*e.g.*, an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), is administered in combination with chemotherapy, and/or immunotherapy. For example, the anti-PD-1 antibody molecule can be used to treat a myeloma, alone or in combination with one or more of: chemotherapy or other anti-cancer agents (*e.g.*, thalidomide analogs, *e.g.*, lenalidomide), an anti-TIM-3 antibody, tumor antigen-pulsed dendritic cells, fusions (*e.g.*, electrofusions) of tumor cells and dendritic cells, or vaccination with immunoglobulin idiotype produced by malignant plasma cells. In one embodiment, the anti-PD-1 antibody molecule is used in combination with an anti-TIM-3 antibody to treat a myeloma, *e.g.*, a multiple myeloma.

In one embodiment, the anti-PD-1 antibody molecule, alone or in combination with another immunomodulator (*e.g.*, an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), is used in combination with chemotherapy to treat a lung cancer, *e.g.*, non-small cell lung cancer. In one embodiment, the anti-PD-1 antibody molecule is used with platinum doublet therapy to treat lung cancer.

In yet another embodiment, the anti-PD-1 antibody molecule, alone or in combination with another immunomodulator (*e.g.*, an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), is used to treat a renal cancer, *e.g.*, renal cell carcinoma (RCC) (*e.g.*, clear cell renal cell carcinoma (CCRCC) or metastatic RCC. The anti-PD-1 antibody molecule can be administered in combination with one or more of: an immune-based strategy (*e.g.*, interleukin-2 or interferon-α), a targeted agent (*e.g.*, a VEGF inhibitor such as a monoclonal antibody to VEGF); a VEGF tyrosine kinase inhibitor such as sunitinib, sorafenib, axitinib and pazopanib; an RNAi inhibitor), or an inhibitor of a downstream mediator of VEGF signaling, *e.g.*, an inhibitor of the mammalian target of rapamycin (mTOR), *e.g.*, everolimus and temsirolimus.

An example of suitable therapeutics for use in combination with the anti-PD-1 antibody molecules described herein, alone or in combination with another immunomodulator (*e.g.*, an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), for treatment of pancreatic cancer includes, but is not limited to, a chemotherapeutic agent, *e.g.*, paclitaxel or a paclitaxel agent (*e.g.*, a paclitaxel formulation such as TAXOL, an albumin-stabilized nanoparticle paclitaxel formulation (*e.g.*, ABRAXANE) or a liposomal paclitaxel formulation); gemcitabine (*e.g.*, gemcitabine alone or in combination with AXP107-11); other chemotherapeutic agents such as oxaliplatin, 5-fluorouracil, capecitabine, rubitecan, epirubicin hydrochloride, NC-6004, cisplatin, docetaxel (*e.g.*, TAXOTERE), mitomycin C, ifosfamide; interferon; tyrosine kinase inhibitor (*e.g.*, EGFR inhibitor (*e.g.*, erlotinib, panitumumab, cetuximab, nimotuzumab); HER2/neu receptor inhibitor (*e.g.*, trastuzumab); dual kinase inhibitor (*e.g.*, bosutinib, saracatinib, lapatinib, vandetanib); multikinase inhibitor (*e.g.*, sorafenib, sunitinib, XL184, pazopanib); VEGF inhibitor (*e.g.*, bevacizumab, AV-951, brivanib); radioimmunotherapy (*e.g.*, XR303); cancer vaccine (*e.g.*, GVAX, survivin peptide); COX-2 inhibitor (*e.g.*, celecoxib); IGF-1 receptor inhibitor (*e.g.*, AMG 479, MK-0646); mTOR inhibitor (*e.g.*, everolimus, temsirolimus); IL-6 inhibitor (*e.g.*, CNTO 328); cyclin-dependent kinase inhibitor (*e.g.*, P276-00, UCN-01); Altered Energy Metabolism-Directed (AEMD) compound (*e.g.*, CPI-613); HDAC inhibitor (*e.g.*, vorinostat);

TRAIL receptor 2 (TR-2) agonist (*e.g.*, conatumumab); MEK inhibitor (*e.g.*, AS703026, selumetinib, GSK1120212); Raf/MEK dual kinase inhibitor (*e.g.*, RO5126766); Notch signaling inhibitor (*e.g.,* MK0752); monoclonal antibody-antibody fusion protein (*e.g.,* L19IL2); curcumin; HSP90 inhibitor (*e.g.*, tanespimycin, STA-9090); rIL-2;, denileukin diftitox; topoisomerase 1 inhibitor (*e.g.,* irinotecan, PEP02); statin (*e.g.*, simvastatin); Factor VIIa inhibitor (*e.g.*, PCI-27483); AKT inhibitor (*e.g.,* RX-0201); hypoxia-activated prodrug (*e.g.*, TH-302); metformin hydrochloride, gamma-secretase inhibitor (*e.g.,* RO4929097); ribonucleotide reductase inhibitor (*e.g.*, 3-AP); immunotoxin (*e.g.*, HuC242-DM4); PARP inhibitor (*e.g.,* KU-0059436, veliparib); CTLA-4 inhbitor (*e.g.*, CP-675,206, ipilimumab); AdV-tk therapy; proteasome inhibitor (*e.g.*, bortezomib (Velcade), NPI-0052); thiazolidinedione (*e.g.*, pioglitazone); NPC-1C; Aurora kinase inhibitor (*e.g.*, R763/AS703569), CTGF inhibitor (*e.g.*, FG-3019); siG12D LODER; and radiation therapy (*e.g.,* tomotherapy, stereotactic radiation, proton therapy), surgery, and a combination thereof. In certain embodiments, a combination of paclitaxel or a paclitaxel agent, and gemcitabine can be used with the anti-PD-1 antibody molecules described herein.

An example of suitable therapeutics for use in combination with the anti-PD-1 antibody molecules, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), for treatment of small cell lung cancer includes, but is not limited to, a chemotherapeutic agent, *e.g.*, etoposide, carboplatin, cisplatin, oxaliplatin, irinotecan, topotecan, gemcitabine, liposomal SN-38, bendamustine, temozolomide, belotecan, NK012, FR901228, flavopiridol); tyrosine kinase inhibitor (*e.g.*, EGFR inhibitor (*e.g.*, erlotinib, gefitinib, cetuximab, panitumumab); multikinase inhibitor (*e.g.*, sorafenib, sunitinib); VEGF inhibitor (*e.g.,* bevacizumab, vandetanib); cancer vaccine (*e.g.,* GVAX); Bcl-2 inhibitor (*e.g.*, oblimersen sodium, ABT-263); proteasome inhibitor (*e.g.*, bortezomib (Velcade), NPI-0052), paclitaxel or a paclitaxel agent; docetaxel; IGF-1 receptor inhibitor (*e.g.*, AMG 479); HGF/SF inhibitor (*e.g.,* AMG 102, MK-0646); chloroquine; Aurora kinase inhibitor (*e.g.,* MLN8237); radioimmunotherapy (*e.g.,* TF2); HSP90 inhibitor (*e.g.*, tanespimycin, STA-9090); mTOR inhibitor (*e.g.*, everolimus); Ep-CAM-/CD3-bispecific antibody (*e.g.,* MT110); CK-2 inhibitor (*e.g.,* CX-4945); HDAC inhibitor (*e.g.,* belinostat); SMO antagonist (*e.g.*, BMS 833923); peptide cancer vaccine, and radiation therapy (*e.g.,* intensity-modulated radiation therapy (IMRT), hypofractionated radiotherapy, hypoxia-guided radiotherapy), surgery, and combinations thereof.

An example of suitable therapeutics for use in combination with the anti-PD-1 antibody molecules, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), for treatment of non-small cell lung cancer includes, but is not limited to, a chemotherapeutic agent, *e.g.*, vinorelbine, cisplatin, docetaxel, pemetrexed disodium, etoposide, gemcitabine, carboplatin, liposomal SN-38, TLK286, temozolomide, topotecan, pemetrexed disodium, azacitidine, irinotecan, tegafur-gimeracil-oteracil potassium, sapacitabine); tyrosine kinase inhibitor (*e.g.*, EGFR inhibitor (*e.g.*, erlotinib, gefitinib, cetuximab, panitumumab, necitumumab, PF-00299804, nimotuzumab, RO5083945), MET inhibitor (*e.g.,* PF-02341066, ARQ 197), PI3K kinase inhibitor (*e.g.,* XL147, GDC-0941), Raf/MEK dual kinase inhibitor (*e.g.,* RO5126766), PI3K/mTOR dual kinase inhibitor (*e.g.*, XL765), SRC inhibitor (*e.g.,* dasatinib), dual inhibitor (*e.g.,* BIBW 2992, GSK1363089, ZD6474, AZD0530, AG-013736, lapatinib, MEHD7945A, linifanib), multikinase inhibitor (*e.g.*, sorafenib, sunitinib, pazopanib, AMG 706, XL184, MGCD265, BMS-690514, R935788), VEGF inhibitor (*e.g.,* endostar, endostatin, bevacizumab, cediranib, BIBF 1120, axitinib, tivozanib, AZD2171), cancer vaccine (*e.g.,* BLP25 liposome vaccine, GVAX, recombinant DNA and adenovirus expressing L523S protein), Bcl-2 inhibitor (*e.g.*, oblimersen sodium), proteasome inhibitor (*e.g.*, bortezomib, carfilzomib, NPI-0052, MLN9708), paclitaxel or a paclitaxel agent, docetaxel, IGF-1 receptor inhibitor (*e.g.*, cixutumumab, MK-0646, OSI 906, CP-751,871, BIIB022), hydroxychloroquine, HSP90 inhibitor (*e.g.*, tanespimycin, STA-9090, AUY922, XL888), mTOR inhibitor (*e.g.*, everolimus, temsirolimus, ridaforolimus), Ep-CAM-/CD3-bispecific antibody (*e.g.*, MT110), CK-2 inhibitor (*e.g.*, CX-4945), HDAC inhibitor (*e.g.*, MS 275, LBH589, vorinostat, valproic acid, FR901228), DHFR inhibitor (*e.g.*, pralatrexate), retinoid (*e.g.*, bexarotene, tretinoin), antibody-drug conjugate (*e.g.*, SGN-15), bisphosphonate (*e.g.*, zoledronic acid), cancer vaccine (*e.g.*, belagenpumatucel-L), low molecular weight heparin (LMWH) (*e.g.*, tinzaparin, enoxaparin), GSK1572932A, melatonin, talactoferrin, dimesna, topoisomerase inhibitor (*e.g.*, amrubicin, etoposide, karenitecin), nelfinavir, cilengitide, ErbB3 inhibitor (*e.g.*, MM-121, U3-1287), survivin inhibitor (*e.g.*, YM155, LY2181308), eribulin mesylate, COX-2 inhibitor (*e.g.*, celecoxib), pegfilgrastim, Polo-like kinase 1 inhibitor (*e.g.*, BI 6727), TRAIL receptor 2 (TR-2) agonist (*e.g.*, CS-1008), CNGRC peptide (SEQ ID NO: 225)-TNF alpha conjugate, dichloroacetate (DCA), HGF inhibitor (*e.g.*, SCH 900105), SAR240550, PPAR-gamma agonist (*e.g.,* CS-7017), gamma-secretase inhibitor (*e.g.*, RO4929097), epigenetic

therapy (*e.g.*, 5-azacitidine), nitroglycerin, MEK inhibitor (*e.g.*, AZD6244), cyclin-dependent kinase inhibitor (*e.g.*, UCN-01), cholesterol-Fus1, antitubulin agent (*e.g.*, E7389), farnesyl-OH-transferase inhibitor (*e.g.*, lonafarnib), immunotoxin (*e.g.*, BB-10901, SS1 (dsFv) PE38), fondaparinux, vascular-disrupting agent (*e.g.*, AVE8062), PD-L1 inhibitor (*e.g.*, MDX-1105, MDX-1106), beta-glucan, NGR-hTNF, EMD 521873, MEK inhibitor (*e.g.*, GSK1120212), epothilone analog (*e.g.*, ixabepilone), kinesin-spindle inhibitor (*e.g.*, 4SC-205), telomere targeting agent (*e.g.*, KML-001), P70 pathway inhibitor (*e.g.*, LY2584702), AKT inhibitor (*e.g.*, MK-2206), angiogenesis inhibitor (*e.g.*, lenalidomide), Notch signaling inhibitor (*e.g.*, OMP-21M18), radiation therapy, surgery, and combinations thereof.

An example of suitable therapeutics for use in combination with the anti-PD-1 antibody molecules, alone or in combination with another immunomodulator (*e.g.*, an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), for treatment of ovarian cancer includes, but is not limited to, a chemotherapeutic agent (*e.g.*, paclitaxel or a paclitaxel agent; docetaxel; carboplatin; gemcitabine; doxorubicin; topotecan; cisplatin; irinotecan, TLK286, ifosfamide, olaparib, oxaliplatin, melphalan, pemetrexed disodium, SJG-136, cyclophosphamide, etoposide, decitabine); ghrelin antagonist (*e.g.*, AEZS-130), immunotherapy (*e.g.*, APC8024, oregovomab, OPT-821), tyrosine kinase inhibitor (*e.g.*, EGFR inhibitor (*e.g.*, erlotinib), dual inhibitor (*e.g.*, E7080), multikinase inhibitor (*e.g.*, AZD0530, JI-101, sorafenib, sunitinib, pazopanib), ON 01910.Na), VEGF inhibitor (*e.g.*, bevacizumab, BIBF 1120, cediranib, AZD2171), PDGFR inhibitor (*e.g.*, IMC-3G3), paclitaxel, topoisomerase inhibitor (*e.g.*, karenitecin, Irinotecan), HDAC inhibitor (*e.g.*, valproate, vorinostat), folate receptor inhibitor (*e.g.*, farletuzumab), angiopoietin inhibitor (*e.g.*, AMG 386), epothilone analog (*e.g.*, ixabepilone), proteasome inhibitor (*e.g.*, carfilzomib), IGF-1 receptor inhibitor (*e.g.*, OSI 906, AMG 479), PARP inhibitor (*e.g.*, veliparib, AG014699, iniparib, MK-4827), Aurora kinase inhibitor (*e.g.*, MLN8237, ENMD-2076), angiogenesis inhibitor (*e.g.*, lenalidomide), DHFR inhibitor (*e.g.*, pralatrexate), radioimmunotherapeutic agnet (*e.g.*, Hu3S193), statin (*e.g.*, lovastatin), topoisomerase 1 inhibitor (*e.g.*, NKTR-102), cancer vaccine (*e.g.*, p53 synthetic long peptides vaccine, autologous OC-DC vaccine), mTOR inhibitor (*e.g.*, temsirolimus, everolimus), BCR/ABL inhibitor (*e.g.*, imatinib), ET-A receptor antagonist (*e.g.*, ZD4054), TRAIL receptor 2 (TR-2) agonist (*e.g.*, CS-1008), HGF/SF inhibitor (*e.g.*, AMG 102), EGEN-001, Polo-like kinase 1 inhibitor (*e.g.*, BI 6727), gamma-secretase inhibitor (*e.g.*, RO4929097), Wee-1 inhibitor (*e.g.*, MK-1775),

antitubulin agent (*e.g.*, vinorelbine, E7389), immunotoxin (*e.g.*, denileukin diftitox), SB-485232, vascular-disrupting agent (*e.g.*, AVE8062), integrin inhibitor (*e.g.*, EMD 525797), kinesin-spindle inhibitor (*e.g.*, 4SC-205), revlimid, HER2 inhibitor (*e.g.*, MGAH22), ErrB3 inhibitor (*e.g.*, MM-121), radiation therapy; and combinations thereof.

In one exemplary embodiment, the anti-PD-1 antibody molecule, alone or in combination with another immunomodulator (*e.g.*, an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), is used to treat a myeloma, alone or in combination with one or more of: chemotherapy or other anti-cancer agents (*e.g.*, thalidomide analogs, *e.g.*, lenalidomide), HSCT (Cook, R. (2008) *J Manag Care Pharm.* 14(7 Suppl):19-25), an anti-TIM-3 antibody (Hallett, WHD *et al.* (2011) *J of American Society for Blood and Marrow Transplantation* 17(8):1133-145), tumor antigen-pulsed dendritic cells, fusions (*e.g.*, electrofusions) of tumor cells and dendritic cells, or vaccination with immunoglobulin idiotype produced by malignant plasma cells (reviewed in Yi, Q. (2009) *Cancer J.* 15(6):502-10).

In yet another embodiment, the anti-PD-1 antibody molecule, alone or in combination with another immunomodulator (*e.g.*, an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), is used to treat a renal cancer, *e.g.*, renal cell carcinoma (RCC) or metastatic RCC. The anti-PD-1 antibody molecule can be administered in combination with one or more of: an immune-based strategy (*e.g.*, interleukin-2 or interferon-α), a targeted agent (*e.g.*, a VEGF inhibitor such as a monoclonal antibody to VEGF, *e.g.*, bevacizumab (Rini, B.I. *et al.* (2010) *J. Clin. Oncol.* 28(13):2137-2143)); a VEGF tyrosine kinase inhibitor such as sunitinib, sorafenib, axitinib and pazopanib (reviewed in Pal. S.K. *et al.* (2014) *Clin. Advances in Hematology & Oncology* 12(2):90-99)); an RNAi inhibitor), or an inhibitor of a downstream mediator of VEGF signaling, *e.g.*, an inhibitor of the mammalian target of rapamycin (mTOR), *e.g.*, everolimus and temsirolimus (Hudes, G. *et al.* (2007) *N. Engl. J. Med.* 356(22):2271-2281, Motzer, R.J. *et al.* (2008) *Lancet* 372: 449-456).

An example of suitable therapeutics for use in combination with the anti-PD-1 antibody molecules described herein, alone or in combination with another immunomodulator (*e.g.*, an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), for treatment of chronic myelogenous leukemia (AML) according to the invention includes, but is not limited to, a chemotherapeutic (*e.g.*, cytarabine, hydroxyurea, clofarabine, melphalan, thiotepa, fludarabine, busulfan, etoposide, cordycepin, pentostatin, capecitabine, azacitidine, cyclophosphamide,

cladribine, topotecan), tyrosine kinase inhibitor (*e.g.*, BCR/ABL inhibitor (*e.g.*, imatinib, nilotinib), ON 01910.Na, dual inhibitor (*e.g.*, dasatinib, bosutinib), multikinase inhibitor (*e.g.*, DCC-2036, ponatinib, sorafenib, sunitinib, RGB-286638)), interferon alfa, steroids, apoptotic agent (*e.g.*, omacetaxine mepesuccinat), immunotherapy (*e.g.*, allogeneic CD4+ memory Th1-like T cells/microparticle-bound anti-CD3/anti-CD28, autologous cytokine induced killer cells (CIK), AHN-12), CD52 targeting agent (*e.g.*, alemtuzumab), HSP90 inhibitor (*e.g.*, tanespimycin, STA-9090, AUY922, XL888), mTOR inhibitor (*e.g.*, everolimus), SMO antagonist (*e.g.*, BMS 833923), ribonucleotide reductase inhibitor (*e.g.*, 3-AP), JAK-2 inhibitor (*e.g.*, INCB018424), Hydroxychloroquine, retinoid (*e.g.*, fenretinide), cyclin-dependent kinase inhibitor (*e.g.*, UCN-01), HDAC inhibitor (*e.g.*, belinostat, vorinostat, JNJ-26481585), PARP inhibitor (*e.g.*, veliparib), MDM2 antagonist (*e.g.*, RO5045337), Aurora B kinase inhibitor (*e.g.*, TAK-901), radioimmunotherapy (*e.g.*, actinium-225-labeled anti-CD33 antibody HuM195), Hedgehog inhibitor (*e.g.*, PF-04449913), STAT3 inhibitor (*e.g.*, OPB-31121), KB004, cancer vaccine (*e.g.*, AG858), bone marrow transplantation, stem cell transplantation, radiation therapy, and combinations thereof.

An example of suitable therapeutics for use in combination with the anti-PD-1 antibody molecules, alone or in combination with another immunomodulator (*e.g.*, an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), for treatment of chronic lymphocytic leukemia (CLL) includes, but is not limited to, a chemotherapeutic agent (*e.g.*, fludarabine, cyclophosphamide, doxorubicin, vincristine, chlorambucil, bendamustine, chlorambucil, busulfan, gemcitabine, melphalan, pentostatin, mitoxantrone, 5-azacytidine, pemetrexed disodium), tyrosine kinase inhibitor (*e.g.*, EGFR inhibitor (*e.g.*, erlotinib), BTK inhibitor (*e.g.*, PCI-32765), multikinase inhibitor (*e.g.*, MGCD265, RGB-286638), CD-20 targeting agent (*e.g.*, rituximab, ofatumumab, RO5072759, LFB-R603), CD52 targeting agent (*e.g.*, alemtuzumab), prednisolone, darbepoetin alfa, lenalidomide, Bcl-2 inhibitor (*e.g.*, ABT-263), immunotherapy (*e.g.*, allogeneic CD4+ memory Th1-like T cells/microparticle-bound anti-CD3/anti-CD28, autologous cytokine induced killer cells (CIK)), HDAC inhibitor (*e.g.*, vorinostat, valproic acid, LBH589, JNJ-26481585, AR-42), XIAP inhibitor (*e.g.*, AEG35156), CD-74 targeting agent (*e.g.*, milatuzumab), mTOR inhibitor (*e.g.*, everolimus), AT-101, immunotoxin (*e.g.*, CAT-8015, anti-Tac(Fv)-PE38 (LMB-2)), CD37 targeting agent (*e.g.*, TRU-016), radioimmunotherapy (*e.g.*, 131-tositumomab), hydroxychloroquine, perifosine, SRC inhibitor (*e.g.*, dasatinib), thalidomide, PI3K delta inhibitor

(*e.g.*, CAL-101), retinoid (*e.g.*, fenretinide), MDM2 antagonist (*e.g.*, RO5045337), plerixafor, Aurora kinase inhibitor (*e.g.*, MLN8237, TAK-901), proteasome inhibitor (*e.g.*, bortezomib), CD-19 targeting agent (*e.g.*, MEDI-551, MOR208), MEK inhibitor (*e.g.*, ABT-348), JAK-2 inhibitor (*e.g.*, INCB018424), hypoxia-activated prodrug (*e.g.*, TH-302), paclitaxel or a paclitaxel agent, HSP90 inhibitor, AKT inhibitor (*e.g.*, MK2206), HMG-CoA inhibitor (*e.g.*, simvastatin), GNKG186, radiation therapy, bone marrow transplantation, stem cell transplantation, and a combination thereof.

An example of suitable therapeutics for use in combination with the anti-PD-1 antibody molecules described herein, alone or in combination with another immunomodulator (*e.g.*, an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), for treatment of acute lymphocytic leukemia (ALL) includes, but is not limited to, a chemotherapeutic agent (*e.g.*, prednisolone, dexamethasone, vincristine, asparaginase, daunorubicin, cyclophosphamide, cytarabine, etoposide, thioguanine, mercaptopurine, clofarabine, liposomal annamycin, busulfan, etoposide, capecitabine, decitabine, azacitidine, topotecan, temozolomide), tyrosine kinase inhibitor (*e.g.*, BCR/ABL inhibitor (*e.g.*, imatinib, nilotinib), ON 01910.Na, multikinase inhibitor (*e.g.*, sorafenib)), CD-20 targeting agent (*e.g.*, rituximab), CD52 targeting agent (*e.g.*, alemtuzumab), HSP90 inhibitor (*e.g.*, STA-9090), mTOR inhibitor (*e.g.*, everolimus, rapamycin), JAK-2 inhibitor (*e.g.*, INCB018424), HER2/neu receptor inhibitor (*e.g.*, trastuzumab), proteasome inhibitor (*e.g.*, bortezomib), methotrexate, asparaginase, CD-22 targeting agent (*e.g.*, epratuzumab, inotuzumab), immunotherapy (*e.g.*, autologous cytokine induced killer cells (CIK), AHN-12), blinatumomab, cyclin-dependent kinase inhibitor (*e.g.*, UCN-01), CD45 targeting agent (*e.g.*, BC8), MDM2 antagonist (*e.g.*, RO5045337), immunotoxin (*e.g.*, CAT-8015, DT2219ARL), HDAC inhibitor (*e.g.*, JNJ-26481585), JVRS-100, paclitaxel or a paclitaxel agent, STAT3 inhibitor (*e.g.*, OPB-31121), PARP inhibitor (*e.g.*, veliparib), EZN-2285, radiation therapy, steroid, bone marrow transplantation, stem cell transplantation, or a combination thereof.

An example of suitable therapeutics for use in combination with the anti-PD-1 antibody molecules described herein, alone or in combination with another immunomodulator (*e.g.*, an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), for treatment of acute myeloid leukemia (AML) includes, but is not limited to, a chemotherapeutic agent (*e.g.*, cytarabine, daunorubicin, idarubicin, clofarabine, decitabine, vosaroxin, azacitidine, clofarabine, ribavirin,

CPX-351, treosulfan, elacytarabine, azacitidine), tyrosine kinase inhibitor (*e.g.,* BCR/ABL inhibitor (*e.g.*, imatinib, nilotinib), ON 01910.Na, multikinase inhibitor (*e.g.,* midostaurin, SU 11248, quizartinib, sorafinib)), immunotoxin (*e.g.,* gemtuzumab ozogamicin), DT388IL3 fusion protein, HDAC inhibitor (*e.g.,* vorinostat, LBH589), plerixafor, mTOR inhibitor (*e.g.*, everolimus), SRC inhibitor (*e.g.,* dasatinib), HSP90 inhbitor (*e.g.,* STA-9090), retinoid (*e.g.,* bexarotene, Aurora kinase inhibitor (*e.g.,* BI 811283), JAK-2 inhibitor (*e.g.,* INCB018424), Polo-like kinase inhibitor (*e.g.,* BI 6727), cenersen, CD45 targeting agent (*e.g.,* BC8), cyclin-dependent kinase inhibitor (*e.g.,* UCN-01), MDM2 antagonist (*e.g.,* RO5045337), mTOR inhibitor (*e.g.,* everolimus), LY573636-sodium, ZRx-101, MLN4924, lenalidomide, immunotherapy (*e.g.,* AHN-12), histamine dihydrochloride, radiation therapy, bone marrow transplantation, stem cell transplantation, and a combination thereof.

An example of suitable therapeutics for use in combination with the anti-PD-1 antibody molecules described herein, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), for treatment of multiple myeloma (MM) includes, but is not limited to, a chemotherapeutic agent (*e.g.*, melphalan, amifostine, cyclophosphamide, doxorubicin, clofarabine, bendamustine, fludarabine, adriamycin, SyB L-0501), thalidomide, lenalidomide, dexamethasone, prednisone, pomalidomide, proteasome inhibitor (*e.g.,* bortezomib, carfilzomib, MLN9708), cancer vaccine (*e.g.*, GVAX), CD-40 targeting agent (*e.g.,* SGN-40, CHIR-12.12), perifosine, zoledronic acid, Immunotherapy (*e.g.,* MAGE-A3, NY-ESO-1 , HuMax-CD38), HDAC inhibitor (*e.g.,* vorinostat, LBH589, AR-42), aplidin, cycline-dependent kinase inhibitor (*e.g.,* PD-0332991, dinaciclib), arsenic trioxide, CB3304, HSP90 inhibitor (*e.g.,* KW-2478), tyrosine kinase inhibitor (*e.g.,* EGFR inhibitor (*e.g.,* cetuximab), multikinase inhibitor (*e.g.*, AT9283)), VEGF inhibitor (*e.g.,* bevacizumab), plerixafor, MEK inhibitor (*e.g.,* AZD6244), IPH2101, atorvastatin, immunotoxin (*e.g.,* BB-10901), NPI-0052, radioimmunotherapeutic (*e.g.,* yttrium Y 90 ibritumomab tiuxetan), STAT3 inhibitor (*e.g.,* OPB-31121), MLN4924, Aurora kinase inhibitor (*e.g.,* ENMD-2076), IMGN901, ACE-041, CK-2 inhibitor (*e.g.,* CX-4945), radiation therapy, bone marrow transplantation, stem cell transplantation, and a combination thereof.

An example of suitable therapeutics for use in combination with the anti-PD-1 antibody molecules, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), for treatment of prostate cancer includes, but is not

limited to, a chemotherapeutic agent (*e.g.*, docetaxel, carboplatin, fludarabine), abiraterone, hormonal therapy (*e.g.*, flutamide, bicalutamide, nilutamide, cyproterone acetate, ketoconazole, aminoglutethimide, abarelix, degarelix, leuprolide, goserelin, triptorelin, buserelin), tyrosine kinase inhibitor (*e.g.*, dual kinase inhibitor (*e.g.*, lapatanib), multikinase inhibitor (*e.g.*, sorafenib, sunitinib)), VEGF inhibitor (*e.g.*, bevacizumab), TAK-700, cancer vaccine (*e.g.*, BPX-101, PEP223), lenalidomide, TOK-001, IGF-1 receptor inhibitor (*e.g.*, cixutumumab), TRC105, Aurora A kinase inhibitor (*e.g.*, MLN8237), proteasome inhibitor (*e.g.*, bortezomib), OGX-011, radioimmunotherapy (*e.g.*, HuJ591-GS), HDAC inhibitor (*e.g.*, valproic acid, SB939, LBH589), hydroxychloroquine, mTOR inhibitor (*e.g.*, everolimus), dovitinib lactate, diindolylmethane, efavirenz, OGX-427, genistein, IMC-3G3, bafetinib, CP-675,206, radiation therapy, surgery, or a combination thereof.

An example of suitable therapeutics for use in combination with the anti-PD-1 antibody molecules, alone or in combination with another immunomodulator (*e.g.*, an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), for treatment of HNSCC includes, but is not limited to, one or both of Compound A8 as described herein (or a compound described in PCT Publication No. WO2010/029082) and cetuximab (*e.g.*, Erbitux, marketed by BMS). In some embodiments, the therapeutic (*e.g.*, the Compound A8 or compound related to A8) is a PI3K modulator, *e.g.*, a PI3K inhibitor. In some embodiments, the therapeutic (*e.g.*, cetuximab) modulates, *e.g.*, inhibits, EGFR. In some embodiments, the cancer has, or is identified as having, elevated levels or activity of PI3K or EGFR compared to a control cell or reference value.

An example of suitable therapeutics for use in combination with the anti-PD-1 antibody molecules, alone or in combination with another immunomodulator (*e.g.*, an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), for treatment of gastric cancer, *e.g.*, MSI-high and/or EBV+ gastric cancer, includes, but is not limited to, Compound A8 as described herein (or a compound described in PCT Publication No. WO2010/029082). In some embodiments, the therapeutic (*e.g.*, the Compound A8 or compound related to A8) is a PI3K modulator, *e.g.*, a PI3K inhibitor. In some embodiments, the cancer has, or is identified as having, elevated levels or activity of PI3K compared to a control cell or reference value.

An example of suitable therapeutics for use in combination with the anti-PD-1 antibody molecules, alone or in combination with another immunomodulator (*e.g.*, an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), for treatment of gastric cancer, *e.g.*, MSI-high and/or

RNF43-inactivated gastric cancer, includes, but is not limited to, Compound A28 as described herein (or a compound described in PCT Publication No. WO2010/101849). In some embodiments, the therapeutic (*e.g.*, the Compound A28 or compound related to A28) is a modulator, *e.g.*, inhibitor, of porcupine. In some embodiments, the cancer has, or is identified as having, elevated levels or activity of porcupine compared to a control cell or reference value.

An example of suitable therapeutics for use in combination with the anti-PD-1 antibody molecules, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), for treatment of GI stromal tumor (GIST), includes, but is not limited to, Compound A16 as described herein (or a compound described in PCT Publication No. WO1999/003854). In some embodiments, the therapeutic (*e.g.*, the Compound A16 or compound related to A16) is a modulator, *e.g.*, inhibitor, of a tyrosine kinase. In some embodiments, the cancer has, or is determined to have, elevated levels or activity of a tyrosine kinase compared to a control cell or reference value.

An example of suitable therapeutics for use in combination with the anti-PD-1 antibody molecules, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), for treatment of NSCLC, *e.g.*, squamous or adenocarcinoma, includes, but is not limited to, one or both of Compound A17 as described herein (or a compound described in US Patent No. 7,767,675 and 8,420,645) and Compound A23 as described herein (or a compound described in PCT Publication No. WO2003/077914). In some embodiments, the compound (*e.g.*, the Compound A17 or compound related to A17) modulates, *e.g.*, inhibits, c-MET. In some embodiments, the compound (*e.g.*, the Compound A23 or compound related to A23) modulates, *e.g.*, inhibits, Alk. In some embodiments, the cancer has, or is determined to have, elevated levels or activity of one or both of c-MET or Alk compared to a control cell or reference value. In some embodiments, the cancer has, or is identified as having, a mutation in EGFR.

An example of suitable therapeutics for use in combination with the anti-PD-1 antibody molecules, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), for treatment of melanoma (*e.g.*, NRAS melanoma) includes, but is not limited to, one or both of Compound A24 as described herein (or a compound described in US Patent Nos. 8,415,355 and 8,685,980) and Compound A34 as described herein (or a compound described in PCT Publication No. WO2003/077914). In some embodiments, the

compound (*e.g.*, the Compound A24 or compound related to A24) modulates, *e.g.*, inhibits, one or more of JAK and CDK4/6. In some embodiments, the compound (*e.g.*, the Compound A34 or compound related to A34) modulates, *e.g.*, inhibits, MEK. In some embodiments, the cancer has, or is identified as having, elevated levels or activity of one or more of JAK, CDK4/6, and MEK compared to a control cell or reference value.

An example of suitable therapeutics for use in combination with the anti-PD-1 antibody molecules, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), for treatment of melanoma (*e.g.*, NRAS melanoma) includes, but is not limited to, one or both of Compound A29 as described herein (or a compound described in PCT Publication No. WO2011/025927) and Compound A34 as described herein (or a compound described in PCT Publication No. WO2003/077914). In some embodiments, the compound (*e.g.*, the Compound A29 or compound related to A29) modulates, *e.g.*, inhibits, BRAF. In some embodiments, the compound (*e.g.*, the Compound A34 or compound related to A34) modulates, *e.g.*, inhibits, MEK. In some embodiments, the cancer has, or is identified as having, elevated levels or activity of one or both of BRAF and MEK compared to a control cell or reference value.

An example of suitable therapeutics for use in combination with the anti-PD-1 antibody molecules, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), for treatment of squamous NSCLC includes, but is not limited to, Compound A5 as described herein (or a compound described in US Patent No. 8,552,002). In some embodiments, the compound (*e.g.*, the Compound A5 or compound related to A5) modulates, *e.g.,* inhibits, FGFR. In some embodiments, the cancer has, or is identified as having, elevated levels or activity of FGFR compared to a control cell or reference value.

An example of suitable therapeutics for use in combination with the anti-PD-1 antibody molecules, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), for treatment of colorectal cancer includes, but is not limited to, one or both of Compound A29 as described herein (or a compound PCT Publication No. WO2011/025927) and cetuximab (*e.g.,* Erbitux, marketed by BMS). In some embodiments, the therapeutic (*e.g.,* the Compound A29 or compound related to A29) modulates, *e.g.,* inhibits, BRAF. In some embodiments, the therapeutic (*e.g.,* cetuximab) modulates, *e.g.,* inhibits EGFR.

In some embodiments, the cancer has, or is identified as having, elevated levels or activity of BRAF or EGFR compared to a control cell or reference value.

This disclosure also provides a method of treating cancer with Compound A8, cetuximab, and a PD-1 antibody molecule (optionally in combination with a TIM-3 antibody molecule or LAG-3 antibody molecule). In some embodiments, the patient is first treated with Compound A8 and cetuximab. This treatment continues for an amount of time, *e.g.*, a predetermined amount of time, *e.g.*, about 1, 2, 4, 6, 8, 10, or 12 months. Next, the PD-1 antibody molecule (optionally in combination with a TIM-3 antibody molecule or LAG-3 antibody molecule) is administered. The PD-1 antibody can optionally be administered in combination with cetuximab.

In some embodiments, the patient is first treated with all three of Compound A8, cetuximab, and a PD-1 antibody molecule (optionally in combination with a TIM-3 antibody molecule or LAG-3 antibody molecule). This treatment continues for an amount of time, *e.g.*, a predetermined amount of time, *e.g.*, about 6, 8, 10, or 12 months. Next, the Compound A8 and/or cetuximab can be tapered off, so that the maintenance phase involves treatment with the PD-1 antibody molecule (*e.g.*, as a monotherapy, or in combination with a TIM-3 antibody molecule or LAG-3 antibody molecule) but not Compound A8 or cetuximab.

In other embodiments, the three compounds (Compound A8, cetuximab, and a PD-1 antibody molecule, optionally in combination with a TIM-3 antibody molecule or LAG-3 antibody molecule) are given sequentially at the outset of the treatment. For instance, Compound A8 and cetuximab can be given first, as described above. Next, the PD-1 antibody molecule (optionally in combination with a TIM-3 antibody molecule or LAG-3 antibody molecule) is added to the regimen. Next, the Compound A8 and/or cetuximab can be tapered off as described above.

Exemplary doses for the three (or more) agent regimens are as follows. The PD-1 antibody molecule can be administered, *e.g.*, at a dose of about 1 to 40 mg/kg, *e.g.*, 1 to 30 mg/kg, *e.g.*, about 5 to 25 mg/kg, about 10 to 20 mg/kg, about 1 to 5 mg/kg, or about 3 mg/kg. In some embodiments, the Compound A8 is administered at a dose of approximately 200-300, 300-400, or 200-400 mg. In some embodiments, the cetuximab is administered at a 400 mg/m2 initial dose as a 120-minute intravenous infusion followed by 250 mg/m2 weekly infused over 60 minutes. In embodiments, one or more of the Compound A8, cetuximab, and PD-1 antibody

molecule is administered at a dose that is lower than the dose at which that agent is typically administered as a monotherapy, *e.g.*, about 0-10%, 10-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, or 80-90% lower than the dose at which that agent is typically administered as a monotherapy. In embodiments, the one or more of the Compound A8, cetuximab, and PD-1 antibody molecule is administered at a dose that is lower than the dose of that agent recited in this paragraph, *e.g.*, about 0-10%, 10-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, or 80-90% lower than the dose of that agent recited in this paragraph. In certain embodiments, the concentration of the Compound A8 that is required to achieve inhibition, *e.g.*, growth inhibition, is lower when the Compound A8 is administered in combination with one or both of the cetuximab and PD-1 antibody molecule than when the Compound A8 is administered individually. In certain embodiments, the concentration of the cetuximab that is required to achieve inhibition, *e.g.,* growth inhibition, is lower when the cetuximab is administered in combination with one or both of the Compound A8 and PD-1 antibody molecule than when the cetuximab is administered individually. In certain embodiments, the concentration of the PD-1 antibody molecule that is required to achieve inhibition, *e.g.,* growth inhibition, is lower when the PD-1 antibody molecule is administered in combination with one or both of the cetuximab and Compound A8 than when the PD-1 antibody molecule is administered individually.

Additionally disclosed herein is a method of treating cancer with the anti-PD-1 antibody molecules, alone or in combination with another immunomodulator (*e.g.,* an anti-LAG-3, anti-PD-L1 or anti-TIM-3 antibody molecule), and a targeted anti-cancer agent, *e.g.*, an agent that targets one or more proteins. In some embodiments, the anti-PD-1 antibody molecule (and optionally other immunomodulator(s)) are administered first, and the targeted anti-cancer agent is administered second. The length of time between administration of the anti-PD-1 antibody molecule and the targeted anti-cancer agent can be, *e.g.*, 10, 20, or 30 minutes, 1, 2, 4, 6, or 12 hours, or 1, 2, 3, 4, 5, 6, or 7 days, or any span of time within this range. In certain embodiments, the anti-PD-1 antibody molecule is administered repeatedly over a period of time (*e.g.*, 1, 2, 3, 4, 5, or 6 days, or 1, 2, 4, 8, 12, 16, or 20 weeks, or any span of time within this range) before the targeted anti-cancer agent is administered. In other embodiments, the anti-PD-1 antibody molecule and the targeted anti-cancer agent are administered at substantially the same time.

Infectious Diseases

Other methods of the invention are used to treat patients that have been exposed to particular toxins or pathogens. Accordingly, another aspect of the invention provides a method of treating an infectious disease in a subject comprising administering to the subject an anti-PD-1 antibody molecule, such that the subject is treated for the infectious disease.

In the treatment of infection (*e.g.*, acute and/or chronic), administration of the anti-PD-1 antibody molecules can be combined with conventional treatments in addition to or in lieu of stimulating natural host immune defenses to infection. Natural host immune defenses to infection include, but are not limited to inflammation, fever, antibody-mediated host defense, T-lymphocyte-mediated host defenses, including lymphokine secretion and cytotoxic T-cells (especially during viral infection), complement mediated lysis and opsonization (facilitated phagocytosis), and phagocytosis. The ability of the anti-PD-1 antibody molecules to reactivate dysfunctional T-cells would be useful to treat chronic infections, in particular those in which cell-mediated immunity is important for complete recovery.

Similar to its application to tumors as discussed above, antibody mediated PD-1 blockade can be used alone, or as an adjuvant, in combination with vaccines, to stimulate the immune response to pathogens, toxins, and self-antigens. Examples of pathogens for which this therapeutic approach may be particularly useful, include pathogens for which there is currently no effective vaccine, or pathogens for which conventional vaccines are less than completely effective. These include, but are not limited to HIV, Hepatitis (A, B, & C), Influenza, Herpes, Giardia, Malaria, Leishmania, *Staphylococcus aureus*, *Pseudomonas Aeruginosa*. PD-1 blockade is particularly useful against established infections by agents such as HIV that present altered antigens over the course of the infections. These novel epitopes are recognized as foreign at the time of anti-human PD-1 administration, thus provoking a strong T cell response that is not dampened by negative signals through PD-1.

Viruses

For infections resulting from viral causes, the anti-PD-1 antibody molecules can be combined by application simultaneous with, prior to or subsequent to application of standard therapies for treating viral infections. Such standard therapies vary depending upon type of virus,

although in almost all cases, administration of human serum containing antibodies (*e.g.*, IgA, IgG) specific to the virus can be effective.

Some examples of pathogenic viruses causing infections treatable by methods include HIV, hepatitis (A, B, or C), herpes virus (*e.g.*, VZV, HSV-1, HAV-6, HSV-II, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus.

In one embodiment, the infection is an influenza infection. Influenza infection can result in fever, cough, myalgia, headache and malaise, which often occur in seasonal epidemics. Influenza is also associated with a number of postinfectious disorders, such as encephalitis, myopericarditis, Goodpasture's syndrome, and Reye's syndrome. Influenza infection also suppresses normal pulmonary antibacterial defenses, such that patient's recovering from influenza have an increased risk of developing bacterial pneumonia. Influenza viral surface proteins show marked antigenic variation, resulting from mutation and recombination. Thus, cytolytic T lymphocytes are the host's primary vehicle for the elimination of virus after infection. Influenza is classified into three primary types: A, B and C. Influenza A is unique in that it infects both humans and many other animals (*e.g.*, pigs, horses, birds and seals) and is the principal cause of pandemic influenza. Also, when a cell is infected by two different influenza A strains, the segmented RNA genomes of two parental virus types mix during replication to create a hybrid replicant, resulting in new epidemic strains. Influenza B does not replicate in animals and thus has less genetic variation and influenza C has only a single serotype.

Most conventional therapies are palliatives of the symptoms resulting from infection, while the host's immune response actually clears the disease. However, certain strains (*e.g.*, influenza A) can cause more serious illness and death. Influenza A may be treated both clinically and prophylactically by the administration of the cyclic amines inhibitors amantadine and rimantadine, which inhibit viral replication. However, the clinical utility of these drugs is limited due to the relatively high incidence of adverse reactions, their narrow anti-viral spectrum (influenza A only), and the propensity of the virus to become resistant. The administration of serum IgG antibody to the major influenza surface proteins, hemagglutinin and neuraminidase can prevent pulmonary infection, whereas mucosal IgA is required to prevent infection of the

upper respiratory tract and trachea. The most effective current treatment for influenza is vaccination with the administration of virus inactivated with formalin or β-propiolactone.

In another embodiment, the infection is a hepatitis infection, *e.g.*, a Hepatitis B or C infection.

Hepatitis B virus (HB-V) is the most infectious known bloodborne pathogen. It is a major cause of acute and chronic heptatis and hepatic carcinoma, as well as life-long, chronic infection. Following infection, the virus replicates in hepatocytes, which also then shed the surface antigen HBsAg. The detection of excessive levels of HBsAg in serum is used a standard method for diagnosing a hepatitis B infection. An acute infection may resolve or it can develop into a chronic persistent infection. Current treatments for chronic HBV include α-interferon, which increases the expression of class I human leukocyte antigen (HLA) on the surface of hepatocytes, thereby facilitating their recognition by cytotoxic T lymphocytes. Additionally, the nucleoside analogs ganciclovir, famciclovir and lamivudine have also shown some efficacy in the treatment of HBV infection in clinical trials. Additional treatments for HBV include pegylated a-interferon, adenfovir, entecavir and telbivudine. While passive immunity can be conferred through parental administration of anti-HBsAg serum antibodies, vaccination with inactivated or recombinant HBsAg also confers resistance to infection. The anti-PD-1 antibody molecules may be combined with conventional treatments for hepatitis B infections for therapeutic advantage.

Hepatitis C virus (HC-V) infection may lead to a chronic form of hepatitis, resulting in cirrosis. While symptoms are similar to infections resulting from Hepatitis B, in distinct contrast to HB-V, infected hosts can be asymptomatic for 10-20 years. The anti-PD-1 antibody molecule can be administered as a monotherapy, or combined with the standard of care for hepatitis C infection. For example, the anti-PD-1 antibody molecule can be administered with one or more of Sovaldi (sofosbuvir) Olysio (simeprevir), plus ribavirin or pegylated interferon. Although regimens that include Incivek (telaprevir) or Victrelis (boceprevir) plus ribavirin and pegylated interferon are also approved, they are associated with increased side effects and longer duration of treatment and are therefore not considered preferred regimens.

Conventional treatment for HC-V infection includes the administration of a combination of α-interferon and ribavirin. A promising potential therapy for HC-V infection is the protease inhibitor telaprevir (VX-960). Additional treatments include: anti-PD-1 antibody (MDX-1106, Medarex), bavituximab (an antibody that binds anionic phospholipid phosphatidylserine in a B2-

glycoprotein I dependent manner, Peregrine Pharmaceuticals), anti-HPV viral coat protein E2 antibod(y)(ies) (*e.g.*, ATL 6865–Ab68+Ab65, XTL Pharmaceuticals) and Civacir® (polyclonal anti-HCV human immune globulin). The anti-PD-L1 antibodies of the invention may be combined with one or more of these treatments for hepatitis C infections for therapeutic advantage. Protease, polymerase and NS5A inhibitors which may be used in combination with the anti-PD-1 antibody molecules to specifically treat Hepatitis C infection include those described in US 2013/0045202, incorporated herein by reference.

In another embodiment, the infection is a measles virus. After an incubation of 9-11 days, hosts infected with the measles virus develop fever, cough, coryza and conjunctivitis. Within 1-2 days, an erythematous, maculopapular rash develop, which quickly spreads over the entire body. Because infection also suppresses cellular immunity, the host is at greater risk for developing bacterial superinfections, including otitis media, pneumonia and postinfectious encephalomyelitis. Acute infection is associated with significant morbidity and mortality, especially in malnourished adolescents.

Treatment for measles includes the passive administration of pooled human IgG, which can prevent infection in non-immune subjects, even if given up to one week after exposure. However, prior immunization with live, attenuated virus is the most effective treatment and prevents disease in more than 95% of those immunized. As there is one serotype of this virus, a single immunization or infection typically results in protection for life from subsequent infection.

In a small proportion of infected hosts, measles can develop into SSPE, which is a chronic progressive neurologic disorder resulting from a persistent infection of the central nervous system. SSPE is caused by clonal variants of measles virus with defects that interfere with virion assembly and budding. For these patients, reactivation of T-cells with the anti-PD-1 antibody molecules so as to facilitate viral clearance would be desirable.

In another embodiment, the infection is HIV. HIV attacks $CD4^+$ cells, including T-lymphocytes, monocyte-macrophages, follicular dendritic cells and Langerhan's cells, and $CD4^+$ helper/inducer cells are depleted. As a result, the host acquires a severe defect in cell-mediated immunity. Infection with HIV results in AIDS in at least 50% of individuals, and is transmitted via sexual contact, administration of infected blood or blood products, artificial insemination with infected semen, exposure to blood-containing needles or syringes and transmission from an infected mother to infant during childbirth.

A host infected with HIV may be asymptomatic, or may develop an acute illness that resembling mononucleosis – fever, headache, sore throat, malaise and rash. Symptoms can progress to progressive immune dysfunction, including persistent fever, night sweats, weight loss, unexplained diarrhea, eczema, psoriasis, seborrheic dermatitis, herpes zoster, oral candidiasis and oral hairy leukoplakia. Opportunistic infections by a host of parasites are common in patients whose infections develop into AIDS.

Treatments for HIV include antiviral therapies including nucleoside analogs, zidovudine (AST) either alone or in combination with didanosine or zalcitabine, dideoxyinosine, dideoxycytidine, lamidvudine, stavudine; reverse transcriptive inhibitors such as delavirdine, nevirapine, loviride, and proteinase inhibitors such as saquinavir, ritonavir, indinavir and nelfinavir. The anti-PD-1 antibody molecules may be combined with conventional treatments for HIV infections for therapeutic advantage.

In another embodiment, the infection is a Cytomegalovirus (CMV). CMV infection is often associated with persistent, latent and recurrent infection. CMV infects and remains latent in monocytes and granulocyte-monocyte progenitor cells. The clinical symptoms of CMV include mononucleosis-like symptoms (*i.e.*, fever, swollen glands, malaise), and a tendancy to develop allergic skin rashes to antibiotics. The virus is spread by direct contact. The virus is shed in the urine, saliva, semen and to a lesser extent in other body fluids. Transmission can also occur from an infected mother to her fetus or newborn and by blood transfusion and organ transplants. CMV infection results in general impairment of cellular immunity, characterized by impaired blastogenic responses to nonspecific mitogens and specific CMV antigens, diminished cytotoxic ability and elevation of CD8 lymphocyte number of $CD4^+$ lymphocytes.

Treatments of CMV infection include the anti-virals ganciclovir, foscarnet and cidovir, but these druges are typically only prescribed in immunocompromised patients. The anti-PD-1 antibody molecules may be combined with conventional treatments for cytomegalovirus infections for therapeutic advantage.

In another embodiment, the infection is Epstein-Barr virus (EBV). EBV can establish persistent and latent infections and primarily attacks B cells. Infection with EBV results in the clinical condition of infectious mononucleosis, which includes fever, sore throat, often with exudate, generalized lymphadenopathy and splenomegaly. Hepatitis is also present, which can develop into jaundice.

While typical treatments for EBV infections are palliative of symptoms, EBV is associated with the development of certain cancers such as Burkitt's lymphoma and nasopharyngeal cancer. Thus, clearance of viral infection before these complications result would be of great benefit. The anti-PD-1 antibody molecules may be combined with conventional treatments for Epstein-Barr virus infections for therapeutic advantage.

In another embodiment, the infection is Herpes simplex virus (HSV). HSV is transmitted by direct contact with an infected host. A direct infection may be asymptomatic, but typically result in blisters containing infectious particles. The disease manifests as cycles of active periods of disease, in which lesions appear and disappear as the viral latently infect the nerve ganglion for subsequent outbreaks. Lesions may be on the face, genitals, eyes and/or hands. In some case, an infection can also cause encephalitis.

Treatments for herpes infections are directed primarily to resolving the symptomatic outbreaks, and include systemic antiviral medicines such as: acyclovir (*e.g.*, Zovirax®), valaciclovir, famciclovir, penciclovir, and topical medications such as docosanol (Abreva®), tromantadine and zilactin. The clearance of latent infections of herpes would be of great clinical benefit. The anti-PD-1 antibody molecules may be combined with conventional treatments for herpes virus infections for therapeutic advantage.

In another embodiment, the infection is Human T-lymphotrophic virus (HTLV-1, HTLV-2). HTLV is transmitted via sexual contact, breast feeding or exposure to contaminated blood. The virus activates a subset of T_H cells called Th1 cells, resulting in their overproliferation and overproduction of Th1 related cytokines (*e.g.*, IFN-γ and TNF-α). This in turn results in a suppression of Th2 lymphocytes and reduction of Th2 cytokine production (*e.g.*, IL-4, IL-5, IL-10 and IL-13), causing a reduction in the ability of an infected host to mount an adequate immune response to invading organisms requiring a Th2-dependent response for clearnance (*e.g.*, parasitic infections, production of mucosal and humoral antibodies).

HTLV infections cause lead to opportunistic infections resulting in bronchiectasis, dermatitis and superinfections with *Staphylococcus* spp. and *Strongyloides* spp. resulting in death from polymicrobial sepsis. HTLV infection can also lead directly to adult T-cell leukemia/lymphoma and progressive demyelinating upper motor neuron disease known as HAM/TSP. The clearance of HTLV latent infections would be of great clinical benefit. The anti-

PD-1 antibody molecules may be combined with conventional treatments for HTLV infections for therapeutic advantage.

In another embodiment, the infection is Human papilloma virus (HPV). HPV primarily affects keratinocytes and occurs in two forms: cutaneous and genital. Transmission is believed to occur through direct contact and/or sexual activity. Both cutaneous and genital HPV infection, can result in warts and latent infections and sometimes recurring infections, which are controlled by host immunity which controls the symptoms and blocks the appearance of warts, but leaves the host capable of transmitting the infection to others.

Infection with HPV can also lead to certain cancers, such as cervical, anal, vulvar, penile and oropharynial cancer. There are no known cures for HPV infection, but current treatment is topical application of Imiquimod, which stimulates the immune system to attack the affected area. The clearance of HPV latent infections would be of great clinical benefit. The anti-PD-L1 antibodies of the invention may be combined with conventional treatments for HPV infections for therapeutic advantage.

Bacterial Infections

Some examples of pathogenic bacteria causing infections treatable by methods of the invention include syphilis, *chlamydia*, *rickettsial bacteria*, *mycobacteria*, *staphylococci*, *streptococci*, *pneumonococci*, *meningococci and conococci*, *klebsiella*, *proteus*, *serratia*, *pseudomonas*, *legionella, diphtheria, salmonella, bacilli*, *cholera, tetanus*, *botulism*, *anthrax, plague, leptospirosis*, *and Lymes disease bacteria*. The anti-PD-1 antibody molecules can be used in combination with existing treatment modalities for the aforesaid infections. For example, Treatments for syphilis include penicillin (*e.g.*, penicillin G.), tetracycline, doxycycline, ceftriaxone and azithromycin.

Lyme disease, caused by *Borrelia burgdorferi* is transmitted into humans through tick bites. The disease manifests initially as a localized rash, followed by flu-like symptoms including malaise, fever, headache, stiff neck and arthralgias. Later manifestations can include migratory and polyarticular arthritis, neurologic and cardiac involvement with cranial nerve palsies and radiculopathy, myocarditis and arrhythmias. Some cases of Lyme disease become persistent, resulting in irreversible damage analogous to tertiary syphilis. Current therapy for Lyme disease includes primarily the administration of antibiotics. Antibiotic-resistant strains may be treated

with hydroxychloroquine or methotrexate. Antibiotic refractory patients with neuropathic pain can be treated with gabapentin. Minocycline may be helpful in late/chronic Lyme disease with neurological or other inflammatory manifestations.

Other forms of borreliois, such as those resulting from *B. recurentis, B. hermsii, B. turicatae, B. parikeri., B. hispanica, B. duttonii* and *B. persica*, as well leptospirosis (*E.g., L. interrogans*), typically resolve spontaneously unless blood titers reach concentrations to cause intrahepatic obstruction.

Fungi and Parasites

Some examples of pathogenic fungi causing infections treatable by methods of the invention include *Candida* (*albicans, krusei, glabrata, tropicalis, etc.*), *Cryptococcus neoformans, Aspergillus* (*fumigatus, niger, etc.*), *Genus Mucorales* (*mucor, absidia, rhizophus*), *Sporothrix schenkii, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Coccidioides immitis* and *Histoplasma capsulatum*.

Some examples of pathogenic parasites causing infections treatable by methods described herein include *Entamoeba histolytica, Balantidium coli, Naegleriafowleri, Acanthamoeba sp., Giardia lambia, Cryptosporidium sp., Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondi, and Nippostrongylus brasiliensis*.

Additional Combination Therapies

Combinations of PD-1 antibody molecules with one or more second therapeutics are provided herein. Many of the combinations in this section are useful in treating cancer, but other indications are also described. This section focuses on combinations of anti-PD-1 antibody molecules, optionally in combination with one or more immunomodulators (*e.g.*, an anti-TIM-3 antibody molecule, an anti-LAG-3 antibody molecule, or an anti-PD-L1 antibody molecule), with one or more of the agents described in Table 7. In the combinations herein below, in one embodiment, the anti-PD-1 antibody molecule includes:

(a) a heavy chain variable region (VH) comprising a VHCDR1 amino acid sequence of SEQ ID NO: 4, a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a light chain variable region (VL) comprising a VLCDR1 amino

acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 33;

(b) a VH comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 32;

(c) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 224, a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 33; or

(d) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 224; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 32.

In the combinations herein below, in another embodiment, the anti-PD-1 antibody molecule comprises (i) a heavy chain variable region (VH) comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1, SEQ ID NO: 4, or SEQ ID NO: 224; a VHCDR2 amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 5; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and (ii) a light chain variable region (VL) comprising a VLCDR1 amino acid sequence of SEQ ID NO: 10 or SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 11 or SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 32 or SEQ ID NO: 33.

In one embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with a PKC inhibitor, Sotrastaurin (Compound A1), or a compound disclosed in PCT Publication No. WO 2005/039549, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the PKC inhibitor is Sotrastaurin (Compound A1) or a compound disclosed in PCT Publication No. WO 2005/039549. In one embodiment, a PD-1 antibody molecule is used in combination with Sotrastaurin (Compound A1), or a

compound as described in PCT Publication No. WO 2005/039549, to treat a disorder such as a cancer, a melanoma, a non-Hodgkin lymphoma, an inflammatory bowel disease, transplant rejection, an ophthalmic disorder, or psoriasis.

In certain embodiments, Sotrastaurin (Compound A1) is administered at a dose of about 20 to 600 mg, *e.g.,* about 200 to about 600 mg, about 50 mg to about 450 mg, about 100 mg to 400 mg, about 150 mg to 350 mg, or about 200 mg to 300 mg, *e.g.,* about 50 mg, 100 mg, 150mg, 200 mg, 300 mg, 400 mg, 500 mg, or 600 mg. The dosing schedule can vary from *e.g.,* every other day to daily, twice or three times a day.

In one embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with a BCR-ABL inhibitor, TASIGNA (Compound A2), or a compound disclosed in PCT Publication No. WO 2004/005281, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the BCR-ABL inhibitor is TASIGNA, or a compound disclosed in PCT Publication No. WO 2004/005281. In one embodiment, a PD-1 antibody molecule is used in combination with TASIGNA (Compound A2), or a compound as described in PCT Publication No. WO 2004/005281, to treat a disorder such as a lymphocytic leukemia, Parkinson's Disease, a neurologic cancer, a melanoma, a digestive/gastrointestinal cancer, a colorectal cancer, a myeloid leukemia, a head and neck cancer, or pulmonary hypertension.

In one embodiment, the BCR-ABL inhibitor or TASIGNA is administered at a dose of about 300 mg (*e.g.*, twice daily, *e.g.*, for newly diagnosed Ph+ CML-CP), or about 400 mg, *e.g.*, twice daily, *e.g.*, for resistant or intolerant Ph+ CML-CP and CML-AP). BCR-ABL inhibitor or a Compound A2 is administered at a dose of about 300-400 mg.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with an HSP90 inhibitor, such as 5-(2,4-dihydroxy-5-isopropylphenyl)-N-ethyl-4-(4-(morpholinomethyl)phenyl)isoxazole-3-carboxamide (Compound A3), or a compound disclosed in PCT Publication No. WO 2010/060937 or WO 2004/072051, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the HSP90 inhibitor is 5-(2,4-dihydroxy-5-isopropylphenyl)-N-ethyl-4-(4-(morpholinomethyl)phenyl)isoxazole-3-carboxamide (Compound A3), or a compound disclosed

in PCT Publication No. WO 2010/060937 or WO 2004/072051. In one embodiment, a PD-1 antibody molecule is used in combination with 5-(2,4-dihydroxy-5-isopropylphenyl)-N-ethyl-4-(4-(morpholinomethyl)phenyl)isoxazole-3-carboxamide (Compound A3), or a compound as described in PCT Publication No. WO 2010/060937 or WO 2004/072051, to treat a disorder such as a cancer, a multiple myeloma, a non-small cell lung cancer, a lymphoma, a gastric cancer, a breast cancer, a digestive/gastrointestinal cancer, a pancreatic cancer, a colorectal cancer, a solid tumor, or a hematopoiesis disorder.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with an inhibitor of PI3K and/or mTOR, Dactolisib (Compound A4) or 8-(6-Methoxy-pyridin-3-yl)-3-methyl-1-(4-piperazin-1-yl-3-trifluoromethyl-phenyl)-1,3-dihydro-imidazo[4,5-c]quinolin-2-one (Compound A41), or a compound disclosed in PCT Publication No. WO 2006/122806, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the PI3K and/or mTOR inhibitor is Dactolisib (Compound A4), 8-(6-Methoxy-pyridin-3-yl)-3-methyl-1-(4-piperazin-1-yl-3-trifluoromethyl-phenyl)-1,3-dihydro-imidazo[4,5-c]quinolin-2-one (Compound A41), or a compound disclosed in PCT Publication No. WO 2006/122806. In one embodiment, a PD-1 antibody molecule is used in combination with Dactolisib (Compound A4), 8-(6-Methoxy-pyridin-3-yl)-3-methyl-1-(4-piperazin-1-yl-3-trifluoromethyl-phenyl)-1,3-dihydro-imidazo[4,5-c]quinolin-2-one (Compound A41), or a compound described in PCT Publication No. WO 2006/122806 , to treat a disorder such as a cancer, a prostate cancer, a leukemia (*e.g.*, lymphocytic leukemia), a breast cancer, a brain cancer, a bladder cancer, a pancreatic cancer, a renal cancer, a solid tumor, or a liver cancer.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with an FGFR inhibitor, 3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-(6-((4-(4-ethylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)-1-methylurea (Compound A5) or a compound disclosed in US Patent 8,552,002, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the FGFR inhibitor is 3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-(6-((4-(4-ethylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)-1-methylurea (Compound A5) or a compound disclosed in US Patent 8,552,002. In one embodiment, a PD-1 antibody molecule is used in combination with Compound A5, or a compound as described in

US 8,552,002, to treat a disorder such as a digestive/gastrointestinal cancer, a hematological cancer, or a solid tumor.

In one embodiment, the FGFR inhibitor or 3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-(6-((4-(4-ethylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)-1-methylurea (Compound A5) is administered at a dose of about 100-125 mg (*e.g.*, per day), *e.g.*, about 100 mg or about 125 mg.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with a PI3K inhibitor, Buparlisib (Compound A6), or a compound disclosed in PCT Publication No. WO 2007/084786, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the PI3K inhibitor is Buparlisib (Compound A6) or a compound disclosed in PCT Publication No. WO 2007/084786. In one embodiment, a PD-1 antibody molecule is used in combination with Buparlisib (Compound A6), or a compound disclosed in PCT Publication No. WO 2007/084786, to treat a disorder such as, a prostate cancer, a non-small cell lung cancer, an endocrine cancer, a leukemia, an ovarian cancer, a melanoma, a bladder cancer, a breast cancer, a female reproductive system cancer, a digestive/gastrointestinal cancer, a colorectal cancer, a glioblastoma multiforme, a solid tumor, a non-Hodgkin lymphoma, a hematopoiesis disorder, or a head and neck cancer.

In one embodiment, the PI3K inhibitor or Buparlisib (Compound A6) is administered at a dose of about 100 mg (*e.g.*, per day).

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with an FGFR inhibitor, 8-(2,6-difluoro-3,5-dimethoxyphenyl)-N-(4-((dimethylamino)methyl)-1H-imidazol-2-yl)quinoxaline-5-carboxamide (Compound A7) or a compound disclosed in PCT Publication No. WO 2009/141386 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the FGFR inhibitor is 8-(2,6-difluoro-3,5-dimethoxyphenyl)-N-(4-((dimethylamino)methyl)-1H-imidazol-2-yl)quinoxaline-5-carboxamide(Compound A7) or a compound disclosed in a PCT Publication No. WO 2009/141386. In one embodiment, the FGFR inhibitor is 8-(2,6-difluoro-3,5-dimethoxyphenyl)-N-(4-((dimethylamino)methyl)-1H-imidazol-2-yl)quinoxaline-5-carboxamide(Compound A7). In one embodiment, a PD-1 antibody molecule is used in combination with 8-(2,6-difluoro-3,5-dimethoxyphenyl)-N-(4-((dimethylamino)methyl)-1H-imidazol-2-yl)quinoxaline-5-

carboxamide(Compound A7), or a compound disclosed in PCT Publication No. WO 2009/141386, to treat a disorder such as a cancer characterized by angiogenesis.

In one embodiment, the FGFR inhibitor or 8-(2,6-difluoro-3,5-dimethoxyphenyl)-N-(4-((dimethylamino)methyl)-1H-imidazol-2-yl)quinoxaline-5-carboxamide (Compound A7) is administered at a dose of *e.g.*, from approximately 3 mg to approximately 5 g, more preferably from approximately 10 mg to approximately 1.5 g per person per day, optionally divided into 1 to 3 single doses which may, for example, be of the same size.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with a PI3K inhibitor, (S)-N1-(4-methyl-5-(2-(1,1,1-trifluoro-2-methylpropan-2-yl)pyridin-4-yl)thiazol-2-yl)pyrrolidine-1,2-dicarboxamide (Compound A8) or a compound disclosed PCT Publication No. WO 2010/029082 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the PI3K inhibitor is (S)-N1-(4-methyl-5-(2-(1,1,1-trifluoro-2-methylpropan-2-yl)pyridin-4-yl)thiazol-2-yl)pyrrolidine-1,2-dicarboxamide (Compound A8) or a compound disclosed PCT Publication No. WO 2010/029082. In one embodiment, a PD-1 antibody molecule is used in combination with (S)-N1-(4-methyl-5-(2-(1,1,1-trifluoro-2-methylpropan-2-yl)pyridin-4-yl)thiazol-2-yl)pyrrolidine-1,2-dicarboxamide (Compound A8), or a compound disclosed PCT Publication No. WO 2010/029082, to treat a disorder such as a gastric cancer, a breast cancer, a pancreatic cancer, a digestive/ gastrointestinal cancer, a solid tumor, and a head and neck cancer.

In one embodiment, the PI3K inhibitor or (S)-N1-(4-methyl-5-(2-(1,1,1-trifluoro-2-methylpropan-2-yl)pyridin-4-yl)thiazol-2-yl)pyrrolidine-1,2-dicarboxamide (Compound A8) is administered at a dose of about 150-300, 200-300, 200-400, or 300-400 mg (*e.g.*, per day), *e.g.*, about 200, 300, or 400 mg.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with an inhibitor of cytochrome P450 (*e.g.*, a CYP17 inhibitor) or a compound disclosed in PCT Publication No. WO 2010/149755, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the cytochrome P450 inhibitor (*e.g.*, the CYP17 inhibitor) is a compound disclosed in PCT Publication No. WO 2010/149755. In one

embodiment, a PD-1 antibody molecule is used in combination with a compound disclosed in PCT Publication No. WO 2010/149755, to treat prostate cancer.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with an HDM2 inhibitor, (S)-1-(4-chlorophenyl)-7-isopropoxy-6-methoxy-2-(4-(methyl(((1r,4S)-4-(4-methyl-3-oxopiperazin-1-yl)cyclohexyl)methyl)amino)phenyl)-1,2-dihydroisoquinolin-3(4H)-one(Compound A10) or a compound disclosed in PCT Publication No. WO 2011/076786 to treat a disorder, *e.g.*, a disorder described herein). In one embodiment, the HDM2 inhibitor is (S)-1-(4-chlorophenyl)-7-isopropoxy-6-methoxy-2-(4-(methyl(((1r,4S)-4-(4-methyl-3-oxopiperazin-1-yl)cyclohexyl)methyl)amino)phenyl)-1,2-dihydroisoquinolin-3(4H)-one (Compound A10) or a compound disclosed in PCT Publication No. WO 2011/076786. In one embodiment, a PD-1 antibody molecule is used in combination with (S)-1-(4-chlorophenyl)-7-isopropoxy-6-methoxy-2-(4-(methyl(((1r,4S)-4-(4-methyl-3-oxopiperazin-1-yl)cyclohexyl)methyl)amino)phenyl)-1,2-dihydroisoquinolin-3(4H)-one (Compound A10), or a compound disclosed in PCTPublication No. WO 2011/076786, to treat a disorder such as a solid tumor.

In one embodiment, the HDM2 inhibitor or (S)-1-(4-chlorophenyl)-7-isopropoxy-6-methoxy-2-(4-(methyl(((1r,4S)-4-(4-methyl-3-oxopiperazin-1-yl)cyclohexyl)methyl)amino)phenyl)-1,2-dihydroisoquinolin-3(4H)-one (Compound A10) is administered at a dose of about 400 to 700 mg, *e.g.*, administered three times weekly, 2 weeks on and one week off. In some embodiments, the dose is about 400, 500, 600, or 700 mg; about 400-500, 500-600, or 600-700 mg, *e.g.*, administered three times weekly.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with an iron chelating agent, Deferasirox (also known as EXJADE; Compound A11), or a compound disclosed in PCT Publication No. WO 1997/049395 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the iron chelating agent is Deferasirox or a compound disclosed in PCT Publication No. WO 1997/049395. In one embodiment, the iron chelating agent is Deferasirox (Compound A11). In one embodiment, a PD-1 antibody molecule is used in combination with Deferasirox (Compound

A11), or a compound disclosed in PCT Publication No. WO 1997/049395, to treat iron overload, hemochromatosis, or myelodysplasia.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with an aromatase inhibitor, Letrozole (also known as FEMARA; Compound A12), or a compound disclosed in US 4,978,672 to treat a disorder, *e.g.,* a disorder described herein. In one embodiment, the aromatase inhibitor is Letrozole (Compound A12) or a compound disclosed in US Patent 4,978,672. In one embodiment, a PD-1 antibody molecule is used in combination with Letrozole (Compound A12), or a compound disclosed in US Patent 4,978,672, to treat a disorder such as a cancer, a leiomyosarcoma, an endometrium cancer, a breast cancer, a female reproductive system cancer, or a hormone deficiency.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with a PI3K inhibitor, *e.g.*, a pan-PI3K inhibitor, (4S,5R)-3-(2'-amino-2-morpholino-4'-(trifluoromethyl)-[4,5'-bipyrimidin]-6-yl)-4-(hydroxymethyl)-5-methyloxazolidin-2-one (Compound A13) or a compound disclosed in PCT Publication No. WO2013/124826 to treat a disorder, *e.g.,* a disorder described herein. In one embodiment, the PI3K inhibitor is (4S,5R)-3-(2'-amino-2-morpholino-4'-(trifluoromethyl)-[4,5'-bipyrimidin]-6-yl)-4-(hydroxymethyl)-5-methyloxazolidin-2-one (Compound A13) or a compound disclosed in PCT Publication No. WO2013/124826. In one embodiment, a PD-1 antibody molecule is used in combination with (4S,5R)-3-(2'-amino-2-morpholino-4'-(trifluoromethyl)-[4,5'-bipyrimidin]-6-yl)-4-(hydroxymethyl)-5-methyloxazolidin-2-one (Compound A13), or a compound disclosed in PCT Publication No. WO2013/124826, to treat a disorder such as a cancer or an advanced solid tumor.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with an inhibitor of p53 and/or a p53/Mdm2 interaction, (S)-5-(5-chloro-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)-6-(4-chlorophenyl)-2-(2,4-dimethoxypyrimidin-5-yl)-1-isopropyl-5,6-dihydropyrrolo[3,4-d]imidazol-4(1H)-one (Compound A14), or a compound disclosed in PCT Publication No. WO2013/111105 to treat a

disorder, *e.g.*, a disorder described herein. In one embodiment, the p53 and/or a p53/Mdm2 interaction inhibitor is (S)-5-(5-chloro-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)-6-(4-chlorophenyl)-2-(2,4-dimethoxypyrimidin-5-yl)-1-isopropyl-5,6-dihydropyrrolo[3,4-d]imidazol-4(1H)-one (Compound A14) or a compound disclosed in PCT Publication No. WO2013/111105. In one embodiment, a PD-1 antibody molecule is used in combination with (S)-5-(5-chloro-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)-6-(4-chlorophenyl)-2-(2,4-dimethoxypyrimidin-5-yl)-1-isopropyl-5,6-dihydropyrrolo[3,4-d]imidazol-4(1H)-one (Compound A14), or a compound disclosed in PCT Publication No. WO2013/111105, to treat a disorder such as a cancer or a soft tissue sarcoma.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with a CSF-1R tyrosine kinase inhibitor, 4-((2-(((1R,2R)-2-hydroxycyclohexyl)amino)benzo[d]thiazol-6-yl)oxy)-N-methylpicolinamide (Compound A15), or a compound disclosed in PCT Publication No. WO 2005/073224 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the CSF-1R tyrosine kinase inhibitor is 4-((2-(((1R,2R)-2-hydroxycyclohexyl)amino)benzo[d]thiazol-6-yl)oxy)-N-methylpicolinamide (Compound A15) or a compound disclosed in PCT Publication No. WO 2005/073224. In one embodiment, a PD-1 antibody molecule is used in combination with 4-((2-(((1R,2R)-2-hydroxycyclohexyl)amino)benzo[d]thiazol-6-yl)oxy)-N-methylpicolinamide (Compound A15) or a compound disclosed in PCT Publication No. WO 2005/073224, to treat a disorder such as cancer.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with an apoptosis inducer and/or an angiogenesis inhibitor, such as Imatinib mesylate (also known as GLEEVEC; Compound A16) or a compound disclosed in PCT Publication No. WO1999/003854 to treat a disorder, *e.g.*, a disorder described. In one embodiment, the apoptosis inducer and/or an angiogenesis inhibitor is Imatinib mesylate (Compound A16) or a compound disclosed in PCT Publication No. WO1999/003854. In one embodiment, a PD-1 antibody molecule is used in combination with Imatinib mesylate (Compound A16), or a compound disclosed in PCT Publication No. WO1999/003854, to treat a disorder such as a cancer, a multiple myeloma, a prostate cancer, a non-small cell lung cancer, a

lymphoma, a gastric cancer, a melanoma, a breast cancer, a pancreatic cancer, a digestive/gastrointestinal cancer, a colorectal cancer, a glioblastoma multiforme, a liver cancer, a head and neck cancer, asthma, multiple sclerosis, allergy, Alzheimer's dementia, amyotrophic lateral sclerosis, or rheumatoid arthritis.

In certain embodiments, Imatinib mesylate (Compound A16) is administered at a dose of about 100 to 1000 mg, *e.g.,* about 200 mg to 800 mg, about 300 mg to 700 mg, or about 400 mg to 600 mg, *e.g.,* about 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, or 700 mg. The dosing schedule can vary from *e.g.,* every other day to daily, twice or three times a day. In one embodiment, Imatinib mesylate is administered at an oral dose from about 100 mg to 600 mg daily, *e.g.*, about 100 mg, 200 mg, 260 mg, 300 mg, 400 mg, or 600 mg daily.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with a JAK inhibitor, 2-fluoro-N-methyl-4-(7-(quinolin-6-ylmethyl)imidazo[1,2-b][1,2,4]triazin-2-yl)benzamide (Compound A17), or a dihydrochloric salt thereof, or a compound disclosed in PCT Publication No. WO 2007/070514, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the JAK inhibitor is 2-fluoro-N-methyl-4-(7-(quinolin-6-ylmethyl)imidazo[1,2-b][1,2,4]triazin-2-yl)benzamide (Compound A17), or a dihydrochloric salt thereof, or a compound disclosed in PCT Publication No. WO 2007/070514. In one embodiment, a PD-1 antibody molecule is used in combination with 2-fluoro-N-methyl-4-(7-(quinolin-6-ylmethyl)imidazo[1,2-b][1,2,4]triazin-2-yl)benzamide (Compound A17), or a dihydrochloric salt thereof, or a compound disclosed in PCT Publication No. WO 2007/070514, to treat a disorder such as colorectal cancer, myeloid leukemia, hematological cancer, autoimmune disease, non-Hodgkin lymphoma, or thrombocythemia.

In one embodiment, the JAK inhibitor or a 2-fluoro-N-methyl-4-(7-(quinolin-6-ylmethyl)imidazo[1,2-b][1,2,4]triazin-2-yl)benzamide (Compound A17), or a dihydrochloric salt thereof is administered at a dose of about 400-600 mg (*e.g.*, per day), *e.g.*, about 400, 500, or 600 mg, or about 400-500 or 500-600 mg.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with a JAK inhibitor, Ruxolitinib Phosphate (also known as JAKAFI; Compound A18) or a compound disclosed in PCT Publication No. WO

2007/070514 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the JAK inhibitor is Ruxolitinib Phosphate (Compound A18) or a compound disclosed in PCT Publication No. WO 2007/070514. In one embodiment, a PD-1 antibody molecule is used in combination with Ruxolitinib Phosphate (Compound A18), or a compound disclosed in PCT Publication No. WO 2007/070514, to treat a disorder such as a prostate cancer, a lymphocytic leukemia, a multiple myeloma, a lymphoma, a lung cancer, a leukemia, cachexia, a breast cancer, a pancreatic cancer, rheumatoid arthritis, psoriasis, a colorectal cancer, a myeloid leukemia, a hematological cancer, an autoimmune disease, a non-Hodgkin lymphoma, or thrombocythemia.

In one embodiment, the JAK inhibitor or Ruxolitinib Phosphate (Compound A18) is administered at a dose of about 15-25 mg, *e.g.*, twice daily. In some embodiments, the dose is about 15, 20, or 25 mg, or about 15-20 or 20-25 mg.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with a deacetylase (DAC) inhibitor, Panobinostat (Compound A19), or a compound disclosed in PCT Publication No. WO 2014/072493 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the DAC inhibitor is Panobinostat (Compound A19) or a compound disclosed in PCT Publication No. WO 2014/072493. In one embodiment, a PD-1 antibody molecule is used in combination with Panobinostat (Compound A19), a compound disclosed in PCT Publication No. WO 2014/072493, to treat a disorder such as a small cell lung cancer, a respiratory/thoracic cancer, a prostate cancer, a multiple myeloma, myelodysplastic syndrome, a bone cancer, a non-small cell lung cancer, an endocrine cancer, a lymphoma, a neurologic cancer, a leukemia, HIV/AIDS, an immune disorder, transplant rejection, a gastric cancer, a melanoma, a breast cancer, a pancreatic cancer, a colorectal cancer, a glioblastoma multiforme, a myeloid leukemia, a hematological cancer, a renal cancer, a non-Hodgkin lymphoma, a head and neck cancer, a hematopoiesis disorders, or a liver cancer.

In one embodiment, the DAC inhibitor or Panobinostat (Compound A19) is administered at a dose of about 20 mg (*e.g.*, per day).

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with an inhibitor of one or more of cytochrome P450

(*e.g.*, 11B2), aldosterone or angiogenesis, Osilodrostat (Compound A20), or a compound disclosed in PCT Publication No. WO2007/024945 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the inhibitor of one or more of cytochrome P450 (*e.g.*, 11B2), aldosterone or angiogenesis is Osilodrostat (Compound A20) or a compound disclosed in PCT Publication No. WO2007/024945. In one embodiment, a PD-1 antibody molecule is used in combination with Osilodrostat (Compound A20), or a compound disclosed in PCT Publication No. WO2007/024945, to treat a disorder such as Cushing's syndrome, hypertension, or heart failure therapy.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with a IAP inhibitor, (S)-N-((S)-1-cyclohexyl-2-((S)-2-(4-(4-fluorobenzoyl)thiazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)-2-(methylamino)propanamide (Compound A21) or a compound disclosed in US 8,552,003 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the IAP inhibitor is (S)-N-((S)-1-cyclohexyl-2-((S)-2-(4-(4-fluorobenzoyl)thiazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)-2-(methylamino)propanamide (Compound A21) or a compound disclosed in US Patent 8,552,003. In one embodiment, a PD-1 antibody molecule is used in combination with (S)-N-((S)-1-cyclohexyl-2-((S)-2-(4-(4-fluorobenzoyl)thiazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)-2-(methylamino)propanamide (Compound A21), or a compound disclosed in US Patent 8,552,003, to treat a disorder such as a multiple myeloma, a breast cancer, an ovarian cancer, a pancreatic cancer, or a hematopoiesis disorder.

In one embodiment, the IAP inhibitor or (S)-N-((S)-1-cyclohexyl-2-((S)-2-(4-(4-fluorobenzoyl)thiazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)-2-(methylamino)propanamide (Compound A21) or a compound disclosed in US 8,552,003 is administered at a dose of approximately 1800 mg, *e.g.*, once weekly.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination a Smoothened (SMO) inhibitor, Sonidegib phosphate (Compound A22), (R)-2-(5-(4-(6-benzyl-4,5-dimethylpyridazin-3-yl)-2-methylpiperazin-1-yl)pyrazin-2-yl)propan-2-ol (Compound A25), or a compound disclosed in PCT Publication No. WO 2007/131201 or WO 2010/007120 to treat a disorder, *e.g.*, a disorder described herein. In

one embodiment, the SMO inhibitor is Sonidegib phosphate (Compound A22), (R)-2-(5-(4-(6-benzyl-4,5-dimethylpyridazin-3-yl)-2-methylpiperazin-1-yl)pyrazin-2-yl)propan-2-ol (Compound A25), or a compound disclosed in PCT Publication No. WO 2007/131201 or WO 2010/007120. In one embodiment, a PD-1 antibody molecule is used in combination with Sonidegib phosphate (Compound A22), (R)-2-(5-(4-(6-benzyl-4,5-dimethylpyridazin-3-yl)-2-methylpiperazin-1-yl)pyrazin-2-yl)propan-2-ol (Compound A25), or a compound disclosed in PCT Publication No. WO 2007/131201 or WO 2010/007120 to treat a disorder such as a cancer, a medulloblastoma, a small cell lung cancer, a prostate cancer, a basal cell carcinoma, a pancreatic cancer, or an inflammation.

In certain embodiments, Sonidegib phosphate (Compound A22) is administered at a dose of about 20 to 500 mg, *e.g.,* about 40 mg to 400 mg, about 50 mg to 300 mg, or about 100 mg to 200 mg, *e.g.,* about 50 mg, 100 mg, 150 mg, 200 mg, 250 mg, or 300 mg. The dosing schedule can vary from *e.g.,* every other day to daily, twice or three times a day.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with an Alk inhibitor, ceritinib (also known as ZYKADIA; Compound A23) or a compound disclosed in PCT Publication No. WO 2007/131201 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the Alk inhibitor is ceritinib (Compound A23) or a compound disclosed in PCT Publication No. WO 2007/131201. In one embodiment, a PD-1 antibody molecule is used in combination with ceritinib (Compound A23), or a compound disclosed in PCT Publication No. WO 2007/131201, to treat a disorder such as non-small cell lung cancer or solid tumors.

In one embodiment, the Alk inhibitor or ceritinib (Compound A23) is administered at a dose of approximately 750 mg, *e.g.*, once daily.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with a JAK and/or CDK4/6 inhibitor, 7-cyclopentyl-N,N-dimethyl-2-((5-(piperazin-1-yl)pyridin-2-yl)amino)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxamide (Compound A24), or a compound disclosed in US Patent 8,415,355 or US Patent 8,685,980 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the JAK and/or CDK4/6 inhibitor is 7-cyclopentyl-N,N-dimethyl-2-((5-(piperazin-1-yl)pyridin-2-

yl)amino)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxamide (Compound A24) or a compound disclosed in US Patent 8,415,355 or US Patent 8,685,980. In one embodiment, a PD-1 antibody molecule is used in combination with 7-cyclopentyl-N,N-dimethyl-2-((5-(piperazin-1-yl)pyridin-2-yl)amino)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxamide (Compound A24), or a compound disclosed in US 8,415,355 or US 8,685,980, to treat a disorder such as a lymphoma, a neurologic cancer, a melanoma, a breast cancer, or a solid tumor.

In one embodiment, the JAK and/or CDK4/6 inhibitor or 7-cyclopentyl-N,N-dimethyl-2-((5-(piperazin-1-yl)pyridin-2-yl)amino)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxamide (Compound A24) is administered at a dose of approximately 200-600 mg, *e.g.*, per day. In one embodiment, the compound is administered at a dose of about 200, 300, 400, 500, or 600 mg, or about 200-300, 300-400, 400-500, or 500-600 mg.

In another embodiment, the antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination a prolactin receptor (PRLR) inhibitor, a human monoclonal antibody molecule (Compound A26) as disclosed in US Patent 7,867,493), to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the PRLR inhibitor is a human monoclonal antibody (Compound A26) disclosed in US 7,867,493. In one embodiment, a PD-1 antibody molecule is used in combination with human monoclonal antibody molecule (Compound A26) described in US Patent 7,867,493 to treat a disorder such as, a cancer, a prostate cancer, or a breast cancer.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with a PIM Kinase inhibitor, N-(4-((1R,3S,5S)-3-amino-5-methylcyclohexyl)pyridin-3-yl)-6-(2,6-difluorophenyl)-5-fluoropicolinamide (Compound A27) or a compound disclosed in PCT Publication No. WO 2010/026124 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the PIM Kinase inhibitor is N-(4-((1R,3S,5S)-3-amino-5-methylcyclohexyl)pyridin-3-yl)-6-(2,6-difluorophenyl)-5-fluoropicolinamide (Compound A27) or a compound disclosed in PCT Publication No. WO 2010/026124. In one embodiment, a PD-1 antibody molecule is used in combination with N-(4-((1R,3S,5S)-3-amino-5-methylcyclohexyl)pyridin-3-yl)-6-(2,6-difluorophenyl)-5-fluoropicolinamide (Compound A27), or a compound disclosed in PCT Publication No. WO

2010/026124, to treat a disorder such as a multiple myeloma, myelodysplastic syndrome, a myeloid leukemia, or a non-Hodgkin lymphoma.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination a Wnt signaling inhibitor, 2-(2',3-dimethyl-[2,4'-bipyridin]-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide (Compound A28) or a compound disclosed in PCT publication No. WO 2010/101849 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the Wnt signaling inhibitor is 2-(2',3-dimethyl-[2,4'-bipyridin]-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide (Compound A28) or a compound disclosed in PCT publication No. WO 2010/101849. In one embodiment, the Wnt signaling inhibitor is 2-(2',3-dimethyl-[2,4'-bipyridin]-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide (Compound A28). In one embodiment, a PD-1 antibody molecule is used in combination with 2-(2',3-dimethyl-[2,4'-bipyridin]-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide (Compound A28), or a compound disclosed in PCT publication No. WO 2010/101849, to treat a disorder such as a solid tumor (*e.g.*, a head and neck cancer, a squamous cell carcinoma, a breast cancer, a pancreatic cancer, or a colon cancer).

In certain embodiments, 2-(2',3-dimethyl-[2,4'-bipyridin]-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide (Compound A28) is administered at a dose of about 1 to 50 mg, *e.g.*, about 2 mg to 45 mg, about 3 mg to 40 mg, about 5 mg to 35 mg, 5 mg to 10 mg, or about 10 mg to 30 mg, *e.g.*, about 2 mg, 5 mg, 10 mg, 20 mg, 30 mg, or 40 mg. The dosing schedule can vary from *e.g.*, every other day to daily, twice or three times a day.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with a BRAF inhibitor, Encorafenib (Compound A29), or a compound disclosed in PCT Publication No. WO 2011/025927 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the BRAF inhibitor is Encorafenib (Compound A29) or a compound disclosed in PCT Publication No. WO 2011/025927. In one embodiment, a PD-1 antibody molecule is used in combination with Encorafenib (Compound A29), or a compound disclosed in PCT Publication No. WO 2011/025927, to treat a disorder such as a non-small cell lung cancer, a melanoma, or a colorectal cancer.

In one embodiment, the BRAF inhibitor or Encorafenib (Compound A29) is administered at a dose of about 200-300, 200-400, or 300-400 mg, *e.g.*, per day. In one embodiment, the compound is administered at a dose of about 200, about 300 or about 400 mg.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination a CDK4/6 inhibitor, 7-cyclopentyl-N,N-dimethyl-2-((5-((1R,6S)-9-methyl-4-oxo-3,9-diazabicyclo[4.2.1]nonan-3-yl)pyridin-2-yl)amino)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxamide (Compound A30), or a compound disclosed in PCT publication No. WO 2011/101409 to treat a disorder, *e.g.,* a disorder described herein. In one embodiment, the CDK4/6 inhibitor is 7-cyclopentyl-N,N-dimethyl-2-((5-((1R,6S)-9-methyl-4-oxo-3,9-diazabicyclo[4.2.1]nonan-3-yl)pyridin-2-yl)amino)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxamide (Compound A30) or a compound disclosed in PCT publication No. WO 2011/101409. In one embodiment, a PD-1 antibody molecule is used in combination with 7-cyclopentyl-N,N-dimethyl-2-((5-((1R,6S)-9-methyl-4-oxo-3,9-diazabicyclo[4.2.1]nonan-3-yl)pyridin-2-yl)amino)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxamide (Compound A30), or a compound disclosed in PCT publication No. WO 2011/101409, to treat a disorder such as a cancer, a mantle cell lymphoma, a liposarcoma, a non-small cell lung cancer, a melanoma, a squamous cell esophageal cancer, or a breast cancer.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with a HER3 inhibitor, Compound A31, or a compound disclosed in PCT Publication No. WO 2012/022814, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the HER3 inhibitor is Compound A31 or a compound disclosed in PCT Publication WO 2012/022814. In one embodiment, a PD-1 antibody molecule is used in combination with Compound A31, or a compound disclosed in PCT Publication WO 2012/022814, to treat a disorder such as a gastric cancer, an esophageal cancer, a head and neck cancer, a squamous cell carcinoma, a stomach cancer, a breast cancer (*e.g.*, metastatic breast cancer), or a digestive/gastrointestinal cancer.

In some embodiments, Compound A31 is a human monoclonal antibody molecule.

In one embodiment, the HER3 inhibitor or Compound A31 is administered at a dose of about 3, 10, 20, or 40 mg/kg, *e.g.*, once weekly (QW). In one embodiment, the compound is administered at a dose of about 3-10, 10-20, or 20-40 mg/kg.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination an FGFR2 and/or FGFR4 inhibitor, Compound A32, or a compound disclosed in a publication PCT Publication No. WO 2014/160160 (*e.g.*, an antibody molecule drug conjugate against an FGFR2 and/or FGFR4, *e.g.*, mAb 12425), to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the FGFR2 and/or FGFR4 inhibitor is Compound A32 or a compound disclosed in a publication PCT Publication No. WO 2014/160160. In one embodiment, a PD-1 antibody molecule is used in combination with Compound A32, or a compound as described in Table 7, to treat a disorder such as a cancer, a gastric cancer, a breast cancer, a rhabdomyosarcoma, a liver cancer, an adrenal cancer, a lung cancer, an esophageal cancer, a colon cancer, or an endometrial cancer.

In some embodiments, Compound A32 is an antibody molecule drug conjugate against an FGFR2 and/or FGFR4, *e.g.*, mAb 12425.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination an M-CSF inhibitor, Compound A33, or a compound disclosed in PCT Publication No. WO 2004/045532 (*e.g.*, an antibody molecule or Fab fragment against M-CSF), to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the M-CSF inhibitor is Compound A33 or a compound disclosed in PCT Publication No. WO 2004/045532. In one embodiment, a PD-1 antibody molecule is used in combination with Compound A33, or a compound as described in PCT Publication No. WO 2004/045532, to treat a disorder such as a cancer, a prostate cancer, a breast cancer, or pigmented villonodular synovitis (PVNS).

In embodiments, Compound A33 is a monoclonal antibody molecule against M-CSF or a fragment (*e.g.*, Fab fragment) thereof. In embodiments, the M-CSF inhibitor or Compound A33 is administered at an average dose of about 10mg/kg.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other

immunomodulators, is used in combination with a MEK inhibitor, Binimetinib (Compound A34), or a compound disclosed in PCT Publication No. WO 2003/077914 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the MEK inhibitor is Binimetinib (Compound A34), or a compound disclosed in PCT Publication No. WO 2003/077914. In one embodiment, a PD-1 antibody molecule is used in combination with Binimetinib (Compound A34), or a compound disclosed in PCT Publication No. WO 2003/077914, to treat a disorder such as a non-small cell lung cancer, a multisystem genetic disorder, a melanoma, an ovarian cancer, a digestive/gastrointestinal cancer, a rheumatoid arthritis, or a colorectal cancer.

In one embodiment, the MEK inhibitor or Binimetinib (Compound A34) is administered at a dose of about 45 mg, *e.g.*, twice daily.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination an inhibitor of one or more of c-KIT, histamine release, Flt3 (*e.g.*, FLK2/STK1) or PKC, Midostaurin (Compound A35) or a compound disclosed in PCT Publication No. WO 2003/037347 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the inhibitor is Midostaurin (Compound A35) or compound disclosed in PCT Publication No. WO 2003/037347. In one embodiment, the inhibitor of one or more of c-KIT, histamine release, Flt3 (*e.g.*, FLK2/STK1) or PKC is Midostaurin. In one embodiment, a PD-1 antibody molecule is used in combination with Midostaurin (Compound A35), or compound disclosed in PCT Publication No. WO 2003/037347, to treat a disorder such as a cancer, a colorectal cancer, a myeloid leukemia, myelodysplastic syndrome, an age-related mascular degeration, a diabetic complication, or a dermatologic disorder.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with a TOR inhibitor (*e.g.*, mTOR inhibitor), Everolimus (also known as AFINITOR; Compound A36) or a Compound disclosed in PCT Publication No. WO 2014/085318 to treat a disorder, *e.g.*, a disorder described herein). In one embodiment, the TOR inhibitor is Everolimus (Compound A36) or a Compound disclosed in PCT Publication No. WO 2014/085318. In one embodiment, a PD-1 antibody molecule is used in combination with Everolimus (Compound A36) to treat a disorder such as an interstitial lung disease, a small cell lung cancer, a respiratory/thoracic cancer, a prostate cancer, a multiple

myeloma, a sarcoma, an age-related macular degeneration,a bone cancer, tuberous sclerosis, a non-small cell lung cancer, an endocrine cancer, a lymphoma, a neurologic disorders, an astrocytoma, a cervical cancer, a neurologic cancer, a leukemia, an immune disorders, transplant rejection, a gastric cancer, a melanoma, epilepsy, a breast cancer, or a bladder cancer.

In one embodiment, the TOR inhibitor or Everolimusis (Compound A36) administered at a dose of about 2.5-20 mg/day. In one embodiment, the compound is administered at a dose of about 2.5, 5, 10, or 20 mg/day, *e.g.*, about 2.5-5, 5-10, or 10-20 mg/day.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination an inhibitor of one or more of VEGFR-2, PDGFRbeta, KIT or Raf kinase C, 1-methyl-5-((2-(5-(trifluoromethyl)-1H-imidazol-2-yl)pyridin-4-yl)oxy)-N-(4-(trifluoromethyl)phenyl)-1H-benzo[d]imidazol-2-amine (Compound A37) or a compound disclosed in PCT Publication No. WO 2007/030377 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the inhibitor of one or more of VEGFR-2, PDGFRbeta, KIT or Raf kinase C is 1-methyl-5-((2-(5-(trifluoromethyl)-1H-imidazol-2-yl)pyridin-4-yl)oxy)-N-(4-(trifluoromethyl)phenyl)-1H-benzo[d]imidazol-2-amine (Compound A37) or a compound disclosed in PCT Publication No. WO 2007/030377. In one embodiment, a PD-1 antibody molecule is used in combination with 1-methyl-5-((2-(5-(trifluoromethyl)-1H-imidazol-2-yl)pyridin-4-yl)oxy)-N-(4-(trifluoromethyl)phenyl)-1H-benzo[d]imidazol-2-amine (Compound A37), or a compound disclosed in PCT Publication No. WO 2007/030377, to treat a disorder such as a cancer, a melanoma, or a solid tumor.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination a somatostatin agonist and/or growth hormone release inhibitor, Pasireotide diaspartate (also known as SIGNIFOR; Compound A38) or a compound disclosed in PCT Publication No. WO2002/010192 or US Patent No. 7,473,761 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the somatostatin agonist and/or growth hormone release inhibitor is Pasireotide diaspartate (Compound A38) or a compound disclosed in PCT Publication No. WO2002/010192 or US Patent No. 7,473,761. In one embodiment, a PD-1 antibody molecule is used in combination with Pasireotide diaspartate (Compound A38), or a compound disclosed in PCT Publication No. WO2002/010192 or US

Patent No. 7,473,761, to treat a disorder such as a prostate cancer, an endocrine cancer, a nurologic cancer, a skin cancer (*e.g.*, a melanoma), a pancreatic cancer, a liver cancer, Cushing's syndrome, a gastrointestinal disorder, acromegaly, a liver and biliary tract disorder, or liver cirrhosis.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination a signal transduction modulator and/or angiogenesis inhibitor, Dovitinib (Compound A39) or a compound disclosed in PCT Publication No. WO 2009/115562 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the signal transduction modulator and/or angiogenesis inhibitor is Dovitinib (Compound A39) or a compound disclosed in PCT Publication No. WO 2009/115562. In one embodiment, a PD-1 antibody molecule is used in combination with Dovitinib (Compound A39), or a compound disclosed in PCT Publication No. WO 2009/115562, to treat a disorder such as a cancer, a respiratory/thoracic cancer, a multiple myeloma, a prostate cancer, a non-small cell lung cancer, an endocrine cancer, or a neurological genetic disorder.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with an EGFR inhibitor, (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide (Compound A40) or a compound disclosed in PCT Publication No. WO 2013/184757 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the EGFR inhibitor is (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide (Compound A40) or a compound disclosed in PCT Publication No. WO 2013/184757. In one embodiment, a PD-1 antibody molecule is used in combination with (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide (Compound A40), or a compound disclosed in PCT Publication No. WO 2013/184757, to treat a disorder such as a cancer, *e.g.*, a solid tumor.

In one embodiment, the EGFR inhibitor or (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide (Compound A40) is administered at a dose of 150-250 mg, *e.g.*, per day. In one embodiment,

the compound is administered at a dose of about 150, 200, or 250 mg, or about 150-200 or 200-250 mg.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination an ALK inhibitor, N^6-(2-isopropoxy-5-methyl-4-(1-methylpiperidin-4-yl)phenyl)-N^4-(2-(isopropylsulfonyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidine-4,6-diamine (Compound A42) or a compound disclosed in PCT Publication No. WO 2008/073687 to treat a disorder, *e.g.,* a disorder described herein. In one embodiment, the ALK inhibitor is N^6-(2-isopropoxy-5-methyl-4-(1-methylpiperidin-4-yl)phenyl)-N^4-(2-(isopropylsulfonyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidine-4,6-diamine (Compound A42) or a compound disclosed in PCT Publication No. WO 2008/073687. In one embodiment, a PD-1 antibody molecule is used in combination with N^6-(2-isopropoxy-5-methyl-4-(1-methylpiperidin-4-yl)phenyl)-N^4-(2-(isopropylsulfonyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidine-4,6-diamine (Compound A42), or a compound disclosed in PCT Publication No. WO 2008/073687, to treat a disorder such as a cancer, an anaplastic large-cell lymphoma (ALCL), a non-small cell lung carcinoma (NSCLC), or a neuroblastoma.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination an IGF-1R inhibitor, 3-(4-(4-((5-chloro-4-((5-methyl-1H-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)-5-fluoro-2-methylphenyl)piperidin-1-yl)thietane 1,1-dioxide (Compound A43), 5-chloro-N^2-(2-fluoro-5-methyl-4-(1-(tetrahydro-2H-pyran-4-yl)piperidin-4-yl)phenyl)-N^4-(5-methyl-1H-pyrazol-3-yl)pyrimidine-2,4-diamine (Compound A44), or 5-chloro-N2-(4-(1-ethylpiperidin-4-yl)-2-fluoro-5-methylphenyl)-N^4-(5-methyl-1H-pyrazol-3-yl)pyrimidine-2,4-diamine (Compound A45) or a compound disclosed in PCT Publication No. WO 2010/002655 to treat a disorder, *e.g.,* a disorder described. In one embodiment, the IGF-1R inhibitor is 3-(4-(4-((5-chloro-4-((5-methyl-1H-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)-5-fluoro-2-methylphenyl)piperidin-1-yl)thietane 1,1-dioxide (Compound A43), 5-chloro-N^2-(2-fluoro-5-methyl-4-(1-(tetrahydro-2H-pyran-4-yl)piperidin-4-yl)phenyl)-N^4-(5-methyl-1H-pyrazol-3-yl)pyrimidine-2,4-diamine (Compound A44), 5-chloro-N2-(4-(1-ethylpiperidin-4-yl)-2-fluoro-5-methylphenyl)-N^4-(5-methyl-1H-pyrazol-3-yl)pyrimidine-2,4-diamine (Compound A45), or a compound disclosed in PCT Publication No.

WO 2010/002655. In one embodiment, a PD-1 antibody molecule is used in combination with 3-(4-(4-((5-chloro-4-((5-methyl-1H-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)-5-fluoro-2-methylphenyl)piperidin-1-yl)thietane 1,1-dioxide (Compound A43), 5-chloro-N^2-(2-fluoro-5-methyl-4-(1-(tetrahydro-2H-pyran-4-yl)piperidin-4-yl)phenyl)-N^4-(5-methyl-1H-pyrazol-3-yl)pyrimidine-2,4-diamine (Compound A44), 5-chloro-N2-(4-(1-ethylpiperidin-4-yl)-2-fluoro-5-methylphenyl)-N^4-(5-methyl-1H-pyrazol-3-yl)pyrimidine-2,4-diamine (Compound A45), or a compound disclosed in PCT Publication No. WO 2010/002655, to treat a disorder such as a cancer or a sarcoma.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination a P-Glycoprotein 1 inhibitor, Valspodar (also known as AMDRAY; Compound A46) or a compound disclosed in EP 296122 to treat a disorder, *e.g.,* a disorder described herein. In one embodiment, the P-Glycoprotein 1 inhibitor is Valspodar (Compound A46) or a compound disclosed in EP 296122. In one embodiment, a PD-1 antibody molecule is used in combination with Valspodar (Compound A46), or a compound disclosed in EP 296122, to treat a disorder such as a cancer or a drug-resistant tumor.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination one or more of a VEGFR inhibitor, Vatalanib succinate (Compound A47) or a compound disclosed in EP 296122 to treat a disorder, *e.g.,* a disorder described herein. In one embodiment, the VEGFR inhibitor is Vatalanib succinate (Compound A47) or a compound disclosed in EP 296122. In one embodiment, a PD-1 antibody molecule is used in combination with Vatalanib succinate (Compound A47), or a compound disclosed in EP 296122, to treat cancer.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with an IDH inhibitor or a compound disclosed in WO2014/141104 to treat a disorder, *e.g.,* a disorder described herein. In one embodiment, the IDH inhibitor is a compound disclosed in PCT Publication No. WO2014/141104. In one embodiment, a PD-1 antibody molecule is used in combination with a compound disclosed in WO2014/141104 to treat a disorder such as a cancer.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with a BCL-ABL inhibitor or a compound disclosed in PCT Publication No. WO2013/171639, WO2013/171640, WO2013/171641, or WO2013/171642 to treat a disorder, *e.g.,* a disorder described herein. In one embodiment, the BCL-ABL inhibitor is a compound disclosed in PCT Publication No. WO2013/171639, WO2013/171640, WO2013/171641, or WO2013/171642. In one embodiment, a PD-1 antibody molecule is used in combination with a compound disclosed in PCT Publication No. WO2013/171639, WO2013/171640, WO2013/171641, or WO2013/171642 to treat a disorder such as a cancer.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with a c-RAF inhibitor or a compound disclosed in PCT Publication No. WO2014/151616 to treat a disorder, *e.g.,* a disorder described herein. In one embodiment, the c-RAF inhibitor is Compound A50 or a compound disclosed in PCT Publication No. WO2014/151616. In one embodiment, a PD-1 antibody molecule is used in combination with a compound disclosed in PCT Publication No. WO2014/151616 to treat a disorder such as a cancer.

In another embodiment, the anti-PD-1 antibody molecule, *e.g.*, an anti-PD-1 antibody molecule as described herein, alone or in combination with one or more other immunomodulators, is used in combination with an ERK1/2 ATP competitive inhibitor or a compound disclosed in International Patent Application No. PCT/US2014/062913 to treat a disorder, *e.g.,* a disorder described herein. In one embodiment, the ERK1/2 ATP competitive inhibitor is a compound disclosed in International Patent Application No. PCT/US2014/062913. In one embodiment, a PD-1 antibody molecule is used in combination with Compound A51 or a compound disclosed in International Patent Application No. PCT/US2014/062913 to treat a disorder such as a cancer.

In some embodiments, the PD-1 antibody molecule is administerd in combination with one or more agents selected from, Compound A8, Compound A17, Compound A23, Compound A24, Compound A27, Compound A29, and Compound A33.

In some embodiments, a PD-1 antibody molecule is administered in combination with an anti-cancer agent having a known activity in an immune cell assay, *e.g.*, in one or more of a huMLR assay, a T cell proliferation assay, and a B-cell proliferation assay. Exemplary assays are described below. Based on the assay, an IC50 for can be calculated for each test agent. In embodiments, the anti-cancer agent has an IC50 of, *e.g.*, 0-1 μM, 1-4 μM, or greater than 4 μM, *e.g.*, 4-10 μM or 4-20 μM. In embodiments, the second therapeutic agent is chosen from one or more of: Compound A9, Compound A16, Compound A17, Compound A21, Compound A22, Compound A25, Compound A28, Compound A48, and Compound 49.

In some embodiments, the Compound A28 (or a compound related to Compound A28) is administered at a dose of approximately 5-10 or 10-30 mg. In some embodiments, the Compound A22 (or compound related to Compound A22) is administered at a dose of about 200 mg. In some embodiments, the Compound A17 (or compound related to Compound A17) is administered at a dose of approximately 400-600 mg. In some embodiments, the Compound A16 (or compound related to Compound A16) is administered at a dose of approximately 400-600 mg PO qDay. In some embodiments, the Compound A29 (or compound related to Compound A29) is administered at a dose of approximately 200-400 or 300-400 mg. In some embodiments, the Compound A24 (or compound related to Compound A24) is administered at a dose of approximately 200-600 mg. In some embodiments, the Compound A23 (ceritinib) (or compound related to ceritinib) is administered at a dose of approximately 750 mg once daily. In some embodiments, the Compound A8 (or compound related to Compound A8) is administered at a dose of approximately 200-400 or 300-400 mg. In some embodiments, the Compound A5 (or compound related to Compound A5) is administered at a dose of approximately 100-125 mg. In some embodiments, the Compound A6 (or compound related to Compound A6) is administered at a dose of about 100 mg. In some embodiments, the Compound A1 (or compound related to Compound A1) is administered at a dose of approximately 200-300 or 200-600 mg. In some embodiments, the Compound A40 (or compound related to Compound A40) is administered at a dose of approximately 150-250 mg. In embodiments, the Compound A10 (or compound related to Compound A10) is administered at a dose of approximately 400 to 700 mg, *e.g.*, administered three times weekly, 2 weeks on and one week off. In embodiments, the BCR-ABL inhibitor is administered at a dose of approximately 20 mg bid-80 mg bid.

Exemplary huMLR assay and B or T cell proliferation assays are provided below.

Human mixed lymphocyte reaction

The Mixed Lymphocyte Reaction (MLR) is a functional assay which measures the proliferative response of lymphocytes from one individual (the responder) to lymphocytes from another individual (the stimulator). To perform an allogeneic MLR, peripheral blood mononuclear cells (PBMC) from three donors were isolated from buffy-coats of unknown HLA type (Kantonspital Blutspendezentrum from Bern and Aarau, Switzerland). The cells were prepared at 2.105 in 0.2mL of culture medium containing RPMI 1640 GlutaMAX™ with 10% fetal calf serum (FCS), 100U penicillin/ 100µg streptomycin, 50µM 2-Mercaptoethanol. Individual 2-way reactions were set up by mixing PBMC from two different donors at a 1:1 ratio and co-cultures were done in triplicates in flat-bottomed 96-well tissue culture plates for 6 days at 37°C, 5% CO2, in presence or not of an 8-point concentration range of test compounds. Cells were pulsed with 3H-TdR (1 µCi/0.2mL) for the last 16h of culture and incorporated radioactivity was used as a measure of cell proliferation. The concentration that inhibited 50% of the maximal huMLR response (IC50) was calculated for each compound. Cyclosporine was used as a positive control of huMLR inhibition.

Human B cell proliferation assay

PBMC were freshly isolated by Ficoll-Paque density gradient from human blood and subjected to negative B-cell isolation. B cells were resuspended in culture medium (RPMI 1640, HEPES, 10% FCS, 50µg/mL gentamicine, 50µM 2-Mercaptoethanol, 1x ITS (Insulin, Transferrin and Sodium Selenite), 1x Non-Essential Amino-Acids) at a concentration of 9.104 per well in a flat-bottom 96-well culture plate. B cell stimulation was performed by human anti-IgM antibody molecule (30ug/mL) and IL-4 (75ng/mL) or by CD40 ligand (3ug/mL) and IL-4 (75ng/mL) in presence or not of a 7-point concentration range of test compounds. After 72h of culture at 37°C, 10% CO2, cells were pulsed with 3H-TdR (1 µCi/well) for the last 6h of culture. B cells were then harvested and the incorporation of thymidine was measured using a scintillation counter. Of each duplicate treatment, the mean was calculated and these data were plotted in XLfit 4 to determine the respective IC50 values.

Human T cell proliferation assay

PBMC were freshly isolated by Ficoll-Paque density gradient from human blood and subjected to negative isolation of T cells. T cells were prepared in culture medium (RPMI 1640, HEPES, 10% FCS, 50μg/mL gentamicine, 50μM 2-Mercaptoethanol, 1x ITS (Insulin, Transferrin and Sodium Selenite), 1x Non-Essential Amino-Acids) at a concentration of 8.104 per well in a flat-bottom 96-well culture plate. T cell stimulation was performed by human anti-CD3 antibody molecule (10ug/mL) or by human anti-CD3 antibody molecule (5μg/mL) and anti-CD28 antibody molecule (1μg/mL) in presence or not of a 7-point concentration range of test compounds. After 72h of culture at 37°C, 10% CO2, cells were pulsed with 3H-TdR (1 μCi/well) for the last 6h of culture. Cell proliferation was measured by the incorporation of thymidine allowing IC50 determination for each tested compound.

Down-Modulators of the Immune System

In an alternative embodiment, the anti-PD-1 antibody molecules disclosed herein are used to produce anti-idiotypic peptides or antibodies (Wallmann, J. *et al.* (2010) "Anti-Ids in Allergy: Timeliness of a Classic Concept," *World Allergy Organiz. J.* 3(6):195-201; Nardi, M. *et al.* (2000) "Antiidiotype Antibody Against Platelet Anti-Gpiiia Contributes To The Regulation Of Thrombocytopenia In HIV-1-ITP Patients," *J. Exp. Med.* 191(12):2093-2100) or mimetics (Zang, Y. C. *et al.* (2003) "Human Anti-Idiotypic T Cells Induced By TCR Peptides Corresponding To A Common CDR3Sequence Motif In Myelin Basic Protein-Reactive T Cells," *Int. Immunol.* 15(9):1073-1080; Loiarro, M. *et al.* (Epub 2010 Apr. 8) "Targeting TLR/IL-1R Signalling In Human Diseases," *Mediators Inflamm.* 2010:674363) of B7-H1 or PD-1. Such molecules serve as surrogates for PD-1, and thus their administration to a subject down-modulates the immune system of such subject by mimicking or facilitating B7-H1-PD-1 binding. Such molecules have utility in the treatment of graft vs. host disease. Similarly, agonist antibodies that i) enhance binding between such antibodies and such receptor/ligand or ii) trigger signal transduction when bound directly to B7-H1 or PD-1, have utility as agonists of B7-H1-PD-1 signaling and thus have utility in the treatment of inflammation and autoimmune disease, by directly or indirectly agonizing receptor activity.

Bispecific antibodies, exhibiting immunospecific binding to both PD-1 and B7-H1 are capable of binding to both APC and T-cells, and thus facilitate the co-localization of APCs and

T-cells. Such co-localization facilitates the ability of such cells to bind together via B7-H1 and PD-1 molecules that are not complexed with antibody, or by co-inhibitory molecules. Such binding provides down modulation of the immune system of the recipient.

Down-modulation of the immune system is desirable in the treatment of inflammatory and auto-immune diseases, and graft vs. host disease (GvHD). Examples of autoimmune disorders that may be treated by administering the antibodies of the present invention include, but are not limited to, alopecia greata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, autoimmune diseases of the adrenal gland, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune oophoritis and orchitis, autoimmune thrombocytopenia, Behcet's disease, bullous pemphigoid, cardiomyopathy, celiac sprue-dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatrical pemphigoid, CREST syndrome, cold agglutinin disease, Crohn's disease, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, glomerulonephritis, Graves' disease, Guillain-Barre, Hashimoto's thyroiditis, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), IgA neuropathy, juvenile arthritis, lichen planus, lupus erthematosus, Meniere's disease, mixed connective tissue disease, multiple sclerosis, Neuromyelitis optica (NMO), type 1 or immune-mediated diabetes mellitus, myasthenia gravis, pemphigus vulgaris, pernicious anemia, polyarteritis nodosa, polychrondritis, polyglandular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynauld's phenomenon, Reiter's syndrome, Rheumatoid arthritis, sarcoidosis, scleroderma, Sjogren's syndrome, stiff-man syndrome, systemic lupus erythematosus, lupus erythematosus, takayasu arteritis, temporal arteristis/giant cell arteritis, transverse myelitis, ulcerative colitis, uveitis, vasculitides such as dermatitis herpetiformis vasculitis, vitiligo, and Wegener's granulomatosis.

Examples of inflammatory disorders which can be prevented, treated or managed in accordance with the methods of the invention include, but are not limited to, asthma, encephilitis, inflammatory bowel disease, chronic obstructive pulmonary disease (COPD), allergic disorders, septic shock, pulmonary fibrosis, undifferentiated spondyloarthropathy, undifferentiated arthropathy, arthritis, inflammatory osteolysis, and chronic inflammation resulting from chronic viral or bacterial infections.

Thus, the antibodies and antigen-binding fragments of the present invention have utility in the treatment of inflammatory and autoimmune diseases.

Diagnostic Uses

In one aspect, the present invention provides a diagnostic method for detecting the presence of a PD-1 protein *in vitro* (*e.g.*, in a biological sample, such as a tissue biopsy, *e.g.*, from a cancerous tissue) or *in vivo* (*e.g., in vivo* imaging in a subject). The method includes: (i) contacting the sample with an antibody molecule described herein , or administering to the subject, the antibody molecule; (optionally) (ii) contacting a reference sample, *e.g.*, a control sample (*e.g.*, a control biological sample, such as plasma, tissue, biopsy) or a control subject)); and (iii) detecting formation of a complex between the antibody molecule, and the sample or subject, or the control sample or subject, wherein a change, *e.g.*, a statistically significant change, in the formation of the complex in the sample or subject relative to the control sample or subject is indicative of the presence of PD-1 in the sample. The antibody molecule can be directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials, as described above and described in more detail below.

The term "sample," as it refers to samples used for detecting polypeptides includes, but is not limited to, cells, cell lysates, proteins or membrane extracts of cells, body fluids, or tissue samples.

Complex formation between the antibody molecule and PD-1can be detected by measuring or visualizing either the binding molecule bound to the PD-1 antigen or unbound binding molecule. Conventional detection assays can be used, *e.g.*, an enzyme-linked immunosorbent assays (ELISA), a radioimmunoassay (RIA) or tissue immunohistochemistry. Alternative to labeling the antibody molecule, the presence of PD-1 can be assayed in a sample by a competition immunoassay utilizing standards labeled with a detectable substance and an unlabeled antibody molecule. In this assay, the biological sample, the labeled standards and the antibody molecule are combined and the amount of labeled standard bound to the unlabeled binding molecule is determined. The amount of PD-1 in the sample is inversely proportional to the amount of labeled standard bound to the antibody molecule.

Nucleic Acids

The invention also features nucleic acids comprising nucleotide sequences that encode heavy and light chain variable regions and CDRs or hypervariable loops of the anti-PD-1 antibody molecules, as described herein. For example, the invention features a first and second nucleic acid encoding heavy and light chain variable regions, respectively, of an anti-PD-1 antibody molecule chosen from one or more of the antibody molecules disclosed herein. The nucleic acid can comprise a nucleotide sequence as set forth in the tables herein, or a sequence substantially identical thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, or which differs by no more than 3, 6, 15, 30, or 45 nucleotides from the sequences shown in the tables herein.

In certain embodiments, the nucleic acid can comprise a nucleotide sequence encoding at least one, two, or three CDRs or hypervariable loops from a heavy chain variable region having an amino acid sequence as set forth in the tables herein, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one or more substitutions, *e.g.*, conserved substitutions). In other embodiments, the nucleic acid can comprise a nucleotide sequence encoding at least one, two, or three CDRs or hypervariable loops from a light chain variable region having an amino acid sequence as set forth in the tables herein, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one or more substitutions, *e.g.*, conserved substitutions). In yet another embodiment, the nucleic acid can comprise a nucleotide sequence encoding at least one, two, three, four, five, or six CDRs or hypervariable loops from heavy and light chain variable regions having an amino acid sequence as set forth in the tables herein, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one or more substitutions, *e.g.*, conserved substitutions).

In certain embodiments, the nucleic acid can comprise a nucleotide sequence encoding at least one, two, or three CDRs or hypervariable loops from a heavy chain variable region having the nucleotide sequence as set forth in the tables herein, a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or capable of hybridizing under the stringency conditions described herein). In another

embodiment, the nucleic acid can comprise a nucleotide sequence encoding at least one, two, or three CDRs or hypervariable loops from a light chain variable region having the nucleotide sequence as set forth in the tables herein, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or capable of hybridizing under the stringency conditions described herein). In yet another embodiment, the nucleic acid can comprise a nucleotide sequence encoding at least one, two, three, four, five, or six CDRs or hypervariable loops from heavy and light chain variable regions having the nucleotide sequence as set forth in the tables herein, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or capable of hybridizing under the stringency conditions described herein).

In another aspect, the application features host cells and vectors containing the nucleic acids described herein. The nucleic acids may be present in a single vector or separate vectors present in the same host cell or separate host cell, as described in more detail hereinbelow.

Vectors

Further provided herein are vectors comprising nucleotide sequences encoding an antibody molecule described herein. In one embodiment, the vectors comprise nucleotides encoding an antibody molecule described herein. In one embodiment, the vectors comprise the nucleotide sequences described herein. The vectors include, but are not limited to, a virus, plasmid, cosmid, lambda phage or a yeast artificial chromosome (YAC).

Numerous vector systems can be employed. For example, one class of vectors utilizes DNA elements which are derived from animal viruses such as, for example, bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (Rous Sarcoma Virus, MMTV or MOMLV) or SV40 virus. Another class of vectors utilizes RNA elements derived from RNA viruses such as Semliki Forest virus, Eastern Equine Encephalitis virus and Flaviviruses.

Additionally, cells which have stably integrated the DNA into their chromosomes may be selected by introducing one or more markers which allow for the selection of transfected host cells. The marker may provide, for example, prototropy to an auxotrophic host, biocide resistance (*e.g.,* antibiotics), or resistance to heavy metals such as copper, or the like. The selectable marker gene can be either directly linked to the DNA sequences to be expressed, or

introduced into the same cell by cotransformation. Additional elements may also be needed for optimal synthesis of mRNA. These elements may include splice signals, as well as transcriptional promoters, enhancers, and termination signals.

Once the expression vector or DNA sequence containing the constructs has been prepared for expression, the expression vectors may be transfected or introduced into an appropriate host cell. Various techniques may be employed to achieve this, such as, for example, protoplast fusion, calcium phosphate precipitation, electroporation, retroviral transduction, viral transfection, gene gun, lipid based transfection or other conventional techniques. In the case of protoplast fusion, the cells are grown in media and screened for the appropriate activity.

Methods and conditions for culturing the resulting transfected cells and for recovering the antibody molecule produced are known to those skilled in the art, and may be varied or optimized depending upon the specific expression vector and mammalian host cell employed, based upon the present description.

Cells

The invention also provides host cells comprising a nucleic acid encoding an antibody molecule as described herein.

In one embodiment, the host cells are genetically engineered to comprise nucleic acids encoding the antibody molecule.

In one embodiment, the host cells are genetically engineered by using an expression cassette. The phrase "expression cassette," refers to nucleotide sequences, which are capable of affecting expression of a gene in hosts compatible with such sequences. Such cassettes may include a promoter, an open reading frame with or without introns, and a termination signal. Additional factors necessary or helpful in effecting expression may also be used, such as, for example, an inducible promoter.

The invention also provides host cells comprising the vectors described herein.

The cell can be, but is not limited to, a eukaryotic cell, a bacterial cell, an insect cell, or a human cell. Suitable eukaryotic cells include, but are not limited to, Vero cells, HeLa cells, COS cells, CHO cells, HEK293 cells, BHK cells and MDCKII cells. Suitable insect cells include, but are not limited to, Sf9 cells.

Table 1. Amino acid and nucleotide sequences for murine, chimeric and humanized antibody molecules. The antibody molecules include murine mAb BAP049, chimeric mAbs BAP049-chi and BAP049-chi-Y, and humanized mAbs BAP049-hum01 to BAP049-hum16 and BAP049-Clone-A to BAP049-Clone-E. The amino acid and nucleotide sequences of the heavy and light chain CDRs, the heavy and light chain variable regions, and the heavy and light chains are shown.

BAP049 HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 6	VH	QVQLQQPGSELVRPGASVKLSCKASGYTFTTYW MHWVRQRPGQGLEWIGNIYPGTGGSNFDEKFKN RTSLTVDTSSTTAYMHLASLTSEDSAVYYCTRW TTGTGAYWGQGTLVTVSA
SEQ ID NO: 7	DNA VH	CAGGTCCAGCTGCAGCAACCTGGGTCTGAGCTG GTGAGGCCTGGAGCTTCAGTGAAGCTGTCCTGC AAGGCGTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGAGGCAGAGGCCTGGACAAGGC CTTGAGTGGATTGGAAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAAAAC AGGACCTCACTGACTGTAGACACATCCTCCACC ACAGCCTACATGCACCTCGCCAGCCTGACATCT GAGGACTCTGCGGTCTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAAGGG ACTCTGGTCACTGTCTCTGCA
SEQ ID NO: 8	VH	QVQLQQSGSELVRPGASVKLSCKASGYTFTTYW MHWVRQRPGQGLEWIGNIYPGTGGSNFDEKFKN RTSLTVDTSSTTAYMHLASLTSEDSAVYYCTRW TTGTGAYWGQGTLVTVSA
SEQ ID NO: 9	DNA VH	CAGGTCCAGCTGCAGCAGTCTGGGTCTGAGCTG GTGAGGCCTGGAGCTTCAGTGAAGCTGTCCTGC AAGGCGTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGAGGCAGAGGCCTGGACAAGGC CTTGAGTGGATTGGAAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAAAAC AGGACCTCACTGACTGTAGACACATCCTCCACC ACAGCCTACATGCACCTCGCCAGCCTGACATCT GAGGACTCTGCGGTCTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAAGGG ACTCTGGTCACTGTCTCTGCA
BAP049 LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 12 (Kabat)	LCDR3	QNDYSYPCT

SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF
SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 15 (Chothia)	LCDR3	DYSYPC
SEQ ID NO: 16	VL	DIVMTQSPSSLTVTAGEKVTMSCKSSQSLLDSG NQKNFLTWYQQKPGQPPKLLIFWASTRESGVPD RFTGSGSVTDFTLTISSVQAEDLAVYYCQNDYS YPCTFGGGTKLEIK
SEQ ID NO: 17	DNA VL	GACATTGTGATGACCCAGTCTCCATCCTCCCTG ACTGTGACAGCAGGAGAGAAGGTCACTATGAGC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCAGGGCAGCCTCCTAAACTGTTGATCTTC TGGGCATCCACTAGGGAATCTGGGGTCCCTGAT CGCTTCACAGGCAGTGGATCTGTAACAGATTTC ACTCTCACCATCAGCAGTGTGCAGGCTGAAGAC CTGGCAGTTTATTACTGTCAGAATGATTATAGT TATCCGTGCACGTTCGGAGGGGGGACCAAGCTG GAAATAAAA
BAP049-chi HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 18	VH	QVQLQQPGSELVRPGASVKLSCKASGYTFTTYW MHWVRQRPGQGLEWIGNIYPGTGGSNFDEKFKN RTSLTVDTSSTTAYMHLASLTSEDSAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 19	DNA VH	CAGGTCCAGCTGCAGCAGCCTGGGTCTGAGCTG GTGAGGCCTGGAGCTTCAGTGAAGCTGTCCTGC AAGGCGTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGAGGCAGAGGCCTGGACAAGGC CTTGAGTGGATTGGAAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAAAAC AGGACCTCACTGACTGTAGACACATCCTCCACC ACAGCCTACATGCACCTCGCCAGCCTGACATCT GAGGACTCTGCGGTCTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 20	HC	QVQLQQPGSELVRPGASVKLSCKASGYTFTTYW MHWVRQRPGQGLEWIGNIYPGTGGSNFDEKFKN RTSLTVDTSSTTAYMHLASLTSEDSAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL

		HNHYTQKSLSLSLGK
SEQ ID NO: 21	DNA HC	CAGGTCCAGCTGCAGCAGCCTGGGTCTGAGCTG GTGAGGCCTGGAGCTTCAGTGAAGCTGTCCTGC AAGGCGTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGAGGCAGAGGCCTGGACAAGGC CTTGAGTGGATTGGAAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAAAAC AGGACCTCACTGACTGTAGACACATCCTCCACC ACAGCCTACATGCACCTCGCCAGCCTGACATCT GAGGACTCTGCGGTCTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA
SEQ ID NO: 22	VH	QVQLQQSGSELVRPGASVKLSCKASGYTFTTYW MHWVRQRPGQGLEWIGNIYPGTGGSNFDEKFKN RTSLTVDTSSTTAYMHLASLTSEDSAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 23	DNA VH	CAGGTCCAGCTGCAGCAGTCTGGGTCTGAGCTG GTGAGGCCTGGAGCTTCAGTGAAGCTGTCCTGC AAGGCGTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGAGGCAGAGGCCTGGACAAGGC CTTGAGTGGATTGGAAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAAAAC AGGACCTCACTGACTGTAGACACATCCTCCACC ACAGCCTACATGCACCTCGCCAGCCTGACATCT GAGGACTCTGCGGTCTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC

		ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 30	HC	QVQLQQSGSELVRPGASVKLSCKASGYTFTTYW MHWVRQRPGQGLEWIGNIYPGTGGSNFDEKFKN RTSLTVDTSSTTAYMHLASLTSEDSAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 31	DNA HC	CAGGTCCAGCTGCAGCAGTCTGGGTCTGAGCTG GTGAGGCCTGGAGCTTCAGTGAAGCTGTCCTGC AAGGCGTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGAGGCAGAGGCCTGGACAAGGC CTTGAGTGGATTGGAAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAAAAC AGGACCTCACTGACTGTAGACACATCCTCCACC ACAGCCTACATGCACCTCGCCAGCCTGACATCT GAGGACTCTGCGGTCTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA

BAP049-chi LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 12 (Kabat)	LCDR3	QNDYSYPCT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF
SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 15 (Chothia)	LCDR3	DYSYPC
SEQ ID NO: 24	VL	DIVMTQSPSSLTVTAGEKVTMSCKSSQSLLDSG NQKNFLTWYQQKPGQPPKLLIFWASTRESGVPD RFTGSGSVTDFTLTISSVQAEDLAVYYCQNDYS YPCTFGQGTKVEIK
SEQ ID NO: 25	DNA VL	GACATTGTGATGACCCAGTCTCCATCCTCCCTG ACTGTGACAGCAGGAGAGAAGGTCACTATGAGC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCAGGGCAGCCTCCTAAACTGTTGATCTTC TGGGCATCCACTAGGGAATCTGGGGTCCCTGAT CGCTTCACAGGCAGTGGATCTGTAACAGATTTC ACTCTCACCATCAGCAGTGTGCAGGCTGAAGAC CTGGCAGTTTATTACTGTCAGAATGATTATAGT TATCCGTGCACGTTCGGCCAAGGGACCAAGGTG GAAATCAAA
SEQ ID NO: 26	LC	DIVMTQSPSSLTVTAGEKVTMSCKSSQSLLDSG NQKNFLTWYQQKPGQPPKLLIFWASTRESGVPD RFTGSGSVTDFTLTISSVQAEDLAVYYCQNDYS YPCTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 27	DNA LC	GACATTGTGATGACCCAGTCTCCATCCTCCCTG ACTGTGACAGCAGGAGAGAAGGTCACTATGAGC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCAGGGCAGCCTCCTAAACTGTTGATCTTC TGGGCATCCACTAGGGAATCTGGGGTCCCTGAT CGCTTCACAGGCAGTGGATCTGTAACAGATTTC ACTCTCACCATCAGCAGTGTGCAGGCTGAAGAC CTGGCAGTTTATTACTGTCAGAATGATTATAGT TATCCGTGCACGTTCGGCCAAGGGACCAAGGTG GAAATCAAACGTACGGTGGCTGCACCATCTGTC TTCATCTTCCCGCCATCTGATGAGCAGTTGAAA TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT AACTTCTATCCCAGAGAGGCCAAAGTACAGTGG AAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTAC GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
BAP049-chi-Y HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY

SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 18	VH	QVQLQQPGSELVRPGASVKLSCKASGYTFTTYW MHWVRQRPGQGLEWIGNIYPGTGGSNFDEKFKN RTSLTVDTSSTTAYMHLASLTSEDSAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 19	DNA VH	CAGGTCCAGCTGCAGCAGCCTGGGTCTGAGCTG GTGAGGCCTGGAGCTTCAGTGAAGCTGTCCTGC AAGGCGTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGAGGCAGAGGCCTGGACAAGGC CTTGAGTGGATTGGAAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAAAAC AGGACCTCACTGACTGTAGACACATCCTCCACC ACAGCCTACATGCACCTCGCCAGCCTGACATCT GAGGACTCTGCGGTCTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 20	HC	QVQLQQPGSELVRPGASVKLSCKASGYTFTTYW MHWVRQRPGQGLEWIGNIYPGTGGSNFDEKFKN RTSLTVDTSSTTAYMHLASLTSEDSAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 21	DNA HC	CAGGTCCAGCTGCAGCAGCCTGGGTCTGAGCTG GTGAGGCCTGGAGCTTCAGTGAAGCTGTCCTGC AAGGCGTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGAGGCAGAGGCCTGGACAAGGC CTTGAGTGGATTGGAAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAAAAC AGGACCTCACTGACTGTAGACACATCCTCCACC ACAGCCTACATGCACCTCGCCAGCCTGACATCT GAGGACTCTGCGGTCTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC

		CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA
SEQ ID NO: 22	VH	QVQLQQSGSELVRPGASVKLSCKASGYTFTTYW MHWVRQRPGQGLEWIGNIYPGTGGSNFDEKFKN RTSLTVDTSSTTAYMHLASLTSEDSAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 23	DNA VH	CAGGTCCAGCTGCAGCAGTCTGGGTCTGAGCTG GTGAGGCCTGGAGCTTCAGTGAAGCTGTCCTGC AAGGCGTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGAGGCAGAGGCCTGGACAAGGC CTTGAGTGGATTGGAAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAAAAC AGGACCTCACTGACTGTAGACACATCCTCCACC ACAGCCTACATGCACCTCGCCAGCCTGACATCT GAGGACTCTGCGGTCTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 30	HC	QVQLQQSGSELVRPGASVKLSCKASGYTFTTYW MHWVRQRPGQGLEWIGNIYPGTGGSNFDEKFKN RTSLTVDTSSTTAYMHLASLTSEDSAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 31	DNA HC	CAGGTCCAGCTGCAGCAGTCTGGGTCTGAGCTG GTGAGGCCTGGAGCTTCAGTGAAGCTGTCCTGC AAGGCGTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGAGGCAGAGGCCTGGACAAGGC CTTGAGTGGATTGGAAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAAAAC AGGACCTCACTGACTGTAGACACATCCTCCACC ACAGCCTACATGCACCTCGCCAGCCTGACATCT GAGGACTCTGCGGTCTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG

		GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA
BAP049-chi-Y LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF
SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 34	VL	DIVMTQSPSSLTVTAGEKVTMSCKSSQSLLDSG NQKNFLTWYQQKPGQPPKLLIFWASTRESGVPD RFTGSGSVTDFTLTISSVQAEDLAVYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 35	DNA VL	GACATTGTGATGACCCAGTCTCCATCCTCCCTG ACTGTGACAGCAGGAGAGAAGGTCACTATGAGC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCAGGGCAGCCTCCTAAACTGTTGATCTTC TGGGCATCCACTAGGGAATCTGGGGTCCCTGAT CGCTTCACAGGCAGTGGATCTGTAACAGATTTC ACTCTCACCATCAGCAGTGTGCAGGCTGAAGAC CTGGCAGTTTATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAA

SEQ ID NO: 36	LC	DIVMTQSPSSLTVTAGEKVTMSCKSSQSLLDSG NQKNFLTWYQQKPGQPPKLLIFWASTRESGVPD RFTGSGSVTDFTLTISSVQAEDLAVYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 37	DNA LC	GACATTGTGATGACCCAGTCTCCATCCTCCCTG ACTGTGACAGCAGGAGAGAAGGTCACTATGAGC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCAGGGCAGCCTCCTAAACTGTTGATCTTC TGGGCATCCACTAGGGAATCTGGGGTCCCTGAT CGCTTCACAGGCAGTGGATCTGTAACAGATTTC ACTCTCACCATCAGCAGTGTGCAGGCTGAAGAC CTGGCAGTTTATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAACGTACGGTGGCTGCACCATCTGTC TTCATCTTCCCGCCATCTGATGAGCAGTTGAAA TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT AACTTCTATCCCAGAGAGGCCAAAGTACAGTGG AAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTAC GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
BAP049-hum01 HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 38	VH	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 39	DNA VH	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCACTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGAGTCACGATTACCGCGGACAAATCCACGAGC ACAGCCTACATGGAGCTGAGCAGCCTGAGATCT GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 40	HC	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS

		GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 41	DNA HC	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCACTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGAGTCACGATTACCGCGGACAAATCCACGAGC ACAGCCTACATGGAGCTGAGCAGCCTGAGATCT GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA
BAP049-hum01 LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF

SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 42	VL	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTEFTLTISSLQPDDFATYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 43	DNA VL	GAAATTGTGTTGACACAGTCTCCAGCCACCCTG TCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCATCA AGGTTCAGCGGCAGTGGATCTGGGACAGAATTC ACTCTCACCATCAGCAGCCTGCAGCCTGATGAT TTTGCAACTTATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAA
SEQ ID NO: 44	LC	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTEFTLTISSLQPDDFATYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 45	DNA LC	GAAATTGTGTTGACACAGTCTCCAGCCACCCTG TCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCATCA AGGTTCAGCGGCAGTGGATCTGGGACAGAATTC ACTCTCACCATCAGCAGCCTGCAGCCTGATGAT TTTGCAACTTATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAACGTACGGTGGCTGCACCATCTGTC TTCATCTTCCCGCCATCTGATGAGCAGTTGAAA TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT AACTTCTATCCCAGAGAGGCCAAAGTACAGTGG AAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTAC GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
BAP049-hum02 HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 38	VH	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN

		RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 39	DNA VH	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCACTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGAGTCACGATTACCGCGGACAAATCCACGAGC ACAGCCTACATGGAGCTGAGCAGCCTGAGATCT GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 40	HC	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 41	DNA HC	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCACTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGAGTCACGATTACCGCGGACAAATCCACGAGC ACAGCCTACATGGAGCTGAGCAGCCTGAGATCT GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG

		GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA
BAP049-hum02 LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF
SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 46	VL	DIQMTQSPSSLSASVGDRVTITCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGIPP RFSGSGYGTDFTLTINNIESEDAAYYFCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 47	DNA VL	GACATCCAGATGACCCAGTCTCCATCCTCCCTG TCTGCATCTGTAGGAGACAGAGTCACCATCACT TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGATCCCACCT CGATTCAGTGGCAGCGGGTATGGAACAGATTTT ACCCTCACAATTAATAACATAGAATCTGAGGAT GCTGCATATTACTTCTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAA
SEQ ID NO: 48	LC	DIQMTQSPSSLSASVGDRVTITCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGIPP RFSGSGYGTDFTLTINNIESEDAAYYFCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 49	DNA LC	GACATCCAGATGACCCAGTCTCCATCCTCCCTG TCTGCATCTGTAGGAGACAGAGTCACCATCACT TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGATCCCACCT CGATTCAGTGGCAGCGGGTATGGAACAGATTTT ACCCTCACAATTAATAACATAGAATCTGAGGAT GCTGCATATTACTTCTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAACGTACGGTGGCTGCACCATCTGTC TTCATCTTCCCGCCATCTGATGAGCAGTTGAAA TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT

		AACTTCTATCCCAGAGAGGCCAAAGTACAGTGG AAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTAC GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
BAP049-hum03 HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 50	VH	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWIRQSPSRGLEWLGNIYPGTGGSNFDEKFKN RFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 51	DNA VH	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGATCAGGCAGTCCCCATCGAGAGGC CTTGAGTGGCTGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGATTCACCATCTCCAGAGACAATTCCAAGAAC ACGCTGTATCTTCAAATGAACAGCCTGAGAGCC GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 52	HC	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWIRQSPSRGLEWLGNIYPGTGGSNFDEKFKN RFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYILPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 53	DNA HC	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGATCAGGCAGTCCCCATCGAGAGGC CTTGAGTGGCTGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGATTCACCATCTCCAGAGACAATTCCAAGAAC ACGCTGTATCTTCAAATGAACAGCCTGAGAGCC GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG

		GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA
BAP049-hum03 LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF
SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 46	VL	DIQMTQSPSSLSASVGDRVTITCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGIPP RFSGSGYGTDFTLTINNIESEDAAYYFCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 47	DNA VL	GACATCCAGATGACCCAGTCTCCATCCTCCCTG TCTGCATCTGTAGGAGACAGAGTCACCATCACT TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGATCCCACCT CGATTCAGTGGCAGCGGGTATGGAACAGATTTT ACCCTCACAATTAATAACATAGAATCTGAGGAT GCTGCATATTACTTCTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAA

SEQ ID NO: 48	LC	DIQMTQSPSSLSASVGDRVTITCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGIPP RFSGSGYGTDFTLTINNIESEDAAYYFCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 49	DNA LC	GACATCCAGATGACCCAGTCTCCATCCTCCCTG TCTGCATCTGTAGGAGACAGAGTCACCATCACT TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGATCCCACCT CGATTCAGTGGCAGCGGGTATGGAACAGATTTT ACCCTCACAATTAATAACATAGAATCTGAGGAT GCTGCATATTACTTCTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAACGTACGGTGGCTGCACCATCTGTC TTCATCTTCCCGCCATCTGATGAGCAGTTGAAA TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT AACTTCTATCCCAGAGAGGCCAAAGTACAGTGG AAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTAC GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
BAP049-hum04 HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 50	VH	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWIRQSPSRGLEWLGNIYPGTGGSNFDEKFKN RFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 51	DNA VH	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGATCAGGCAGTCCCCATCGAGAGGC CTTGAGTGGCTGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGATTCACCATCTCCAGAGACAATTCCAAGAAC ACGCTGTATCTTCAAATGAACAGCCTGAGAGCC GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 52	HC	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWIRQSPSRGLEWLGNIYPGTGGSNFDEKFKN RFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS

		GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 53	DNA HC	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGATCAGGCAGTCCCCATCGAGAGGC CTTGAGTGGCTGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGATTCACCATCTCCAGAGACAATTCCAAGAAC ACGCTGTATCTTCAAATGAACAGCCTGAGAGCC GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA
BAP049-hum04 LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF

SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 54	VL	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGKAPKLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLQPEDIATYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 55	DNA VL	GAAATTGTGTTGACACAGTCTCCAGCCACCCTG TCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTATCAGCAG AAACCAGGGAAAGCTCCTAAGCTCCTGATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCATCA AGGTTCAGTGGAAGTGGATCTGGGACAGATTTT ACTTTCACCATCAGCAGCCTGCAGCCTGAAGAT ATTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAA
SEQ ID NO: 56	LC	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGKAPKLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLQPEDIATYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 57	DNA LC	GAAATTGTGTTGACACAGTCTCCAGCCACCCTG TCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTATCAGCAG AAACCAGGGAAAGCTCCTAAGCTCCTGATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCATCA AGGTTCAGTGGAAGTGGATCTGGGACAGATTTT ACTTTCACCATCAGCAGCCTGCAGCCTGAAGAT ATTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAACGTACGGTGGCTGCACCATCTGTC TTCATCTTCCCGCCATCTGATGAGCAGTTGAAA TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT AACTTCTATCCCAGAGAGGCCAAAGTACAGTGG AAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTAC GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
BAP049-hum05 HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 38	VH	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN

		RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 39	DNA VH	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCACTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGAGTCACGATTACCGCGGACAAATCCACGAGC ACAGCCTACATGGAGCTGAGCAGCCTGAGATCT GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 40	HC	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 41	DNA HC	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCACTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGAGTCACGATTACCGCGGACAAATCCACGAGC ACAGCCTACATGGAGCTGAGCAGCCTGAGATCT GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG

		GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA
BAP049-hum05 LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF
SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 54	VL	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGKAPKLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLQPEDIATYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 55	DNA VL	GAAATTGTGTTGACACAGTCTCCAGCCACCCTG TCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTATCAGCAG AAACCAGGGAAAGCTCCTAAGCTCCTGATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCATCA AGGTTCAGTGGAAGTGGATCTGGGACAGATTTT ACTTTCACCATCAGCAGCCTGCAGCCTGAAGAT ATTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAA
SEQ ID NO: 56	LC	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGKAPKLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLQPEDIATYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 57	DNA LC	GAAATTGTGTTGACACAGTCTCCAGCCACCCTG TCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTATCAGCAG AAACCAGGGAAAGCTCCTAAGCTCCTGATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCATCA AGGTTCAGTGGAAGTGGATCTGGGACAGATTTT ACTTTCACCATCAGCAGCCTGCAGCCTGAAGAT ATTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAACGTACGGTGGCTGCACCATCTGTC TTCATCTTCCCGCCATCTGATGAGCAGTTGAAA TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT

		AACTTCTATCCCAGAGAGGCCAAAGTACAGTGG AAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTAC GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
BAP049-hum06 HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 38	VH	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 39	DNA VH	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCACTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGAGTCACGATTACCGCGGACAAATCCACGAGC ACAGCCTACATGGAGCTGAGCAGCCTGAGATCT GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 40	HC	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYILPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 41	DNA HC	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCACTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGAGTCACGATTACCGCGGACAAATCCACGAGC ACAGCCTACATGGAGCTGAGCAGCCTGAGATCT GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG

		GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA
BAP049-hum06 LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF
SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 58	VL	DIVMTQTPLSLPVTPGEPASISCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 59	DNA VL	GATATTGTGATGACCCAGACTCCACTCTCCCTG CCCGTCACCCCTGGAGAGCCGGCCTCCATCTCC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAA

SEQ ID NO: 60	LC	DIVMTQTPLSLPVTPGEPASISCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 61	DNA LC	GATATTGTGATGACCCAGACTCCACTCTCCCTG CCCGTCACCCCTGGAGAGCCGGCCTCCATCTCC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAACGTACGGTGGCTGCACCATCTGTC TTCATCTTCCCGCCATCTGATGAGCAGTTGAAA TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT AACTTCTATCCCAGAGAGGCCAAAGTACAGTGG AAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTAC GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
BAP049-hum07 HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 38	VH	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 39	DNA VH	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCACTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGAGTCACGATTACCGCGGACAAATCCACGAGC ACAGCCTACATGGAGCTGAGCAGCCTGAGATCT GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 40	HC	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS

		GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 41	DNA HC	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCACTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGAGTCACGATTACCGCGGACAAATCCACGAGC ACAGCCTACATGGAGCTGAGCAGCCTGAGATCT GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA
BAP049-hum07 LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF

SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 62	VL	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGKAPKLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 63	DNA VL	GAAATTGTGTTGACACAGTCTCCAGCCACCCTG TCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTATCAGCAG AAACCAGGGAAAGCTCCTAAGCTCCTGATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAA
SEQ ID NO: 64	LC	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGKAPKLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 65	DNA LC	GAAATTGTGTTGACACAGTCTCCAGCCACCCTG TCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTATCAGCAG AAACCAGGGAAAGCTCCTAAGCTCCTGATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAACGTACGGTGGCTGCACCATCTGTC TTCATCTTCCCGCCATCTGATGAGCAGTTGAAA TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT AACTTCTATCCCAGAGAGGCCAAAGTACAGTGG AAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTAC GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
BAP049-hum08 HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 50	VH	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWIRQSPSRGLEWLGNIYPGTGGSNFDEKFKN

		RFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 51	DNA VH	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGATCAGGCAGTCCCCATCGAGAGGC CTTGAGTGGCTGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGATTCACCATCTCCAGAGACAATTCCAAGAAC ACGCTGTATCTTCAAATGAACAGCCTGAGAGCC GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 52	HC	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWIRQSPSRGLEWLGNIYPGTGGSNFDEKFKN RFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 53	DNA HC	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGATCAGGCAGTCCCCATCGAGAGGC CTTGAGTGGCTGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGATTCACCATCTCCAGAGACAATTCCAAGAAC ACGCTGTATCTTCAAATGAACAGCCTGAGAGCC GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG

		GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA
BAP049-hum08 LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF
SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 66	VL	EIVLTQSPDFQSVTPKEKVTITCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 67	DNA VL	GAAATTGTGCTGACTCAGTCTCCAGACTTTCAG TCTGTGACTCCAAAGGAGAAAGTCACCATCACC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAA
SEQ ID NO: 68	LC	EIVLTQSPDFQSVTPKEKVTITCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 69	DNA LC	GAAATTGTGCTGACTCAGTCTCCAGACTTTCAG TCTGTGACTCCAAAGGAGAAAGTCACCATCACC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAACGTACGGTGGCTGCACCATCTGTC TTCATCTTCCCGCCATCTGATGAGCAGTTGAAA TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT

		AACTTCTATCCCAGAGAGGCCAAAGTACAGTGG AAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTAC GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
BAP049-hum09 HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 38	VH	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 39	DNA VH	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCACTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGAGTCACGATTACCGCGGACAAATCCACGAGC ACAGCCTACATGGAGCTGAGCAGCCTGAGATCT GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 40	HC	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYILPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 41	DNA HC	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCACTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGAGTCACGATTACCGCGGACAAATCCACGAGC ACAGCCTACATGGAGCTGAGCAGCCTGAGATCT GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG

		GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA
BAP049-hum09 LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF
SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 66	VL	EIVLTQSPDFQSVTPKEKVTITCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 67	DNA VL	GAAATTGTGCTGACTCAGTCTCCAGACTTTCAG TCTGTGACTCCAAAGGAGAAAGTCACCATCACC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAA

SEQ ID NO: 68	LC	EIVLTQSPDFQSVTPKEKVTITCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 69	DNA LC	GAAATTGTGCTGACTCAGTCTCCAGACTTTCAG TCTGTGACTCCAAAGGAGAAAGTCACCATCACC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAACGTACGGTGGCTGCACCATCTGTC TTCATCTTCCCGCCATCTGATGAGCAGTTGAAA TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT AACTTCTATCCCAGAGAGGCCAAAGTACAGTGG AAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTAC GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
BAP049-hum10 HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 50	VH	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWIRQSPSRGLEWLGNIYPGTGGSNFDEKFKN RFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 51	DNA VH	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGATCAGGCAGTCCCCATCGAGAGGC CTTGAGTGGCTGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGATTCACCATCTCCAGAGACAATTCCAAGAAC ACGCTGTATCTTCAAATGAACAGCCTGAGAGCC GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 52	HC	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWIRQSPSRGLEWLGNIYPGTGGSNFDEKFKN RFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS

		GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 53	DNA HC	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGATCAGGCAGTCCCCATCGAGAGGC CTTGAGTGGCTGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGATTCACCATCTCCAGAGACAATTCCAAGAAC ACGCTGTATCTTCAAATGAACAGCCTGAGAGCC GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA
BAP049-hum10 LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF

SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 70	VL	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 71	DNA VL	GAAATTGTGTTGACACAGTCTCCAGCCACCCTG TCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAA
SEQ ID NO: 72	LC	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 73	DNA LC	GAAATTGTGTTGACACAGTCTCCAGCCACCCTG TCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAACGTACGGTGGCTGCACCATCTGTC TTCATCTTCCCGCCATCTGATGAGCAGTTGAAA TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT AACTTCTATCCCAGAGAGGCCAAAGTACAGTGG AAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTAC GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
BAP049-hum11 HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 38	VH	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN

		RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 39	DNA VH	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCACTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGAGTCACGATTACCGCGGACAAATCCACGAGC ACAGCCTACATGGAGCTGAGCAGCCTGAGATCT GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 40	HC	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 41	DNA HC	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCACTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGAGTCACGATTACCGCGGACAAATCCACGAGC ACAGCCTACATGGAGCTGAGCAGCCTGAGATCT GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG

		GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA
BAP049-hum11 LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF
SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 70	VL	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 71	DNA VL	GAAATTGTGTTGACACAGTCTCCAGCCACCCTG TCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAA
SEQ ID NO: 72	LC	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 73	DNA LC	GAAATTGTGTTGACACAGTCTCCAGCCACCCTG TCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAACGTACGGTGGCTGCACCATCTGTC TTCATCTTCCCGCCATCTGATGAGCAGTTGAAA TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT

		AACTTCTATCCCAGAGAGGCCAAAGTACAGTGG AAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTAC GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
BAP049-hum12 HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 38	VH	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 39	DNA VH	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCACTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGAGTCACGATTACCGCGGACAAATCCACGAGC ACAGCCTACATGGAGCTGAGCAGCCTGAGATCT GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 40	HC	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYILPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 41	DNA HC	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCACTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGAGTCACGATTACCGCGGACAAATCCACGAGC ACAGCCTACATGGAGCTGAGCAGCCTGAGATCT GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG

		GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA
BAP049-hum12 LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF
SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 74	VL	DIQMTQSPSSLSASVGDRVTITCKSSQSLLDSG NQKNFLTWYLQKPGQSPQLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 75	DNA VL	GACATCCAGATGACCCAGTCTCCATCCTCCCTG TCTGCATCTGTAGGAGACAGAGTCACCATCACT TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCTGCAG AAGCCAGGGCAGTCTCCACAGCTCCTGATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAA

SEQ ID NO: 76	LC	DIQMTQSPSSLSASVGDRVTITCKSSQSLLDSG NQKNFLTWYLQKPGQSPQLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 77	DNA LC	GACATCCAGATGACCCAGTCTCCATCCTCCCTG TCTGCATCTGTAGGAGACAGAGTCACCATCACT TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCTGCAG AAGCCAGGGCAGTCTCCACAGCTCCTGATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAACGTACGGTGGCTGCACCATCTGTC TTCATCTTCCCGCCATCTGATGAGCAGTTGAAA TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT AACTTCTATCCCAGAGAGGCCAAAGTACAGTGG AAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTAC GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
BAP049-hum13 HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 38	VH	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 39	DNA VH	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCACTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGAGTCACGATTACCGCGGACAAATCCACGAGC ACAGCCTACATGGAGCTGAGCAGCCTGAGATCT GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 40	HC	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS

		GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 41	DNA HC	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCACTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGAGTCACGATTACCGCGGACAAATCCACGAGC ACAGCCTACATGGAGCTGAGCAGCCTGAGATCT GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA
BAP049-hum13 LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF

SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 78	VL	DVVMTQSPLSLPVTLGQPASISCKSSQSLLDSG NQKNFLTWYQQKPGKAPKLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 79	DNA VL	GATGTTGTGATGACTCAGTCTCCACTCTCCCTG CCCGTCACCCTTGGACAGCCGGCCTCCATCTCC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTAACCTGGTATCAGCAG AAACCAGGGAAAGCTCCTAAGCTCCTGATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAA
SEQ ID NO: 80	LC	DVVMTQSPLSLPVTLGQPASISCKSSQSLLDSG NQKNFLTWYQQKPGKAPKLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 81	DNA LC	GATGTTGTGATGACTCAGTCTCCACTCTCCCTG CCCGTCACCCTTGGACAGCCGGCCTCCATCTCC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTAACCTGGTATCAGCAG AAACCAGGGAAAGCTCCTAAGCTCCTGATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAACGTACGGTGGCTGCACCATCTGTC TTCATCTTCCCGCCATCTGATGAGCAGTTGAAA TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT AACTTCTATCCCAGAGAGGCCAAAGTACAGTGG AAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTAC GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
BAP049-hum14 HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 82	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYW MHWIRQSPSRGLEWLGNIYPGTGGSNFDEKFKN

		RFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 83	DNA VH	CAGGTTCAGCTGGTGCAGTCTGGAGCTGAGGTG AAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGC AAGGCTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGATCAGGCAGTCCCCATCGAGAGGC CTTGAGTGGCTGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGATTCACCATCTCCAGAGACAATTCCAAGAAC ACGCTGTATCTTCAAATGAACAGCCTGAGAGCC GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTACTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 84	HC	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYW MHWIRQSPSRGLEWLGNIYPGTGGSNFDEKFKN RFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 85	DNA HC	CAGGTTCAGCTGGTGCAGTCTGGAGCTGAGGTG AAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGC AAGGCTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGATCAGGCAGTCCCCATCGAGAGGC CTTGAGTGGCTGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGATTCACCATCTCCAGAGACAATTCCAAGAAC ACGCTGTATCTTCAAATGAACAGCCTGAGAGCC GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTACTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG

		GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA
BAP049-hum14 LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF
SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 70	VL	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 71	DNA VL	GAAATTGTGTTGACACAGTCTCCAGCCACCCTG TCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAA
SEQ ID NO: 72	LC	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 73	DNA LC	GAAATTGTGTTGACACAGTCTCCAGCCACCCTG TCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAACGTACGGTGGCTGCACCATCTGTC TTCATCTTCCCGCCATCTGATGAGCAGTTGAAA TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT

		AACTTCTATCCCAGAGAGGCCAAAGTACAGTGG AAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTAC GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
BAP049-hum15 HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 82	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYW MHWIRQSPSRGLEWLGNIYPGTGGSNFDEKFKN RFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 83	DNA VH	CAGGTTCAGCTGGTGCAGTCTGGAGCTGAGGTG AAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGC AAGGCTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGATCAGGCAGTCCCCATCGAGAGGC CTTGAGTGGCTGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGATTCACCATCTCCAGAGACAATTCCAAGAAC ACGCTGTATCTTCAAATGAACAGCCTGAGAGCC GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTACTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 84	HC	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYW MHWIRQSPSRGLEWLGNIYPGTGGSNFDEKFKN RFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYILPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 85	DNA HC	CAGGTTCAGCTGGTGCAGTCTGGAGCTGAGGTG AAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGC AAGGCTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGATCAGGCAGTCCCCATCGAGAGGC CTTGAGTGGCTGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGATTCACCATCTCCAGAGACAATTCCAAGAAC ACGCTGTATCTTCAAATGAACAGCCTGAGAGCC GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTACTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG

		GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA
BAP049-hum15 LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF
SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 66	VL	EIVLTQSPDFQSVTPKEKVTITCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 67	DNA VL	GAAATTGTGCTGACTCAGTCTCCAGACTTTCAG TCTGTGACTCCAAAGGAGAAAGTCACCATCACC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAA

SEQ ID NO: 68	LC	EIVLTQSPDFQSVTPKEKVTITCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 69	DNA LC	GAAATTGTGCTGACTCAGTCTCCAGACTTTCAG TCTGTGACTCCAAAGGAGAAAGTCACCATCACC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAACGTACGGTGGCTGCACCATCTGTC TTCATCTTCCCGCCATCTGATGAGCAGTTGAAA TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT AACTTCTATCCCAGAGAGGCCAAAGTACAGTGG AAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTAC GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
BAP049-hum16 HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 86	VH	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQAPGQGLEWMGNIYPGTGGSNFDEKFKN RFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 87	DNA VH	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCCCTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGATTCACCATCTCCAGAGACAATTCCAAGAAC ACGCTGTATCTTCAAATGAACAGCCTGAGAGCC GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCC
SEQ ID NO: 88	HC	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQAPGQGLEWMGNIYPGTGGSNFDEKFKN RFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS

		GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 89	DNA HC	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTG AAAAAGCCCGGGGAGTCTCTGAGGATCTCCTGT AAGGGTTCTGGCTACACATTCACCACTTACTGG ATGCACTGGGTGCGACAGGCCCCTGGACAAGGG CTTGAGTGGATGGGTAATATTTATCCTGGTACT GGTGGTTCTAACTTCGATGAGAAGTTCAAGAAC AGATTCACCATCTCCAGAGACAATTCCAAGAAC ACGCTGTATCTTCAAATGAACAGCCTGAGAGCC GAGGACACGGCCGTGTATTACTGTACAAGATGG ACTACTGGGACGGGAGCTTATTGGGGCCAGGGC ACCACCGTGACCGTGTCCTCCGCTTCCACCAAG GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC TGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGACCTAC ACCTGCAACGTAGATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCGTGCCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCATGATCTCCCGGACC CCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGCAAG GTGTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAG GAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCTACAGCAGGCTA ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA
BAP049-hum16 LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF

SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 66	VL	EIVLTQSPDFQSVTPKEKVTITCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 67	DNA VL	GAAATTGTGCTGACTCAGTCTCCAGACTTTCAG TCTGTGACTCCAAAGGAGAAAGTCACCATCACC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAA
SEQ ID NO: 68	LC	EIVLTQSPDFQSVTPKEKVTITCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 69	DNA LC	GAAATTGTGCTGACTCAGTCTCCAGACTTTCAG TCTGTGACTCCAAAGGAGAAAGTCACCATCACC TGCAAGTCCAGTCAGAGTCTGTTAGACAGTGGA AATCAAAAGAACTTCTTGACCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT TGGGCATCCACTAGGGAATCTGGGGTCCCCTCG AGGTTCAGTGGCAGTGGATCTGGGACAGATTTC ACCTTTACCATCAGTAGCCTGGAAGCTGAAGAT GCTGCAACATATTACTGTCAGAATGATTATAGT TATCCGTACACGTTCGGCCAAGGGACCAAGGTG GAAATCAAACGTACGGTGGCTGCACCATCTGTC TTCATCTTCCCGCCATCTGATGAGCAGTTGAAA TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT AACTTCTATCCCAGAGAGGCCAAAGTACAGTGG AAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTAC GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
BAP049-Clone-A HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 38	VH	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN

		RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 90	DNA VH	GAAGTGCAGCTGGTGCAGTCTGGCGCCGAAGTG AAGAAGCCTGGCGAGTCCCTGCGGATCTCCTGC AAGGGCTCTGGCTACACCTTCACCACCTACTGG ATGCACTGGGTGCGACAGGCTACCGGCCAGGGC CTGGAATGGATGGGCAACATCTATCCTGGCACC GGCGGCTCCAACTTCGACGAGAAGTTCAAGAAC AGAGTGACCATCACCGCCGACAAGTCCACCTCC ACCGCCTACATGGAACTGTCCTCCCTGAGATCC GAGGACACCGCCGTGTACTACTGCACCCGGTGG ACAACCGGCACAGGCGCTTATTGGGGCCAGGGC ACCACAGTGACCGTGTCCTCT
SEQ ID NO: 91	HC	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLG
SEQ ID NO: 92	DNA HC	GAAGTGCAGCTGGTGCAGTCTGGCGCCGAAGTG AAGAAGCCTGGCGAGTCCCTGCGGATCTCCTGC AAGGGCTCTGGCTACACCTTCACCACCTACTGG ATGCACTGGGTGCGACAGGCTACCGGCCAGGGC CTGGAATGGATGGGCAACATCTATCCTGGCACC GGCGGCTCCAACTTCGACGAGAAGTTCAAGAAC AGAGTGACCATCACCGCCGACAAGTCCACCTCC ACCGCCTACATGGAACTGTCCTCCCTGAGATCC GAGGACACCGCCGTGTACTACTGCACCCGGTGG ACAACCGGCACAGGCGCTTATTGGGGCCAGGGC ACCACAGTGACCGTGTCCTCTGCTTCTACCAAG GGGCCCAGCGTGTTCCCCCTGGCCCCCTGCTCC AGAAGCACCAGCGAGAGCACAGCCGCCCTGGGC TGCCTGGTGAAGGACTACTTCCCCGAGCCCGTG ACCGTGTCCTGGAACAGCGGAGCCCTGACCAGC GGCGTGCACACCTTCCCCGCCGTGCTGCAGAGC AGCGGCCTGTACAGCCTGAGCAGCGTGGTGACC GTGCCCAGCAGCAGCCTGGGCACCAAGACCTAC ACCTGTAACGTGGACCACAAGCCCAGCAACACC AAGGTGGACAAGAGGGTGGAGAGCAAGTACGGC CCACCCTGCCCCCCCTGCCCAGCCCCCGAGTTC CTGGGCGGACCCAGCGTGTTCCTGTTCCCCCCC AAGCCCAAGGACACCCTGATGATCAGCAGAACC CCCGAGGTGACCTGTGTGGTGGTGGACGTGTCC CAGGAGGACCCCGAGGTCCAGTTCAACTGGTAC GTGGACGGCGTGGAGGTGCACAACGCCAAGACC AAGCCCAGAGAGGAGCAGTTTAACAGCACCTAC CGGGTGGTGTCCGTGCTGACCGTGCTGCACCAG GACTGGCTGAACGGCAAAGAGTACAAGTGTAAG

		GTCTCCAACAAGGGCCTGCCAAGCAGCATCGAA AAGACCATCAGCAAGGCCAAGGGCCAGCCTAGA GAGCCCCAGGTCTACACCCTGCCACCCAGCCAA GAGGAGATGACCAAGAACCAGGTGTCCCTGACC TGTCTGGTGAAGGGCTTCTACCCAAGCGACATC GCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAG AACAACTACAAGACCACCCCCCCAGTGCTGGAC AGCGACGGCAGCTTCTTCCTGTACAGCAGGCTG ACCGTGGACAAGTCCAGATGGCAGGAGGGCAAC GTCTTTAGCTGCTCCGTGATGCACGAGGCCCTG CACAACCACTACACCCAGAAGAGCCTGAGCCTG TCCCTGGGC
BAP049-Clone-A LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF
SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 42	VL	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTEFTLTISSLQPDDFATYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 93	DNA VL	GAGATCGTGCTGACCCAGTCCCCTGCCACCCTG TCACTGTCTCCAGGCGAGAGAGCTACCCTGTCC TGCAAGTCCTCCCAGTCCCTGCTGGACTCCGGC AACCAGAAGAACTTCCTGACCTGGTATCAGCAG AAGCCCGGCCAGGCCCCCAGACTGCTGATCTAC TGGGCCTCCACCCGGGAATCTGGCGTGCCCTCT AGATTCTCCGGCTCCGGCTCTGGCACCGAGTTT ACCCTGACCATCTCCAGCCTGCAGCCCGACGAC TTCGCCACCTACTACTGCCAGAACGACTACTCC TACCCCTACACCTTCGGCCAGGGCACCAAGGTG GAAATCAAG
SEQ ID NO: 44	LC	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTEFTLTISSLQPDDFATYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 94	DNA LC	GAGATCGTGCTGACCCAGTCCCCTGCCACCCTG TCACTGTCTCCAGGCGAGAGAGCTACCCTGTCC TGCAAGTCCTCCCAGTCCCTGCTGGACTCCGGC AACCAGAAGAACTTCCTGACCTGGTATCAGCAG AAGCCCGGCCAGGCCCCCAGACTGCTGATCTAC TGGGCCTCCACCCGGGAATCTGGCGTGCCCTCT AGATTCTCCGGCTCCGGCTCTGGCACCGAGTTT ACCCTGACCATCTCCAGCCTGCAGCCCGACGAC TTCGCCACCTACTACTGCCAGAACGACTACTCC TACCCCTACACCTTCGGCCAGGGCACCAAGGTG GAAATCAAGCGTACGGTGGCCGCTCCCAGCGTG TTCATCTTCCCCCCAAGCGACGAGCAGCTGAAG AGCGGCACCGCCAGCGTGGTGTGTCTGCTGAAC

		AACTTCTACCCCAGGGAGGCCAAGGTGCAGTGG AAGGTGGACAACGCCCTGCAGAGCGGCAACAGC CAGGAGAGCGTCACCGAGCAGGACAGCAAGGAC TCCACCTACAGCCTGAGCAGCACCCTGACCCTG AGCAAGGCCGACTACGAGAAGCACAAGGTGTAC GCCTGTGAGGTGACCCACCAGGGCCTGTCCAGC CCCGTGACCAAGAGCTTCAACAGGGGCGAGTGC
BAP049-Clone-B HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 38	VH	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 95	DNA VH	GAGGTGCAGCTGGTGCAGTCAGGCGCCGAAGTG AAGAAGCCCGGCGAGTCACTGAGAATTAGCTGT AAAGGTTCAGGCTACACCTTCACTACCTACTGG ATGCACTGGGTCCGCCAGGCTACCGGTCAAGGC CTCGAGTGGATGGGTAATATCTACCCCGGCACC GGCGGCTCTAACTTCGACGAGAAGTTTAAGAAT AGAGTGACTATCACCGCCGATAAGTCTACTAGC ACCGCCTATATGGAACTGTCTAGCCTGAGATCA GAGGACACCGCCGTCTACTACTGCACTAGGTGG ACTACCGGCACAGGCGCCTACTGGGGTCAAGGC ACTACCGTGACCGTGTCTAGC
SEQ ID NO: 91	HC	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYILPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLG
SEQ ID NO: 96	DNA HC	GAGGTGCAGCTGGTGCAGTCAGGCGCCGAAGTG AAGAAGCCCGGCGAGTCACTGAGAATTAGCTGT AAAGGTTCAGGCTACACCTTCACTACCTACTGG ATGCACTGGGTCCGCCAGGCTACCGGTCAAGGC CTCGAGTGGATGGGTAATATCTACCCCGGCACC GGCGGCTCTAACTTCGACGAGAAGTTTAAGAAT AGAGTGACTATCACCGCCGATAAGTCTACTAGC ACCGCCTATATGGAACTGTCTAGCCTGAGATCA GAGGACACCGCCGTCTACTACTGCACTAGGTGG ACTACCGGCACAGGCGCCTACTGGGGTCAAGGC ACTACCGTGACCGTGTCTAGCGCTAGCACTAAG

		GGCCCGTCCGTGTTCCCCCTGGCACCTTGTAGC CGGAGCACTAGCGAATCCACCGCTGCCCTCGGC TGCCTGGTCAAGGATTACTTCCCGGAGCCCGTG ACCGTGTCCTGGAACAGCGGAGCCCTGACCTCC GGAGTGCACACCTTCCCCGCTGTGCTGCAGAGC TCCGGGCTGTACTCGCTGTCGTCGGTGGTCACG GTGCCTTCATCTAGCCTGGGTACCAAGACCTAC ACTTGCAACGTGGACCACAAGCCTTCCAACACT AAGGTGGACAAGCGCGTCGAATCGAAGTACGGC CCACCGTGCCCGCCTTGTCCCGCGCCGGAGTTC CTCGGCGGTCCCTCGGTCTTTCTGTTCCCACCG AAGCCCAAGGACACTTTGATGATTTCCCGCACC CCTGAAGTGACATGCGTGGTCGTGGACGTGTCA CAGGAAGATCCGGAGGTGCAGTTCAATTGGTAC GTGGATGGCGTCGAGGTGCACAACGCCAAAACC AAGCCGAGGGAGGAGCAGTTCAACTCCACTTAC CGCGTCGTGTCCGTGCTGACGGTGCTGCATCAG GACTGGCTGAACGGGAAGGAGTACAAGTGCAAA GTGTCCAACAAGGGACTTCCTAGCTCAATCGAA AAGACCATCTCGAAAGCCAAGGGACAGCCCCGG GAACCCCAAGTGTATACCCTGCCACCGAGCCAG GAAGAAATGACTAAGAACCAAGTCTCATTGACT TGCCTTGTGAAGGGCTTCTACCCATCGGATATC GCCGTGGAATGGGAGTCCAACGGCCAGCCGGAA AACAACTACAAGACCACCCCTCCGGTGCTGGAC TCAGACGGATCCTTCTTCCTCTACTCGCGGCTG ACCGTGGATAAGAGCAGATGGCAGGAGGGAAAT GTGTTCAGCTGTTCTGTGATGCATGAAGCCCTG CACAACCACTACACTCAGAAGTCCCTGTCCCTC TCCCTGGGA
BAP049-Clone-B LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF
SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 54	VL	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGKAPKLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLQPEDIATYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 97	DNA VL	GAGATCGTCCTGACTCAGTCACCCGCTACCCTG AGCCTGAGCCCTGGCGAGCGGGCTACACTGAGC TGTAAATCTAGTCAGTCACTGCTGGATAGCGGT AATCAGAAGAACTTCCTGACCTGGTATCAGCAG AAGCCCGGTAAAGCCCCTAAGCTGCTGATCTAC TGGGCCTCTACTAGAGAATCAGGCGTGCCCTCT AGGTTTAGCGGTAGCGGTAGTGGCACCGACTTC ACCTTCACTATCTCTAGCCTGCAGCCCGAGGAT ATCGCTACCTACTACTGTCAGAACGACTATAGC TACCCCTACACCTTCGGTCAAGGCACTAAGGTC GAGATTAAG

SEQ ID NO: 56	LC	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGKAPKLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLQPEDIATYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 98	DNA LC	GAGATCGTCCTGACTCAGTCACCCGCTACCCTG AGCCTGAGCCCTGGCGAGCGGGCTACACTGAGC TGTAAATCTAGTCAGTCACTGCTGGATAGCGGT AATCAGAAGAACTTCCTGACCTGGTATCAGCAG AAGCCCGGTAAAGCCCCTAAGCTGCTGATCTAC TGGGCCTCTACTAGAGAATCAGGCGTGCCCTCT AGGTTTAGCGGTAGCGGTAGTGGCACCGACTTC ACCTTCACTATCTCTAGCCTGCAGCCCGAGGAT ATCGCTACCTACTACTGTCAGAACGACTATAGC TACCCCTACACCTTCGGTCAAGGCACTAAGGTC GAGATTAAGCGTACGGTGGCCGCTCCCAGCGTG TTCATCTTCCCCCCCAGCGACGAGCAGCTGAAG AGCGGCACCGCCAGCGTGGTGTGCCTGCTGAAC AACTTCTACCCCCGGGAGGCCAAGGTGCAGTGG AAGGTGGACAACGCCCTGCAGAGCGGCAACAGC CAGGAGAGCGTCACCGAGCAGGACAGCAAGGAC TCCACCTACAGCCTGAGCAGCACCCTGACCCTG AGCAAGGCCGACTACGAGAAGCATAAGGTGTAC GCCTGCGAGGTGACCCACCAGGGCCTGTCCAGC CCCGTGACCAAGAGCTTCAACAGGGGCGAGTGC
BAP049-Clone-C HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 38	VH	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 90	DNA VH	GAAGTGCAGCTGGTGCAGTCTGGCGCCGAAGTG AAGAAGCCTGGCGAGTCCCTGCGGATCTCCTGC AAGGGCTCTGGCTACACCTTCACCACCTACTGG ATGCACTGGGTGCGACAGGCTACCGGCCAGGGC CTGGAATGGATGGGCAACATCTATCCTGGCACC GGCGGCTCCAACTTCGACGAGAAGTTCAAGAAC AGAGTGACCATCACCGCCGACAAGTCCACCTCC ACCGCCTACATGGAACTGTCCTCCCTGAGATCC GAGGACACCGCCGTGTACTACTGCACCCGGTGG ACAACCGGCACAGGCGCTTATTGGGGCCAGGGC ACCACAGTGACCGTGTCCTCT
SEQ ID NO: 91	HC	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS

		GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLG
SEQ ID NO: 92	DNA HC	GAAGTGCAGCTGGTGCAGTCTGGCGCCGAAGTG AAGAAGCCTGGCGAGTCCCTGCGGATCTCCTGC AAGGGCTCTGGCTACACCTTCACCACCTACTGG ATGCACTGGGTGCGACAGGCTACCGGCCAGGGC CTGGAATGGATGGGCAACATCTATCCTGGCACC GGCGGCTCCAACTTCGACGAGAAGTTCAAGAAC AGAGTGACCATCACCGCCGACAAGTCCACCTCC ACCGCCTACATGGAACTGTCCTCCCTGAGATCC GAGGACACCGCCGTGTACTACTGCACCCGGTGG ACAACCGGCACAGGCGCTTATTGGGGCCAGGGC ACCACAGTGACCGTGTCCTCTGCTTCTACCAAG GGGCCCAGCGTGTTCCCCCTGGCCCCCTGCTCC AGAAGCACCAGCGAGAGCACAGCCGCCCTGGGC TGCCTGGTGAAGGACTACTTCCCCGAGCCCGTG ACCGTGTCCTGGAACAGCGGAGCCCTGACCAGC GGCGTGCACACCTTCCCCGCCGTGCTGCAGAGC AGCGGCCTGTACAGCCTGAGCAGCGTGGTGACC GTGCCCAGCAGCAGCCTGGGCACCAAGACCTAC ACCTGTAACGTGGACCACAAGCCCAGCAACACC AAGGTGGACAAGAGGGTGGAGAGCAAGTACGGC CCACCCTGCCCCCCCTGCCCAGCCCCCGAGTTC CTGGGCGGACCCAGCGTGTTCCTGTTCCCCCCC AAGCCCAAGGACACCCTGATGATCAGCAGAACC CCCGAGGTGACCTGTGTGGTGGTGGACGTGTCC CAGGAGGACCCCGAGGTCCAGTTCAACTGGTAC GTGGACGGCGTGGAGGTGCACAACGCCAAGACC AAGCCCAGAGAGGAGCAGTTTAACAGCACCTAC CGGGTGGTGTCCGTGCTGACCGTGCTGCACCAG GACTGGCTGAACGGCAAAGAGTACAAGTGTAAG GTCTCCAACAAGGGCCTGCCAAGCAGCATCGAA AAGACCATCAGCAAGGCCAAGGGCCAGCCTAGA GAGCCCCAGGTCTACACCCTGCCACCCAGCCAA GAGGAGATGACCAAGAACCAGGTGTCCCTGACC TGTCTGGTGAAGGGCTTCTACCCAAGCGACATC GCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAG AACAACTACAAGACCACCCCCCCAGTGCTGGAC AGCGACGGCAGCTTCTTCCTGTACAGCAGGCTG ACCGTGGACAAGTCCAGATGGCAGGAGGGCAAC GTCTTTAGCTGCTCCGTGATGCACGAGGCCCTG CACAACCACTACACCCAGAAGAGCCTGAGCCTG TCCCTGGGC
BAP049-Clone-C LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF

SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 66	VL	EIVLTQSPDFQSVTPKEKVTITCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 99	DNA VL	GAGATCGTGCTGACCCAGTCCCCCGACTTCCAG TCCGTGACCCCCAAAGAAAAAGTGACCATCACA TGCAAGTCCTCCCAGTCCCTGCTGGACTCCGGC AACCAGAAGAACTTCCTGACCTGGTATCAGCAG AAGCCCGGCCAGGCCCCCAGACTGCTGATCTAC TGGGCCTCCACCCGGGAATCTGGCGTGCCCTCT AGATTCTCCGGCTCCGGCTCTGGCACCGACTTT ACCTTCACCATCTCCAGCCTGGAAGCCGAGGAC GCCGCCACCTACTACTGCCAGAACGACTACTCC TACCCCTACACCTTCGGCCAGGGCACCAAGGTG GAAATCAAG
SEQ ID NO: 68	LC	EIVLTQSPDFQSVTPKEKVTITCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 100	DNA LC	GAGATCGTGCTGACCCAGTCCCCCGACTTCCAG TCCGTGACCCCCAAAGAAAAAGTGACCATCACA TGCAAGTCCTCCCAGTCCCTGCTGGACTCCGGC AACCAGAAGAACTTCCTGACCTGGTATCAGCAG AAGCCCGGCCAGGCCCCCAGACTGCTGATCTAC TGGGCCTCCACCCGGGAATCTGGCGTGCCCTCT AGATTCTCCGGCTCCGGCTCTGGCACCGACTTT ACCTTCACCATCTCCAGCCTGGAAGCCGAGGAC GCCGCCACCTACTACTGCCAGAACGACTACTCC TACCCCTACACCTTCGGCCAGGGCACCAAGGTG GAAATCAAGCGTACGGTGGCCGCTCCCAGCGTG TTCATCTTCCCCCCAAGCGACGAGCAGCTGAAG AGCGGCACCGCCAGCGTGGTGTGTCTGCTGAAC AACTTCTACCCCAGGGAGGCCAAGGTGCAGTGG AAGGTGGACAACGCCCTGCAGAGCGGCAACAGC CAGGAGAGCGTCACCGAGCAGGACAGCAAGGAC TCCACCTACAGCCTGAGCAGCACCCTGACCCTG AGCAAGGCCGACTACGAGAAGCACAAGGTGTAC GCCTGTGAGGTGACCCACCAGGGCCTGTCCAGC CCCGTGACCAAGAGCTTCAACAGGGGCGAGTGC
BAP049-Clone-D HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 50	VH	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWIRQSPSRGLEWLGNIYPGTGGSNFDEKFKN

		RFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 101	DNA VH	GAAGTGCAGCTGGTGCAGTCTGGCGCCGAAGTG AAGAAGCCTGGCGAGTCCCTGCGGATCTCCTGC AAGGGCTCTGGCTACACCTTCACCACCTACTGG ATGCACTGGATCCGGCAGTCCCCCTCTAGGGGC CTGGAATGGCTGGGCAACATCTACCCTGGCACC GGCGGCTCCAACTTCGACGAGAAGTTCAAGAAC AGGTTCACCATCTCCCGGGACAACTCCAAGAAC ACCCTGTACCTGCAGATGAACTCCCTGCGGGCC GAGGACACCGCCGTGTACTACTGTACCAGATGG ACCACCGGAACCGGCGCCTATTGGGGCCAGGGC ACAACAGTGACCGTGTCCTCC
SEQ ID NO: 102	HC	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWIRQSPSRGLEWLGNIYPGTGGSNFDEKFKN RFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLG
SEQ ID NO: 103	DNA HC	GAAGTGCAGCTGGTGCAGTCTGGCGCCGAAGTG AAGAAGCCTGGCGAGTCCCTGCGGATCTCCTGC AAGGGCTCTGGCTACACCTTCACCACCTACTGG ATGCACTGGATCCGGCAGTCCCCCTCTAGGGGC CTGGAATGGCTGGGCAACATCTACCCTGGCACC GGCGGCTCCAACTTCGACGAGAAGTTCAAGAAC AGGTTCACCATCTCCCGGGACAACTCCAAGAAC ACCCTGTACCTGCAGATGAACTCCCTGCGGGCC GAGGACACCGCCGTGTACTACTGTACCAGATGG ACCACCGGAACCGGCGCCTATTGGGGCCAGGGC ACAACAGTGACCGTGTCCTCCGCTTCTACCAAG GGGCCCAGCGTGTTCCCCCTGGCCCCCTGCTCC AGAAGCACCAGCGAGAGCACAGCCGCCCTGGGC TGCCTGGTGAAGGACTACTTCCCCGAGCCCGTG ACCGTGTCCTGGAACAGCGGAGCCCTGACCAGC GGCGTGCACACCTTCCCCGCCGTGCTGCAGAGC AGCGGCCTGTACAGCCTGAGCAGCGTGGTGACC GTGCCCAGCAGCAGCCTGGGCACCAAGACCTAC ACCTGTAACGTGGACCACAAGCCCAGCAACACC AAGGTGGACAAGAGGGTGGAGAGCAAGTACGGC CCACCCTGCCCCCCCTGCCCAGCCCCCGAGTTC CTGGGCGGACCCAGCGTGTTCCTGTTCCCCCCC AAGCCCAAGGACACCCTGATGATCAGCAGAACC CCCGAGGTGACCTGTGTGGTGGTGGACGTGTCC CAGGAGGACCCCGAGGTCCAGTTCAACTGGTAC GTGGACGGCGTGGAGGTGCACAACGCCAAGACC AAGCCCAGAGAGGAGCAGTTTAACAGCACCTAC CGGGTGGTGTCCGTGCTGACCGTGCTGCACCAG GACTGGCTGAACGGCAAAGAGTACAAGTGTAAG

		GTCTCCAACAAGGGCCTGCCAAGCAGCATCGAA AAGACCATCAGCAAGGCCAAGGGCCAGCCTAGA GAGCCCCAGGTCTACACCCTGCCACCCAGCCAA GAGGAGATGACCAAGAACCAGGTGTCCCTGACC TGTCTGGTGAAGGGCTTCTACCCAAGCGACATC GCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAG AACAACTACAAGACCACCCCCCCAGTGCTGGAC AGCGACGGCAGCTTCTTCCTGTACAGCAGGCTG ACCGTGGACAAGTCCAGATGGCAGGAGGGCAAC GTCTTTAGCTGCTCCGTGATGCACGAGGCCCTG CACAACCACTACACCCAGAAGAGCCTGAGCCTG TCCCTGGGC
BAP049-Clone-D LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF
SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 70	VL	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 104	DNA VL	GAGATCGTGCTGACCCAGTCCCCTGCCACCCTG TCACTGTCTCCAGGCGAGAGAGCTACCCTGTCC TGCAAGTCCTCCCAGTCCCTGCTGGACTCCGGC AACCAGAAGAACTTCCTGACCTGGTATCAGCAG AAGCCCGGCCAGGCCCCCAGACTGCTGATCTAC TGGGCCTCCACCCGGGAATCTGGCGTGCCCTCT AGATTCTCCGGCTCCGGCTCTGGCACCGACTTT ACCTTCACCATCTCCAGCCTGGAAGCCGAGGAC GCCGCCACCTACTACTGCCAGAACGACTACTCC TACCCCTACACCTTCGGCCAGGGCACCAAGGTG GAAATCAAG
SEQ ID NO: 72	LC	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 105	DNA LC	GAGATCGTGCTGACCCAGTCCCCTGCCACCCTG TCACTGTCTCCAGGCGAGAGAGCTACCCTGTCC TGCAAGTCCTCCCAGTCCCTGCTGGACTCCGGC AACCAGAAGAACTTCCTGACCTGGTATCAGCAG AAGCCCGGCCAGGCCCCCAGACTGCTGATCTAC TGGGCCTCCACCCGGGAATCTGGCGTGCCCTCT AGATTCTCCGGCTCCGGCTCTGGCACCGACTTT ACCTTCACCATCTCCAGCCTGGAAGCCGAGGAC GCCGCCACCTACTACTGCCAGAACGACTACTCC TACCCCTACACCTTCGGCCAGGGCACCAAGGTG GAAATCAAGCGTACGGTGGCCGCTCCCAGCGTG TTCATCTTCCCCCCAAGCGACGAGCAGCTGAAG AGCGGCACCGCCAGCGTGGTGTGTCTGCTGAAC

		AACTTCTACCCCAGGGAGGCCAAGGTGCAGTGG AAGGTGGACAACGCCCTGCAGAGCGGCAACAGC CAGGAGAGCGTCACCGAGCAGGACAGCAAGGAC TCCACCTACAGCCTGAGCAGCACCCTGACCCTG AGCAAGGCCGACTACGAGAAGCACAAGGTGTAC GCCTGTGAGGTGACCCACCAGGGCCTGTCCAGC CCCGTGACCAAGAGCTTCAACAGGGGCGAGTGC
BAP049-Clone-E HC		
SEQ ID NO: 1 (Kabat)	HCDR1	TYWMH
SEQ ID NO: 2 (Kabat)	HCDR2	NIYPGTGGSNFDEKFKN
SEQ ID NO: 3 (Kabat)	HCDR3	WTTGTGAY
SEQ ID NO: 4 (Chothia)	HCDR1	GYTFTTY
SEQ ID NO: 5 (Chothia)	HCDR2	YPGTGG
SEQ ID NO: 3 (Chothia)	HCDR3	WTTGTGAY
SEQ ID NO: 38	VH	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSS
SEQ ID NO: 95	DNA VH	GAGGTGCAGCTGGTGCAGTCAGGCGCCGAAGTG AAGAAGCCCGGCGAGTCACTGAGAATTAGCTGT AAAGGTTCAGGCTACACCTTCACTACCTACTGG ATGCACTGGGTCCGCCAGGCTACCGGTCAAGGC CTCGAGTGGATGGGTAATATCTACCCCGGCACC GGCGGCTCTAACTTCGACGAGAAGTTTAAGAAT AGAGTGACTATCACCGCCGATAAGTCTACTAGC ACCGCCTATATGGAACTGTCTAGCCTGAGATCA GAGGACACCGCCGTCTACTACTGCACTAGGTGG ACTACCGGCACAGGCGCCTACTGGGGTCAAGGC ACTACCGTGACCGTGTCTAGC
SEQ ID NO: 91	HC	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYW MHWVRQATGQGLEWMGNIYPGTGGSNFDEKFKN RVTITADKSTSTAYMELSSLRSEDTAVYYCTRW TTGTGAYWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYILPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLG
SEQ ID NO: 96	DNA HC	GAGGTGCAGCTGGTGCAGTCAGGCGCCGAAGTG AAGAAGCCCGGCGAGTCACTGAGAATTAGCTGT AAAGGTTCAGGCTACACCTTCACTACCTACTGG ATGCACTGGGTCCGCCAGGCTACCGGTCAAGGC CTCGAGTGGATGGGTAATATCTACCCCGGCACC GGCGGCTCTAACTTCGACGAGAAGTTTAAGAAT AGAGTGACTATCACCGCCGATAAGTCTACTAGC ACCGCCTATATGGAACTGTCTAGCCTGAGATCA GAGGACACCGCCGTCTACTACTGCACTAGGTGG ACTACCGGCACAGGCGCCTACTGGGGTCAAGGC ACTACCGTGACCGTGTCTAGCGCTAGCACTAAG

		GGCCCGTCCGTGTTCCCCCTGGCACCTTGTAGC CGGAGCACTAGCGAATCCACCGCTGCCCTCGGC TGCCTGGTCAAGGATTACTTCCCGGAGCCCGTG ACCGTGTCCTGGAACAGCGGAGCCCTGACCTCC GGAGTGCACACCTTCCCCGCTGTGCTGCAGAGC TCCGGGCTGTACTCGCTGTCGTCGGTGGTCACG GTGCCTTCATCTAGCCTGGGTACCAAGACCTAC ACTTGCAACGTGGACCACAAGCCTTCCAACACT AAGGTGGACAAGCGCGTCGAATCGAAGTACGGC CCACCGTGCCCGCCTTGTCCCGCGCCGGAGTTC CTCGGCGGTCCCTCGGTCTTTCTGTTCCCACCG AAGCCCAAGGACACTTTGATGATTTCCCGCACC CCTGAAGTGACATGCGTGGTCGTGGACGTGTCA CAGGAAGATCCGGAGGTGCAGTTCAATTGGTAC GTGGATGGCGTCGAGGTGCACAACGCCAAAACC AAGCCGAGGGAGGAGCAGTTCAACTCCACTTAC CGCGTCGTGTCCGTGCTGACGGTGCTGCATCAG GACTGGCTGAACGGGAAGGAGTACAAGTGCAAA GTGTCCAACAAGGGACTTCCTAGCTCAATCGAA AAGACCATCTCGAAAGCCAAGGGACAGCCCCGG GAACCCCAAGTGTATACCCTGCCACCGAGCCAG GAAGAAATGACTAAGAACCAAGTCTCATTGACT TGCCTTGTGAAGGGCTTCTACCCATCGGATATC GCCGTGGAATGGGAGTCCAACGGCCAGCCGGAA AACAACTACAAGACCACCCCTCCGGTGCTGGAC TCAGACGGATCCTTCTTCCTCTACTCGCGGCTG ACCGTGGATAAGAGCAGATGGCAGGAGGGAAAT GTGTTCAGCTGTTCTGTGATGCATGAAGCCCTG CACAACCACTACACTCAGAAGTCCCTGTCCCTC TCCCTGGGA
BAP049-Clone-E LC		
SEQ ID NO: 10 (Kabat)	LCDR1	KSSQSLLDSGNQKNFLT
SEQ ID NO: 11 (Kabat)	LCDR2	WASTRES
SEQ ID NO: 32 (Kabat)	LCDR3	QNDYSYPYT
SEQ ID NO: 13 (Chothia)	LCDR1	SQSLLDSGNQKNF
SEQ ID NO: 14 (Chothia)	LCDR2	WAS
SEQ ID NO: 33 (Chothia)	LCDR3	DYSYPY
SEQ ID NO: 70	VL	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIK
SEQ ID NO: 106	DNA VL	GAGATCGTCCTGACTCAGTCACCCGCTACCCTG AGCCTGAGCCCTGGCGAGCGGGCTACACTGAGC TGTAAATCTAGTCAGTCACTGCTGGATAGCGGT AATCAGAAGAACTTCCTGACCTGGTATCAGCAG AAGCCCGGTCAAGCCCCTAGACTGCTGATCTAC TGGGCCTCTACTAGAGAATCAGGCGTGCCCTCT AGGTTTAGCGGTAGCGGTAGTGGCACCGACTTC ACCTTCACTATCTCTAGCCTGGAAGCCGAGGAC GCCGCTACCTACTACTGTCAGAACGACTATAGC TACCCCTACACCTTCGGTCAAGGCACTAAGGTC GAGATTAAG

SEQ ID NO: 72	LC	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSG NQKNFLTWYQQKPGQAPRLLIYWASTRESGVPS RFSGSGSGTDFTFTISSLEAEDAATYYCQNDYS YPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 107	DNA LC	GAGATCGTCCTGACTCAGTCACCCGCTACCCTG AGCCTGAGCCCTGGCGAGCGGGCTACACTGAGC TGTAAATCTAGTCAGTCACTGCTGGATAGCGGT AATCAGAAGAACTTCCTGACCTGGTATCAGCAG AAGCCCGGTCAAGCCCCTAGACTGCTGATCTAC TGGGCCTCTACTAGAGAATCAGGCGTGCCCTCT AGGTTTAGCGGTAGCGGTAGTGGCACCGACTTC ACCTTCACTATCTCTAGCCTGGAAGCCGAGGAC GCCGCTACCTACTACTGTCAGAACGACTATAGC TACCCCTACACCTTCGGTCAAGGCACTAAGGTC GAGATTAAGCGTACGGTGGCCGCTCCCAGCGTG TTCATCTTCCCCCCCAGCGACGAGCAGCTGAAG AGCGGCACCGCCAGCGTGGTGTGCCTGCTGAAC AACTTCTACCCCCGGGAGGCCAAGGTGCAGTGG AAGGTGGACAACGCCCTGCAGAGCGGCAACAGC CAGGAGAGCGTCACCGAGCAGGACAGCAAGGAC TCCACCTACAGCCTGAGCAGCACCCTGACCCTG AGCAAGGCCGACTACGAGAAGCATAAGGTGTAC GCCTGCGAGGTGACCCACCAGGGCCTGTCCAGC CCCGTGACCAAGAGCTTCAACAGGGGCGAGTGC
BAP049 HC		
SEQ ID NO: 108 (Kabat)	HCDR1	ACTTACTGGATGCAC
SEQ ID NO: 109 (Kabat)	HCDR2	AATATTTATCCTGGTACTGGTGGTTCTAACTTC GATGAGAAGTTCAAGAAC
SEQ ID NO: 110 (Kabat)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
SEQ ID NO: 111 (Chothia)	HCDR1	GGCTACACATTCACCACTTAC
SEQ ID NO: 112 (Chothia)	HCDR2	TATCCTGGTACTGGTGGT
SEQ ID NO: 110 (Chothia)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
BAP049 LC		
SEQ ID NO: 113 (Kabat)	LCDR1	AAGTCCAGTCAGAGTCTGTTAGACAGTGGAAAT CAAAAGAACTTCTTGACC
SEQ ID NO: 114 (Kabat)	LCDR2	TGGGCATCCACTAGGGAATCT
SEQ ID NO: 115 (Kabat)	LCDR3	CAGAATGATTATAGTTATCCGTGCACG
SEQ ID NO: 116 (Chothia)	LCDR1	AGTCAGAGTCTGTTAGACAGTGGAAATCAAAAG AACTTC
SEQ ID NO: 117 (Chothia)	LCDR2	TGGGCATCC
SEQ ID NO: 118 (Chothia)	LCDR3	GATTATAGTTATCCGTGC
BAP049-chi HC		
SEQ ID NO: 108 (Kabat)	HCDR1	ACTTACTGGATGCAC
SEQ ID NO: 109 (Kabat)	HCDR2	AATATTTATCCTGGTACTGGTGGTTCTAACTTC GATGAGAAGTTCAAGAAC
SEQ ID NO: 110 (Kabat)	HCDR3	TGGACTACTGGGACGGGAGCTTAT

SEQ ID NO: 111 (Chothia)	HCDR1	GGCTACACATTCACCACTTAC
SEQ ID NO: 112 (Chothia)	HCDR2	TATCCTGGTACTGGTGGT
SEQ ID NO: 110 (Chothia)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
BAP049-chi LC		
SEQ ID NO: 113 (Kabat)	LCDR1	AAGTCCAGTCAGAGTCTGTTAGACAGTGGAAAT CAAAAGAACTTCTTGACC
SEQ ID NO: 114 (Kabat)	LCDR2	TGGGCATCCACTAGGGAATCT
SEQ ID NO: 115 (Kabat)	LCDR3	CAGAATGATTATAGTTATCCGTGCACG
SEQ ID NO: 116 (Chothia)	LCDR1	AGTCAGAGTCTGTTAGACAGTGGAAATCAAAAG AACTTC
SEQ ID NO: 117 (Chothia)	LCDR2	TGGGCATCC
SEQ ID NO: 118 (Chothia)	LCDR3	GATTATAGTTATCCGTGC
BAP049-chi Y HC		
SEQ ID NO: 108 (Kabat)	HCDR1	ACTTACTGGATGCAC
SEQ ID NO: 109 (Kabat)	HCDR2	AATATTTATCCTGGTACTGGTGGTTCTAACTTC GATGAGAAGTTCAAGAAC
SEQ ID NO: 110 (Kabat)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
SEQ ID NO: 111 (Chothia)	HCDR1	GGCTACACATTCACCACTTAC
SEQ ID NO: 112 (Chothia)	HCDR2	TATCCTGGTACTGGTGGT
SEQ ID NO: 110 (Chothia)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
BAP049-chi Y LC		
SEQ ID NO: 113 (Kabat)	LCDR1	AAGTCCAGTCAGAGTCTGTTAGACAGTGGAAAT CAAAAGAACTTCTTGACC
SEQ ID NO: 114 (Kabat)	LCDR2	TGGGCATCCACTAGGGAATCT
SEQ ID NO: 119 (Kabat)	LCDR3	CAGAATGATTATAGTTATCCGTACACG
SEQ ID NO: 116 (Chothia)	LCDR1	AGTCAGAGTCTGTTAGACAGTGGAAATCAAAAG AACTTC
SEQ ID NO: 117 (Chothia)	LCDR2	TGGGCATCC
SEQ ID NO: 120 (Chothia)	LCDR3	GATTATAGTTATCCGTAC
BAP049-hum01 HC		
SEQ ID NO: 108 (Kabat)	HCDR1	ACTTACTGGATGCAC
SEQ ID NO: 109 (Kabat)	HCDR2	AATATTTATCCTGGTACTGGTGGTTCTAACTTC GATGAGAAGTTCAAGAAC
SEQ ID NO: 110 (Kabat)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
SEQ ID NO: 111 (Chothia)	HCDR1	GGCTACACATTCACCACTTAC
SEQ ID NO: 112 (Chothia)	HCDR2	TATCCTGGTACTGGTGGT
SEQ ID NO: 110 (Chothia)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
BAP049-hum01 LC		
SEQ ID NO: 113 (Kabat)	LCDR1	AAGTCCAGTCAGAGTCTGTTAGACAGTGGAAAT CAAAAGAACTTCTTGACC
SEQ ID NO: 114 (Kabat)	LCDR2	TGGGCATCCACTAGGGAATCT
SEQ ID NO: 119 (Kabat)	LCDR3	CAGAATGATTATAGTTATCCGTACACG
SEQ ID NO: 116 (Chothia)	LCDR1	AGTCAGAGTCTGTTAGACAGTGGAAATCAAAAG AACTTC
SEQ ID NO: 117 (Chothia)	LCDR2	TGGGCATCC

SEQ ID NO: 120 (Chothia)	LCDR3	GATTATAGTTATCCGTAC
BAP049-hum02 HC		
SEQ ID NO: 108 (Kabat)	HCDR1	ACTTACTGGATGCAC
SEQ ID NO: 109 (Kabat)	HCDR2	AATATTTATCCTGGTACTGGTGGTTCTAACTTC GATGAGAAGTTCAAGAAC
SEQ ID NO: 110 (Kabat)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
SEQ ID NO: 111 (Chothia)	HCDR1	GGCTACACATTCACCACTTAC
SEQ ID NO: 112 (Chothia)	HCDR2	TATCCTGGTACTGGTGGT
SEQ ID NO: 110 (Chothia)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
BAP049-hum02 LC		
SEQ ID NO: 113 (Kabat)	LCDR1	AAGTCCAGTCAGAGTCTGTTAGACAGTGGAAAT CAAAAGAACTTCTTGACC
SEQ ID NO: 114 (Kabat)	LCDR2	TGGGCATCCACTAGGGAATCT
SEQ ID NO: 119 (Kabat)	LCDR3	CAGAATGATTATAGTTATCCGTACACG
SEQ ID NO: 116 (Chothia)	LCDR1	AGTCAGAGTCTGTTAGACAGTGGAAATCAAAAG AACTTC
SEQ ID NO: 117 (Chothia)	LCDR2	TGGGCATCC
SEQ ID NO: 120 (Chothia)	LCDR3	GATTATAGTTATCCGTAC
BAP049-hum03 HC		
SEQ ID NO: 108 (Kabat)	HCDR1	ACTTACTGGATGCAC
SEQ ID NO: 109 (Kabat)	HCDR2	AATATTTATCCTGGTACTGGTGGTTCTAACTTC GATGAGAAGTTCAAGAAC
SEQ ID NO: 110 (Kabat)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
SEQ ID NO: 111 (Chothia)	HCDR1	GGCTACACATTCACCACTTAC
SEQ ID NO: 112 (Chothia)	HCDR2	TATCCTGGTACTGGTGGT
SEQ ID NO: 110 (Chothia)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
BAP049-hum03 LC		
SEQ ID NO: 113 (Kabat)	LCDR1	AAGTCCAGTCAGAGTCTGTTAGACAGTGGAAAT CAAAAGAACTTCTTGACC
SEQ ID NO: 114 (Kabat)	LCDR2	TGGGCATCCACTAGGGAATCT
SEQ ID NO: 119 (Kabat)	LCDR3	CAGAATGATTATAGTTATCCGTACACG
SEQ ID NO: 116 (Chothia)	LCDR1	AGTCAGAGTCTGTTAGACAGTGGAAATCAAAAG AACTTC
SEQ ID NO: 117 (Chothia)	LCDR2	TGGGCATCC
SEQ ID NO: 120 (Chothia)	LCDR3	GATTATAGTTATCCGTAC
BAP049-hum04 HC		
SEQ ID NO: 108 (Kabat)	HCDR1	ACTTACTGGATGCAC
SEQ ID NO: 109 (Kabat)	HCDR2	AATATTTATCCTGGTACTGGTGGTTCTAACTTC GATGAGAAGTTCAAGAAC
SEQ ID NO: 110 (Kabat)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
SEQ ID NO: 111 (Chothia)	HCDR1	GGCTACACATTCACCACTTAC
SEQ ID NO: 112 (Chothia)	HCDR2	TATCCTGGTACTGGTGGT
SEQ ID NO: 110 (Chothia)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
BAP049-hum04 LC		

SEQ ID NO: 113 (Kabat)	LCDR1	AAGTCCAGTCAGAGTCTGTTAGACAGTGGAAAT CAAAAGAACTTCTTGACC
SEQ ID NO: 114 (Kabat)	LCDR2	TGGGCATCCACTAGGGAATCT
SEQ ID NO: 119 (Kabat)	LCDR3	CAGAATGATTATAGTTATCCGTACACG
SEQ ID NO: 116 (Chothia)	LCDR1	AGTCAGAGTCTGTTAGACAGTGGAAATCAAAAG AACTTC
SEQ ID NO: 117 (Chothia)	LCDR2	TGGGCATCC
SEQ ID NO: 120 (Chothia)	LCDR3	GATTATAGTTATCCGTAC
BAP049-hum05 HC		
SEQ ID NO: 108 (Kabat)	HCDR1	ACTTACTGGATGCAC
SEQ ID NO: 109 (Kabat)	HCDR2	AATATTTATCCTGGTACTGGTGGTTCTAACTTC GATGAGAAGTTCAAGAAC
SEQ ID NO: 110 (Kabat)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
SEQ ID NO: 111 (Chothia)	HCDR1	GGCTACACATTCACCACTTAC
SEQ ID NO: 112 (Chothia)	HCDR2	TATCCTGGTACTGGTGGT
SEQ ID NO: 110 (Chothia)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
BAP049-hum05 LC		
SEQ ID NO: 113 (Kabat)	LCDR1	AAGTCCAGTCAGAGTCTGTTAGACAGTGGAAAT CAAAAGAACTTCTTGACC
SEQ ID NO: 114 (Kabat)	LCDR2	TGGGCATCCACTAGGGAATCT
SEQ ID NO: 119 (Kabat)	LCDR3	CAGAATGATTATAGTTATCCGTACACG
SEQ ID NO: 116 (Chothia)	LCDR1	AGTCAGAGTCTGTTAGACAGTGGAAATCAAAAG AACTTC
SEQ ID NO: 117 (Chothia)	LCDR2	TGGGCATCC
SEQ ID NO: 120 (Chothia)	LCDR3	GATTATAGTTATCCGTAC
BAP049-hum06 HC		
SEQ ID NO: 108 (Kabat)	HCDR1	ACTTACTGGATGCAC
SEQ ID NO: 109 (Kabat)	HCDR2	AATATTTATCCTGGTACTGGTGGTTCTAACTTC GATGAGAAGTTCAAGAAC
SEQ ID NO: 110 (Kabat)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
SEQ ID NO: 111 (Chothia)	HCDR1	GGCTACACATTCACCACTTAC
SEQ ID NO: 112 (Chothia)	HCDR2	TATCCTGGTACTGGTGGT
SEQ ID NO: 110 (Chothia)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
BAP049-hum06 LC		
SEQ ID NO: 113 (Kabat)	LCDR1	AAGTCCAGTCAGAGTCTGTTAGACAGTGGAAAT CAAAAGAACTTCTTGACC
SEQ ID NO: 114 (Kabat)	LCDR2	TGGGCATCCACTAGGGAATCT
SEQ ID NO: 119 (Kabat)	LCDR3	CAGAATGATTATAGTTATCCGTACACG
SEQ ID NO: 116 (Chothia)	LCDR1	AGTCAGAGTCTGTTAGACAGTGGAAATCAAAAG AACTTC
SEQ ID NO: 117 (Chothia)	LCDR2	TGGGCATCC
SEQ ID NO: 120 (Chothia)	LCDR3	GATTATAGTTATCCGTAC
BAP049-hum07 HC		
SEQ ID NO: 108 (Kabat)	HCDR1	ACTTACTGGATGCAC
SEQ ID NO: 109 (Kabat)	HCDR2	AATATTTATCCTGGTACTGGTGGTTCTAACTTC GATGAGAAGTTCAAGAAC

SEQ ID NO: 110 (Kabat)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
SEQ ID NO: 111 (Chothia)	HCDR1	GGCTACACATTCACCACTTAC
SEQ ID NO: 112 (Chothia)	HCDR2	TATCCTGGTACTGGTGGT
SEQ ID NO: 110 (Chothia)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
BAP049-hum07 LC		
SEQ ID NO: 113 (Kabat)	LCDR1	AAGTCCAGTCAGAGTCTGTTAGACAGTGGAAAT CAAAAGAACTTCTTGACC
SEQ ID NO: 114 (Kabat)	LCDR2	TGGGCATCCACTAGGGAATCT
SEQ ID NO: 119 (Kabat)	LCDR3	CAGAATGATTATAGTTATCCGTACACG
SEQ ID NO: 116 (Chothia)	LCDR1	AGTCAGAGTCTGTTAGACAGTGGAAATCAAAAG AACTTC
SEQ ID NO: 117 (Chothia)	LCDR2	TGGGCATCC
SEQ ID NO: 120 (Chothia)	LCDR3	GATTATAGTTATCCGTAC
BAP049-hum08 HC		
SEQ ID NO: 108 (Kabat)	HCDR1	ACTTACTGGATGCAC
SEQ ID NO: 109 (Kabat)	HCDR2	AATATTTATCCTGGTACTGGTGGTTCTAACTTC GATGAGAAGTTCAAGAAC
SEQ ID NO: 110 (Kabat)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
SEQ ID NO: 111 (Chothia)	HCDR1	GGCTACACATTCACCACTTAC
SEQ ID NO: 112 (Chothia)	HCDR2	TATCCTGGTACTGGTGGT
SEQ ID NO: 110 (Chothia)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
BAP049-hum08 LC		
SEQ ID NO: 113 (Kabat)	LCDR1	AAGTCCAGTCAGAGTCTGTTAGACAGTGGAAAT CAAAAGAACTTCTTGACC
SEQ ID NO: 114 (Kabat)	LCDR2	TGGGCATCCACTAGGGAATCT
SEQ ID NO: 119 (Kabat)	LCDR3	CAGAATGATTATAGTTATCCGTACACG
SEQ ID NO: 116 (Chothia)	LCDR1	AGTCAGAGTCTGTTAGACAGTGGAAATCAAAAG AACTTC
SEQ ID NO: 117 (Chothia)	LCDR2	TGGGCATCC
SEQ ID NO: 120 (Chothia)	LCDR3	GATTATAGTTATCCGTAC
BAP049-hum09 HC		
SEQ ID NO: 108 (Kabat)	HCDR1	ACTTACTGGATGCAC
SEQ ID NO: 109 (Kabat)	HCDR2	AATATTTATCCTGGTACTGGTGGTTCTAACTTC GATGAGAAGTTCAAGAAC
SEQ ID NO: 110 (Kabat)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
SEQ ID NO: 111 (Chothia)	HCDR1	GGCTACACATTCACCACTTAC
SEQ ID NO: 112 (Chothia)	HCDR2	TATCCTGGTACTGGTGGT
SEQ ID NO: 110 (Chothia)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
BAP049-hum09 LC		
SEQ ID NO: 113 (Kabat)	LCDR1	AAGTCCAGTCAGAGTCTGTTAGACAGTGGAAAT CAAAAGAACTTCTTGACC
SEQ ID NO: 114 (Kabat)	LCDR2	TGGGCATCCACTAGGGAATCT
SEQ ID NO: 119 (Kabat)	LCDR3	CAGAATGATTATAGTTATCCGTACACG
SEQ ID NO: 116 (Chothia)	LCDR1	AGTCAGAGTCTGTTAGACAGTGGAAATCAAAAG AACTTC

SEQ ID NO: 117 (Chothia)	LCDR2	TGGGCATCC
SEQ ID NO: 120 (Chothia)	LCDR3	GATTATAGTTATCCGTAC
BAP049-hum10 HC		
SEQ ID NO: 108 (Kabat)	HCDR1	ACTTACTGGATGCAC
SEQ ID NO: 109 (Kabat)	HCDR2	AATATTTATCCTGGTACTGGTGGTTCTAACTTC GATGAGAAGTTCAAGAAC
SEQ ID NO: 110 (Kabat)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
SEQ ID NO: 111 (Chothia)	HCDR1	GGCTACACATTCACCACTTAC
SEQ ID NO: 112 (Chothia)	HCDR2	TATCCTGGTACTGGTGGT
SEQ ID NO: 110 (Chothia)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
BAP049-hum10 LC		
SEQ ID NO: 113 (Kabat)	LCDR1	AAGTCCAGTCAGAGTCTGTTAGACAGTGGAAAT CAAAAGAACTTCTTGACC
SEQ ID NO: 114 (Kabat)	LCDR2	TGGGCATCCACTAGGGAATCT
SEQ ID NO: 119 (Kabat)	LCDR3	CAGAATGATTATAGTTATCCGTACACG
SEQ ID NO: 116 (Chothia)	LCDR1	AGTCAGAGTCTGTTAGACAGTGGAAATCAAAAG AACTTC
SEQ ID NO: 117 (Chothia)	LCDR2	TGGGCATCC
SEQ ID NO: 120 (Chothia)	LCDR3	GATTATAGTTATCCGTAC
BAP049-hum11 HC		
SEQ ID NO: 108 (Kabat)	HCDR1	ACTTACTGGATGCAC
SEQ ID NO: 109 (Kabat)	HCDR2	AATATTTATCCTGGTACTGGTGGTTCTAACTTC GATGAGAAGTTCAAGAAC
SEQ ID NO: 110 (Kabat)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
SEQ ID NO: 111 (Chothia)	HCDR1	GGCTACACATTCACCACTTAC
SEQ ID NO: 112 (Chothia)	HCDR2	TATCCTGGTACTGGTGGT
SEQ ID NO: 110 (Chothia)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
BAP049-hum11 LC		
SEQ ID NO: 113 (Kabat)	LCDR1	AAGTCCAGTCAGAGTCTGTTAGACAGTGGAAAT CAAAAGAACTTCTTGACC
SEQ ID NO: 114 (Kabat)	LCDR2	TGGGCATCCACTAGGGAATCT
SEQ ID NO: 119 (Kabat)	LCDR3	CAGAATGATTATAGTTATCCGTACACG
SEQ ID NO: 116 (Chothia)	LCDR1	AGTCAGAGTCTGTTAGACAGTGGAAATCAAAAG AACTTC
SEQ ID NO: 117 (Chothia)	LCDR2	TGGGCATCC
SEQ ID NO: 120 (Chothia)	LCDR3	GATTATAGTTATCCGTAC
BAP049-hum12 HC		
SEQ ID NO: 108 (Kabat)	HCDR1	ACTTACTGGATGCAC
SEQ ID NO: 109 (Kabat)	HCDR2	AATATTTATCCTGGTACTGGTGGTTCTAACTTC GATGAGAAGTTCAAGAAC
SEQ ID NO: 110 (Kabat)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
SEQ ID NO: 111 (Chothia)	HCDR1	GGCTACACATTCACCACTTAC
SEQ ID NO: 112 (Chothia)	HCDR2	TATCCTGGTACTGGTGGT
SEQ ID NO: 110 (Chothia)	HCDR3	TGGACTACTGGGACGGGAGCTTAT

BAP049-hum12 LC		
SEQ ID NO: 113 (Kabat)	LCDR1	AAGTCCAGTCAGAGTCTGTTAGACAGTGGAAAT CAAAAGAACTTCTTGACC
SEQ ID NO: 114 (Kabat)	LCDR2	TGGGCATCCACTAGGGAATCT
SEQ ID NO: 119 (Kabat)	LCDR3	CAGAATGATTATAGTTATCCGTACACG
SEQ ID NO: 116 (Chothia)	LCDR1	AGTCAGAGTCTGTTAGACAGTGGAAATCAAAAG AACTTC
SEQ ID NO: 117 (Chothia)	LCDR2	TGGGCATCC
SEQ ID NO: 120 (Chothia)	LCDR3	GATTATAGTTATCCGTAC
BAP049-hum13 HC		
SEQ ID NO: 108 (Kabat)	HCDR1	ACTTACTGGATGCAC
SEQ ID NO: 109 (Kabat)	HCDR2	AATATTTATCCTGGTACTGGTGGTTCTAACTTC GATGAGAAGTTCAAGAAC
SEQ ID NO: 110 (Kabat)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
SEQ ID NO: 111 (Chothia)	HCDR1	GGCTACACATTCACCACTTAC
SEQ ID NO: 112 (Chothia)	HCDR2	TATCCTGGTACTGGTGGT
SEQ ID NO: 110 (Chothia)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
BAP049-hum13 LC		
SEQ ID NO: 121 (Kabat)	LCDR1	AAGTCCAGTCAGAGTCTGTTAGACAGTGGAAAT CAAAAGAACTTCTTAACC
SEQ ID NO: 114 (Kabat)	LCDR2	TGGGCATCCACTAGGGAATCT
SEQ ID NO: 119 (Kabat)	LCDR3	CAGAATGATTATAGTTATCCGTACACG
SEQ ID NO: 116 (Chothia)	LCDR1	AGTCAGAGTCTGTTAGACAGTGGAAATCAAAAG AACTTC
SEQ ID NO: 117 (Chothia)	LCDR2	TGGGCATCC
SEQ ID NO: 120 (Chothia)	LCDR3	GATTATAGTTATCCGTAC
BAP049-hum14 HC		
SEQ ID NO: 108 (Kabat)	HCDR1	ACTTACTGGATGCAC
SEQ ID NO: 109 (Kabat)	HCDR2	AATATTTATCCTGGTACTGGTGGTTCTAACTTC GATGAGAAGTTCAAGAAC
SEQ ID NO: 223 (Kabat)	HCDR3	TGGACTACTGGGACGGGAGCTTAC
SEQ ID NO: 111 (Chothia)	HCDR1	GGCTACACATTCACCACTTAC
SEQ ID NO: 112 (Chothia)	HCDR2	TATCCTGGTACTGGTGGT
SEQ ID NO: 223 (Chothia)	HCDR3	TGGACTACTGGGACGGGAGCTTAC
BAP049-hum14 LC		
SEQ ID NO: 113 (Kabat)	LCDR1	AAGTCCAGTCAGAGTCTGTTAGACAGTGGAAAT CAAAAGAACTTCTTGACC
SEQ ID NO: 114 (Kabat)	LCDR2	TGGGCATCCACTAGGGAATCT
SEQ ID NO: 119 (Kabat)	LCDR3	CAGAATGATTATAGTTATCCGTACACG
SEQ ID NO: 116 (Chothia)	LCDR1	AGTCAGAGTCTGTTAGACAGTGGAAATCAAAAG AACTTC
SEQ ID NO: 117 (Chothia)	LCDR2	TGGGCATCC
SEQ ID NO: 120 (Chothia)	LCDR3	GATTATAGTTATCCGTAC
BAP049-hum15 HC		
SEQ ID NO: 108 (Kabat)	HCDR1	ACTTACTGGATGCAC

SEQ ID NO: 109 (Kabat)	HCDR2	AATATTTATCCTGGTACTGGTGGTTCTAACTTC GATGAGAAGTTCAAGAAC
SEQ ID NO: 223 (Kabat)	HCDR3	TGGACTACTGGGACGGGAGCTTAC
SEQ ID NO: 111 (Chothia)	HCDR1	GGCTACACATTCACCACTTAC
SEQ ID NO: 112 (Chothia)	HCDR2	TATCCTGGTACTGGTGGT
SEQ ID NO: 223 (Chothia)	HCDR3	TGGACTACTGGGACGGGAGCTTAC
BAP049-hum15 LC		
SEQ ID NO: 113 (Kabat)	LCDR1	AAGTCCAGTCAGAGTCTGTTAGACAGTGGAAAT CAAAAGAACTTCTTGACC
SEQ ID NO: 114 (Kabat)	LCDR2	TGGGCATCCACTAGGGAATCT
SEQ ID NO: 119 (Kabat)	LCDR3	CAGAATGATTATAGTTATCCGTACACG
SEQ ID NO: 116 (Chothia)	LCDR1	AGTCAGAGTCTGTTAGACAGTGGAAATCAAAAG AACTTC
SEQ ID NO: 117 (Chothia)	LCDR2	TGGGCATCC
SEQ ID NO: 120 (Chothia)	LCDR3	GATTATAGTTATCCGTAC
BAP049-hum16 HC		
SEQ ID NO: 108 (Kabat)	HCDR1	ACTTACTGGATGCAC
SEQ ID NO: 109 (Kabat)	HCDR2	AATATTTATCCTGGTACTGGTGGTTCTAACTTC GATGAGAAGTTCAAGAAC
SEQ ID NO: 110 (Kabat)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
SEQ ID NO: 111 (Chothia)	HCDR1	GGCTACACATTCACCACTTAC
SEQ ID NO: 112 (Chothia)	HCDR2	TATCCTGGTACTGGTGGT
SEQ ID NO: 110 (Chothia)	HCDR3	TGGACTACTGGGACGGGAGCTTAT
BAP049-hum16 LC		
SEQ ID NO: 113 (Kabat)	LCDR1	AAGTCCAGTCAGAGTCTGTTAGACAGTGGAAAT CAAAAGAACTTCTTGACC
SEQ ID NO: 114 (Kabat)	LCDR2	TGGGCATCCACTAGGGAATCT
SEQ ID NO: 119 (Kabat)	LCDR3	CAGAATGATTATAGTTATCCGTACACG
SEQ ID NO: 116 (Chothia)	LCDR1	AGTCAGAGTCTGTTAGACAGTGGAAATCAAAAG AACTTC
SEQ ID NO: 117 (Chothia)	LCDR2	TGGGCATCC
SEQ ID NO: 120 (Chothia)	LCDR3	GATTATAGTTATCCGTAC
BAP049-Clone-A HC		
SEQ ID NO: 122 (Kabat)	HCDR1	ACCTACTGGATGCAC
SEQ ID NO: 123 (Kabat)	HCDR2	AACATCTATCCTGGCACCGGCGGCTCCAACTTC GACGAGAAGTTCAAGAAC
SEQ ID NO: 124 (Kabat)	HCDR3	TGGACAACCGGCACAGGCGCTTAT
SEQ ID NO: 125 (Chothia)	HCDR1	GGCTACACCTTCACCACCTAC
SEQ ID NO: 126 (Chothia)	HCDR2	TATCCTGGCACCGGCGGC
SEQ ID NO: 124 (Chothia)	HCDR3	TGGACAACCGGCACAGGCGCTTAT
BAP049-Clone-A LC		
SEQ ID NO: 127 (Kabat)	LCDR1	AAGTCCTCCCAGTCCCTGCTGGACTCCGGCAAC CAGAAGAACTTCCTGACC
SEQ ID NO: 128 (Kabat)	LCDR2	TGGGCCTCCACCCGGGAATCT
SEQ ID NO: 129 (Kabat)	LCDR3	CAGAACGACTACTCCTACCCCTACACC

SEQ ID NO: 130 (Chothia)	LCDR1	TCCCAGTCCCTGCTGGACTCCGGCAACCAGAAG AACTTC
SEQ ID NO: 131 (Chothia)	LCDR2	TGGGCCTCC
SEQ ID NO: 132 (Chothia)	LCDR3	GACTACTCCTACCCCTAC
BAP049-Clone-B HC		
SEQ ID NO: 133 (Kabat)	HCDR1	ACCTACTGGATGCAC
SEQ ID NO: 134 (Kabat)	HCDR2	AATATCTACCCCGGCACCGGCGGCTCTAACTTC GACGAGAAGTTTAAGAAT
SEQ ID NO: 135 (Kabat)	HCDR3	TGGACTACCGGCACAGGCGCCTAC
SEQ ID NO: 136 (Chothia)	HCDR1	GGCTACACCTTCACTACCTAC
SEQ ID NO: 137 (Chothia)	HCDR2	TACCCCGGCACCGGCGGC
SEQ ID NO: 135 (Chothia)	HCDR3	TGGACTACCGGCACAGGCGCCTAC
BAP049-Clone-B LC		
SEQ ID NO: 138 (Kabat)	LCDR1	AAATCTAGTCAGTCACTGCTGGATAGCGGTAAT CAGAAGAACTTCCTGACC
SEQ ID NO: 139 (Kabat)	LCDR2	TGGGCCTCTACTAGAGAATCA
SEQ ID NO: 140 (Kabat)	LCDR3	CAGAACGACTATAGCTACCCCTACACC
SEQ ID NO: 141 (Chothia)	LCDR1	AGTCAGTCACTGCTGGATAGCGGTAATCAGAAG AACTTC
SEQ ID NO: 142 (Chothia)	LCDR2	TGGGCCTCT
SEQ ID NO: 143 (Chothia)	LCDR3	GACTATAGCTACCCCTAC
BAP049-Clone-C HC		
SEQ ID NO: 122 (Kabat)	HCDR1	ACCTACTGGATGCAC
SEQ ID NO: 123 (Kabat)	HCDR2	AACATCTATCCTGGCACCGGCGGCTCCAACTTC GACGAGAAGTTCAAGAAC
SEQ ID NO: 124 (Kabat)	HCDR3	TGGACAACCGGCACAGGCGCTTAT
SEQ ID NO: 125 (Chothia)	HCDR1	GGCTACACCTTCACCACCTAC
SEQ ID NO: 126 (Chothia)	HCDR2	TATCCTGGCACCGGCGGC
SEQ ID NO: 124 (Chothia)	HCDR3	TGGACAACCGGCACAGGCGCTTAT
BAP049-Clone-C LC		
SEQ ID NO: 127 (Kabat)	LCDR1	AAGTCCTCCCAGTCCCTGCTGGACTCCGGCAAC CAGAAGAACTTCCTGACC
SEQ ID NO: 128 (Kabat)	LCDR2	TGGGCCTCCACCCGGGAATCT
SEQ ID NO: 129 (Kabat)	LCDR3	CAGAACGACTACTCCTACCCCTACACC
SEQ ID NO: 130 (Chothia)	LCDR1	TCCCAGTCCCTGCTGGACTCCGGCAACCAGAAG AACTTC
SEQ ID NO: 131 (Chothia)	LCDR2	TGGGCCTCC
SEQ ID NO: 132 (Chothia)	LCDR3	GACTACTCCTACCCCTAC
BAP049-Clone-D HC		
SEQ ID NO: 122 (Kabat)	HCDR1	ACCTACTGGATGCAC
SEQ ID NO: 144 (Kabat)	HCDR2	AACATCTACCCTGGCACCGGCGGCTCCAACTTC GACGAGAAGTTCAAGAAC
SEQ ID NO: 145 (Kabat)	HCDR3	TGGACCACCGGAACCGGCGCCTAT
SEQ ID NO: 125 (Chothia)	HCDR1	GGCTACACCTTCACCACCTAC
SEQ ID NO: 146 (Chothia)	HCDR2	TACCCTGGCACCGGCGGC

SEQ ID NO: 145 (Chothia)	HCDR3	TGGACCACCGGAACCGGCGCCTAT
BAP049-Clone-D LC		
SEQ ID NO: 127 (Kabat)	LCDR1	AAGTCCTCCCAGTCCCTGCTGGACTCCGGCAAC CAGAAGAACTTCCTGACC
SEQ ID NO: 128 (Kabat)	LCDR2	TGGGCCTCCACCCGGGAATCT
SEQ ID NO: 129 (Kabat)	LCDR3	CAGAACGACTACTCCTACCCCTACACC
SEQ ID NO: 130 (Chothia)	LCDR1	TCCCAGTCCCTGCTGGACTCCGGCAACCAGAAG AACTTC
SEQ ID NO: 131 (Chothia)	LCDR2	TGGGCCTCC
SEQ ID NO: 132 (Chothia)	LCDR3	GACTACTCCTACCCCTAC
BAP049-Clone-E HC		
SEQ ID NO: 133 (Kabat)	HCDR1	ACCTACTGGATGCAC
SEQ ID NO: 134 (Kabat)	HCDR2	AATATCTACCCCGGCACCGGCGGCTCTAACTTC GACGAGAAGTTTAAGAAT
SEQ ID NO: 135 (Kabat)	HCDR3	TGGACTACCGGCACAGGCGCCTAC
SEQ ID NO: 136 (Chothia)	HCDR1	GGCTACACCTTCACTACCTAC
SEQ ID NO: 137 (Chothia)	HCDR2	TACCCCGGCACCGGCGGC
SEQ ID NO: 135 (Chothia)	HCDR3	TGGACTACCGGCACAGGCGCCTAC
BAP049-Clone-E LC		
SEQ ID NO: 138 (Kabat)	LCDR1	AAATCTAGTCAGTCACTGCTGGATAGCGGTAAT CAGAAGAACTTCCTGACC
SEQ ID NO: 139 (Kabat)	LCDR2	TGGGCCTCTACTAGAGAATCA
SEQ ID NO: 140 (Kabat)	LCDR3	CAGAACGACTATAGCTACCCCTACACC
SEQ ID NO: 141 (Chothia)	LCDR1	AGTCAGTCACTGCTGGATAGCGGTAATCAGAAG AACTTC
SEQ ID NO: 142 (Chothia)	LCDR2	TGGGCCTCT
SEQ ID NO: 143 (Chothia)	LCDR3	GACTATAGCTACCCCTAC

Table 2. Amino acid and nucleotide sequences of the heavy and light chain framework regions for humanized mAbs BAP049-hum01 to BAP049-hum16 and BAP049-Clone-A to BAP049-Clone-E

	Amino Acid Sequence	**Nucleotide Sequence**
VHFW1 (type a)	EVQLVQSGAEVKKPGESLRISCKGS (SEQ ID NO: 147)	GAAGTGCAGCTGGTGCAGTCTGGAGCAGAGGTGAAAAA GCCCGGGGAGTCTCTGAGGATCTCCTGTAAGGGTTCT (SEQ ID NO: 148) GAAGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAA GCCTGGCGAGTCCCTGCGGATCTCCTGCAAGGGCTCT (SEQ ID NO: 149) GAGGTGCAGCTGGTGCAGTCAGGCGCCGAAGTGAAGAA GCCCGGCGAGTCACTGAGAATTAGCTGTAAAGGTTCA (SEQ ID NO: 150)
VHFW1 (type b)	QVQLVQSGAEVKKPGASVKVSCKAS (SEQ ID NO: 151)	CAGGTTCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAA GCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGCTTCT (SEQ ID NO: 152)

VHFW2 (type a)	WVRQATGQGLEWMG (SEQ ID NO: 153)	TGGGTGCGACAGGCCACTGGACAAGGGCTTGAGTGGAT GGGT (SEQ ID NO: 154) TGGGTGCGACAGGCTACCGGCCAGGGCCTGGAATGGAT GGGC (SEQ ID NO: 155) TGGGTCCGCCAGGCTACCGGTCAAGGCCTCGAGTGGAT GGGT (SEQ ID NO: 156)
VHFW2 (type b)	WIRQSPSRGLEWLG (SEQ ID NO: 157)	TGGATCAGGCAGTCCCCATCGAGAGGCCTTGAGTGGCT GGGT (SEQ ID NO: 158) TGGATCCGGCAGTCCCCCTCTAGGGGCCTGGAATGGCT GGGC (SEQ ID NO: 159)
VHFW2 (type c)	WVRQAPGQGLEWMG (SEQ ID NO: 160)	TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGAT GGGT (SEQ ID NO: 161)
VHFW3 (type a)	RVTITADKSTSTAYMELSSLRSEDTAVY YCTR (SEQ ID NO: 162)	AGAGTCACGATTACCGCGGACAAATCCACGAGCACAGC CTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGG CCGTGTATTACTGTACAAGA (SEQ ID NO: 163) AGAGTGACCATCACCGCCGACAAGTCCACCTCCACCGC CTACATGGAACTGTCCTCCCTGAGATCCGAGGACACCG CCGTGTACTACTGCACCCGG (SEQ ID NO: 164) AGAGTGACTATCACCGCCGATAAGTCTACTAGCACCGC CTATATGGAACTGTCTAGCCTGAGATCAGAGGACACCG CCGTCTACTACTGCACTAGG (SEQ ID NO: 165)
VHFW3 (type b)	RFTISRDNSKNTLYLQMNSLRAEDTAVY YCTR (SEQ ID NO: 166)	AGATTCACCATCTCCAGAGACAATTCCAAGAACACGCT GTATCTTCAAATGAACAGCCTGAGAGCCGAGGACACGG CCGTGTATTACTGTACAAGA (SEQ ID NO: 167) AGGTTCACCATCTCCCGGGACAACTCCAAGAACACCCT GTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCG CCGTGTACTACTGTACCAGA (SEQ ID NO: 168)
VHFW4	WGQGTTVTVSS (SEQ ID NO: 169)	TGGGGCCAGGGCACCACCGTGACCGTGTCCTCC (SEQ ID NO: 170) TGGGGCCAGGGCACCACAGTGACCGTGTCCTCT (SEQ ID NO: 171) TGGGGTCAAGGCACTACCGTGACCGTGTCTAGC (SEQ ID NO: 172) TGGGGCCAGGGCACAACAGTGACCGTGTCCTCC (SEQ ID NO: 173)
VLFW1 (type a)	EIVLTQSPDFQSVTPKEKVTITC (SEQ ID NO: 174)	GAAATTGTGCTGACTCAGTCTCCAGACTTTCAGTCTGT GACTCCAAAGGAGAAAGTCACCATCACCTGC (SEQ ID NO: 175) GAGATCGTGCTGACCCAGTCCCCCGACTTCCAGTCCGT GACCCCCAAAGAAAAAGTGACCATCACATGC (SEQ ID NO: 176)

VLFW1 (type b)	EIVLTQSPATLSLSPGERATLSC (SEQ ID NO: 177)	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTT GTCTCCAGGGGAAAGAGCCACCCTCTCCTGC (SEQ ID NO: 178) GAGATCGTGCTGACCCAGTCCCCTGCCACCCTGTCACT GTCTCCAGGCGAGAGAGCTACCCTGTCCTGC (SEQ ID NO: 179) GAGATCGTCCTGACTCAGTCACCCGCTACCCTGAGCCT GAGCCCTGGCGAGCGGGCTACACTGAGCTGT (SEQ ID NO: 180)
VLFW1 (type c)	DIVMTQTPLSLPVTPGEPASISC (SEQ ID NO: 181)	GATATTGTGATGACCCAGACTCCACTCTCCCTGCCCGT CACCCCTGGAGAGCCGGCCTCCATCTCCTGC (SEQ ID NO: 182)
VLFW1 (type d)	DVVMTQSPLSLPVTLGQPASISC (SEQ ID NO: 183)	GATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGT CACCCTTGGACAGCCGGCCTCCATCTCCTGC (SEQ ID NO: 184)
VLFW1 (type e)	DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 185)	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGC ATCTGTAGGAGACAGAGTCACCATCACTTGC (SEQ ID NO: 186)
VLFW2 (type a)	WYQQKPGQAPRLLIY (SEQ ID NO: 187)	TGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCT CATCTAT (SEQ ID NO: 188) TGGTATCAGCAGAAGCCCGGCCAGGCCCCCAGACTGCT GATCTAC (SEQ ID NO: 189) TGGTATCAGCAGAAGCCCGGTCAAGCCCCTAGACTGCT GATCTAC (SEQ ID NO: 190)
VLFW2 (type b)	WYQQKPGKAPKLLIY (SEQ ID NO: 191)	TGGTATCAGCAGAAACCAGGGAAAGCTCCTAAGCTCCT GATCTAT (SEQ ID NO: 192) TGGTATCAGCAGAAGCCCGGTAAAGCCCCTAAGCTGCT GATCTAC (SEQ ID NO: 193)
VLFW2 (type c)	WYLQKPGQSPQLLIY (SEQ ID NO: 194)	TGGTACCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCT GATCTAT (SEQ ID NO: 195)
VLFW3 (type a)	GVPSRFSGSGSGTDFTFTISSLEAEDAA TYYC (SEQ ID NO: 196)	GGGGTCCCCTCGAGGTTCAGTGGCAGTGGATCTGGGAC AGATTTCACCTTTACCATCAGTAGCCTGGAAGCTGAAG ATGCTGCAACATATTACTGT (SEQ ID NO: 197) GGCGTGCCCTCTAGATTCTCCGGCTCCGGCTCTGGCAC CGACTTTACCTTCACCATCTCCAGCCTGGAAGCCGAGG ACGCCGCCACCTACTACTGC (SEQ ID NO: 198) GGCGTGCCCTCTAGGTTTAGCGGTAGCGGTAGTGGCAC CGACTTCACCTTCACTATCTCTAGCCTGGAAGCCGAGG ACGCCGCTACCTACTACTGT (SEQ ID NO: 199)
VLFW3 (type b)	GIPPRFSGSGYGTDFTLTINNIESEDAA YYFC (SEQ ID NO: 200)	GGGATCCCACCTCGATTCAGTGGCAGCGGGTATGGAAC AGATTTTACCCTCACAATTAATAACATAGAATCTGAGG ATGCTGCATATTACTTCTGT (SEQ ID NO: 201)
VLFW3	GVPSRFSGSGSGTEFTLTISSLQPDDFA	GGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGAC

(type c)	TYYC (SEQ ID NO: 202)	AGAATTCACTCTCACCATCAGCAGCCTGCAGCCTGATG ATTTTGCAACTTATTACTGT (SEQ ID NO: 203) GGCGTGCCCTCTAGATTCTCCGGCTCCGGCTCTGGCAC CGAGTTTACCCTGACCATCTCCAGCCTGCAGCCCGACG ACTTCGCCACCTACTACTGC (SEQ ID NO: 204)
VLFW3 (type d)	GVPSRFSGSGSGTDFTFTISSLQPEDIA TYYC (SEQ ID NO: 205)	GGGGTCCCATCAAGGTTCAGTGGAAGTGGATCTGGGAC AGATTTTACTTTCACCATCAGCAGCCTGCAGCCTGAAG ATATTGCAACATATTACTGT (SEQ ID NO: 206) GGCGTGCCCTCTAGGTTTAGCGGTAGCGGTAGTGGCAC CGACTTCACCTTCACTATCTCTAGCCTGCAGCCCGAGG ATATCGCTACCTACTACTGT (SEQ ID NO: 207)
VLFW4	FGQGTKVEIK (SEQ ID NO: 208)	TTCGGCCAAGGGACCAAGGTGGAAATCAAA (SEQ ID NO: 209) TTCGGCCAGGGCACCAAGGTGGAAATCAAG (SEQ ID NO: 210) TTCGGTCAAGGCACTAAGGTCGAGATTAAG (SEQ ID NO: 211)

Table 3. Constant region amino acid sequences of human IgG heavy chains and human kappa light chain

HC	**IgG4 (S228P) mutant constant region amino acid sequence (EU Numbering)** ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES KYGPPCPPCP APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE ALHNHYTQKS LSLSLGK (SEQ ID NO: 212)
LC	**Human kappa constant region amino acid sequence** RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG NSQESVTEQD SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK SFNRGEC (SEQ ID NO: 213)
HC	**IgG4 (S228P) mutant constant region amino acid sequence lacing C-terminal lysine (K) (EU Numbering)** ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES KYGPPCPPCP APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE ALHNHYTQKS LSLSLG (SEQ ID NO: 214)

HC	**IgG1 wild type** ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKRVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK (SEQ ID NO: 215)
HC	**IgG1 (N297A) mutant constant region amino acid sequence (EU Numbering)** ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKRVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYA STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK (SEQ ID NO: 216)
HC	**IgG1 (D265A, P329A) mutant constant region amino acid sequence (EU Numbering)** ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKRVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVAVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LAAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK (SEQ ID NO: 217)
HC	**IgG1 (L234A, L235A) mutant constant region amino acid sequence (EU Numbering)** ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKRVEP KSCDKTHTCP PCPAPEAAGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK (SEQ ID NO: 218)

Table 4. Amino acid sequences of the heavy and light chain leader sequences for humanized mAbs BAP049-Clone-A to BAP049-Clone-E

BAP049-Clone-A	HC	MEWSWVFLFFLSVTTGVHS (SEQ ID NO: 219)
	LC	MSVPTQVLGLLLLWLTDARC (SEQ ID NO: 220)
BAP049-Clone-B	HC	MAWVWTLPFLMAAAQSVQA (SEQ ID NO: 221)
	LC	MSVLTQVLALLLLWLTGTRC (SEQ ID NO: 222)
BAP049-Clone-C	HC	MEWSWVFLFFLSVTTGVHS (SEQ ID NO: 219)
	LC	MSVPTQVLGLLLLWLTDARC (SEQ ID NO: 220)

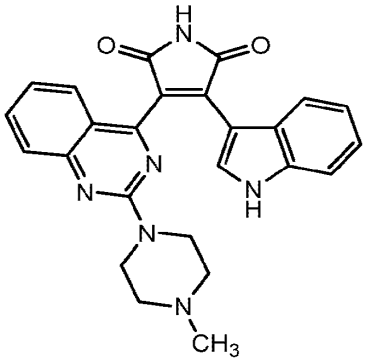
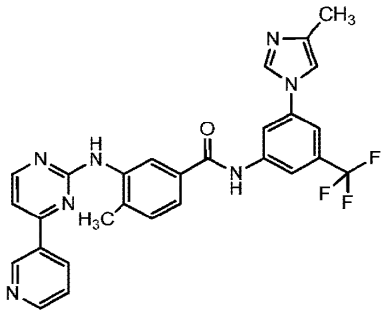
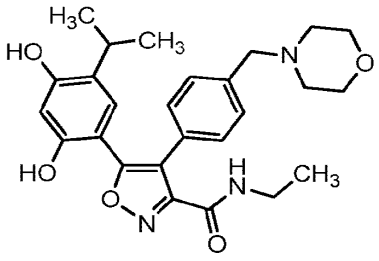
BAP049-Clone-D	HC	MEWSWVFLFFLSVTTGVHS (SEQ ID NO: 219)
	LC	MSVPTQVLGLLLLWLTDARC (SEQ ID NO: 220)
BAP049-Clone-E	HC	MAWVWTLPFLMAAAQSVQA (SEQ ID NO: 221)
	LC	MSVLTQVLALLLLWLTGTRC (SEQ ID NO: 222)

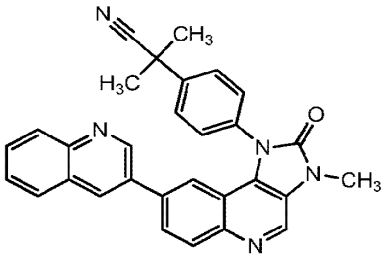
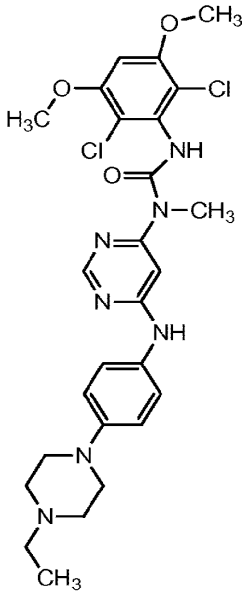
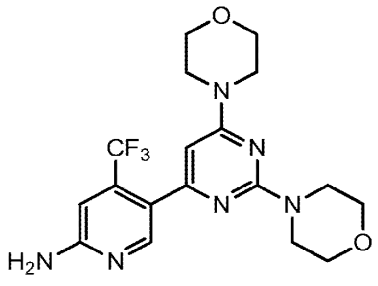
Table 5. See Examples.

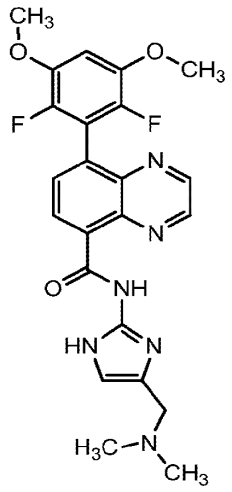
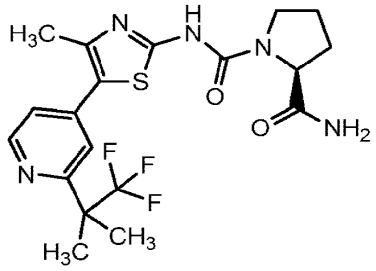
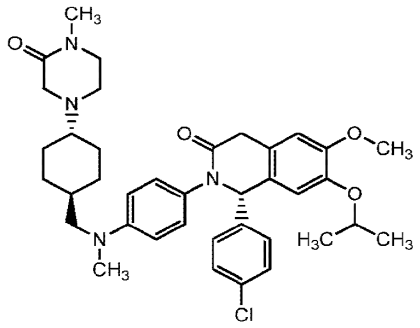
Table 6. See Examples.

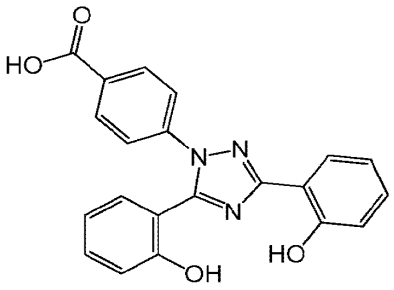
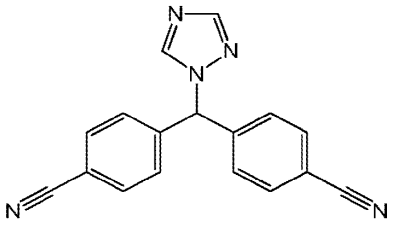
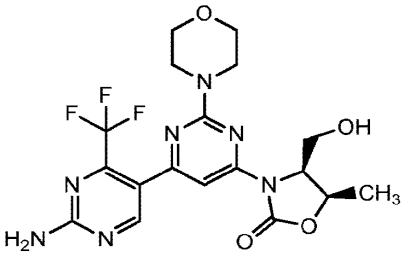
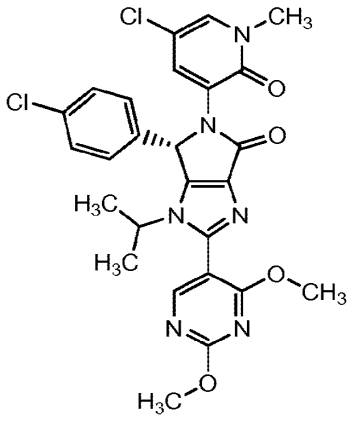
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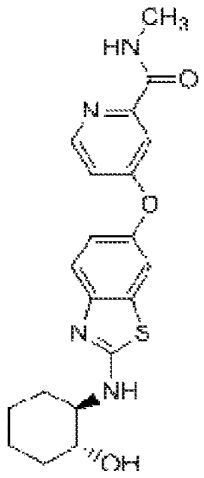
Table 7. Selected therapeutic agents that can be administered in combination with the anti-PD-1 antibody molecules, *e.g.*, as a single agent or in combination with other immunomodulators described herein. Each publication listed in this Table is herein incorporated by reference in its entirety, including all structural formulae therein.

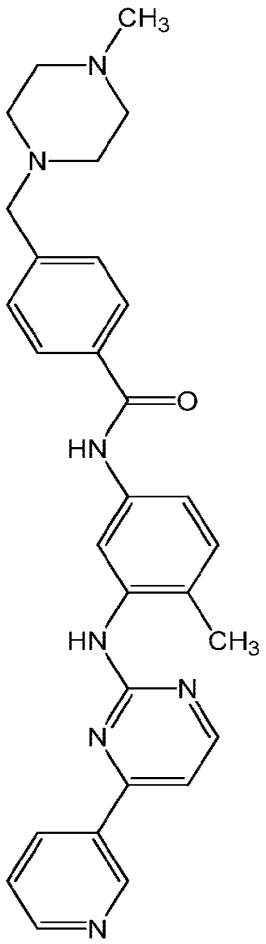
Compound Designation	Generic Name Tradename	Compound Structure	Patents / Patent Application Publications
A1	Sotrastaurin		EP 1682103 US 2007/142401 WO 2005/039549
A2	Nilotinib HCl monohydrate TASIGNA®	 HCl • H_2O	WO 2004/005281 US 7,169,791
A3			WO 2010/060937 WO 2004/072051 EP 1611112 US 8,450,310

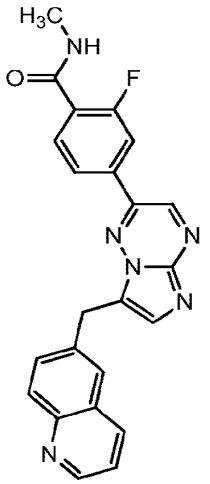
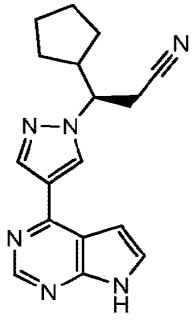
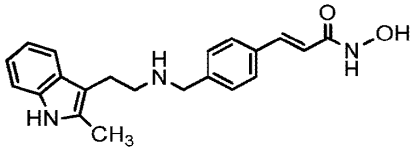
A4	Dactolisib		WO 2006/122806
A5			US 8,552,002
A6	Buparlisib		WO 2007/084786

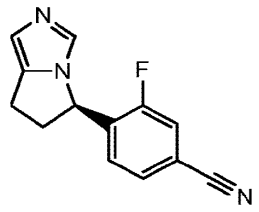
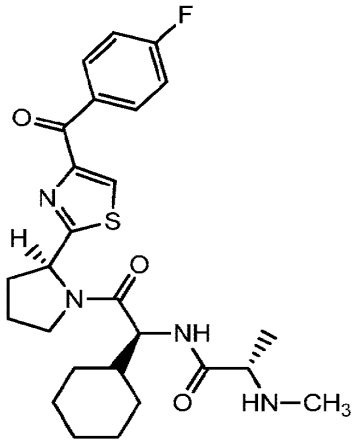
A7			WO 2009/141386 US 2010/0105667
A8			WO 2010/029082
A9		CYP17 inhibitor	WO 2010/149755 US 8,263,635 B2 EP 2445903 B1
A10			WO 2011/076786

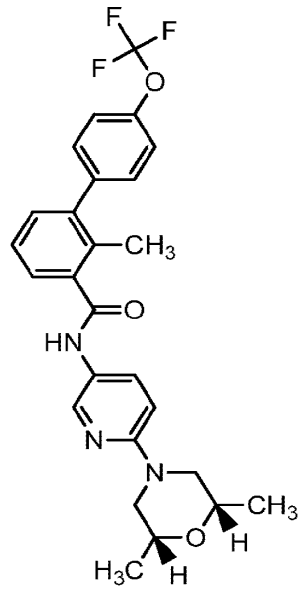
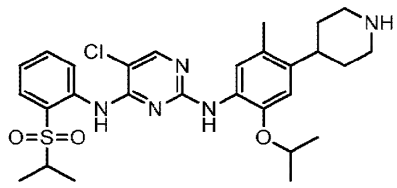
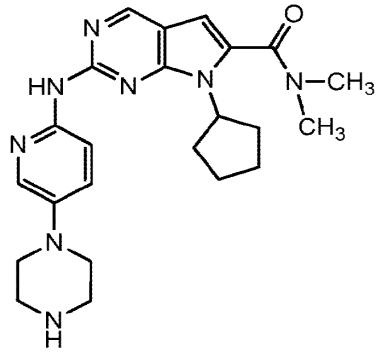
A11	Deferasirox EXJADE®		WO 1997/049395
A12	Letrozole FEMARA®		US 4,978,672
A13			WO 2013/124826 US 2013/0225574
A14			WO 2013/111105

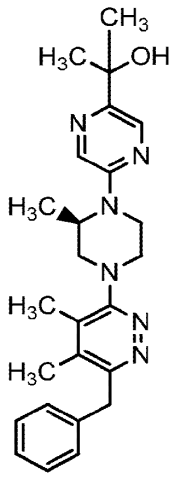
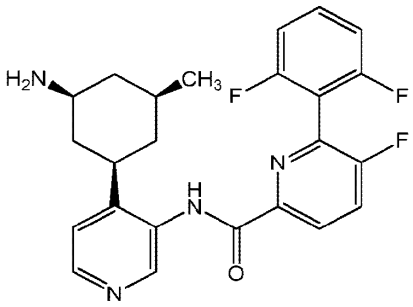
A15			WO 2005/073224

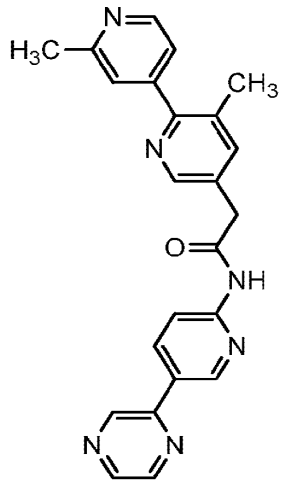
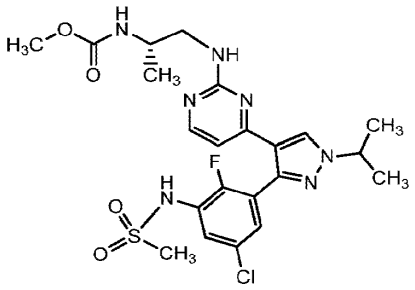
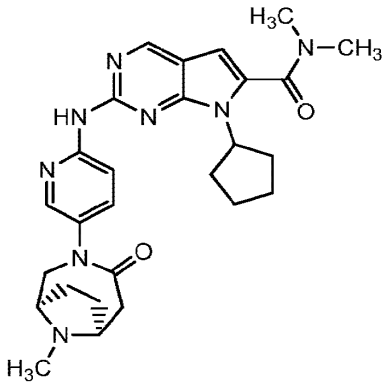
A16	Imatinib mesylate GLEEVEC®	 Mesylate	WO 1999/003854
A17			EP 2099447 US 7,767,675 US 8,420,645

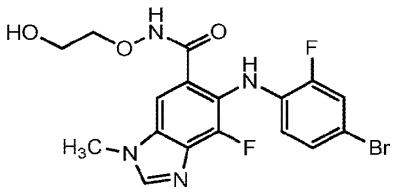
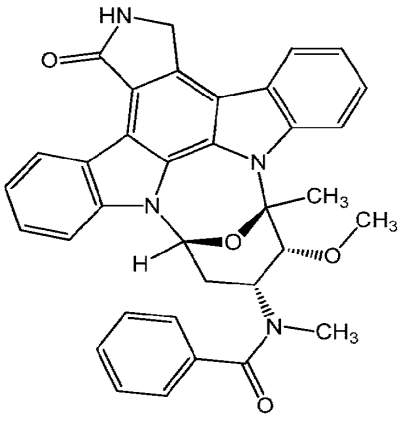
		 Dihydrochloric salt	
A18	Ruxolitinib Phosphate JAKAFI®	 H_3PO_4	WO 2007/070514 EP 2474545 US 7,598,257 WO 2014/018632
A19	Panobinostat		WO 2014/072493 WO 2002/022577 EP 1870399

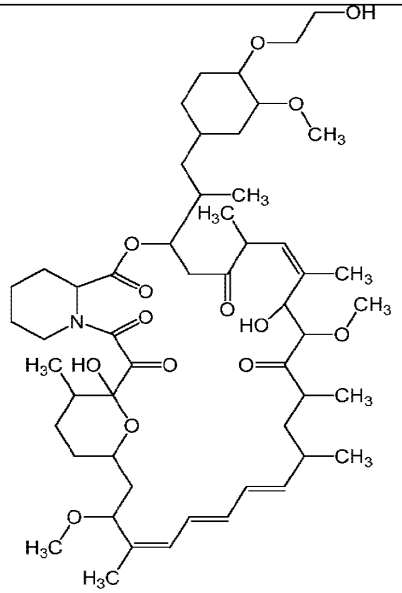
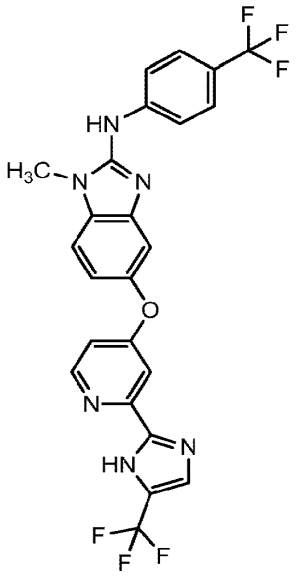
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A21			WO 2008/016893 EP 2051990 US 8,546,336

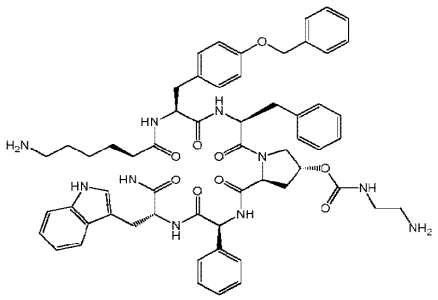
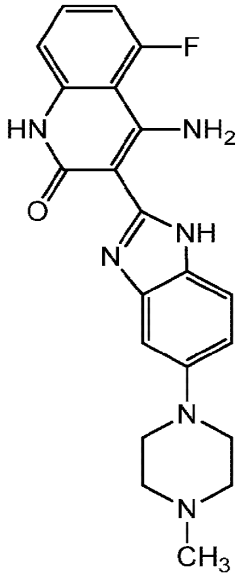
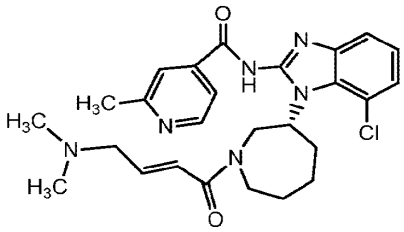
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A23	ceritinib ZYKADIA™		WO 2008/073687 US 8,039,479
A24			US 8,415,355 US 8,685,980

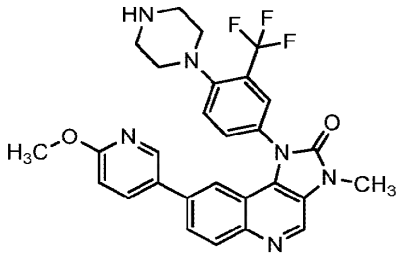
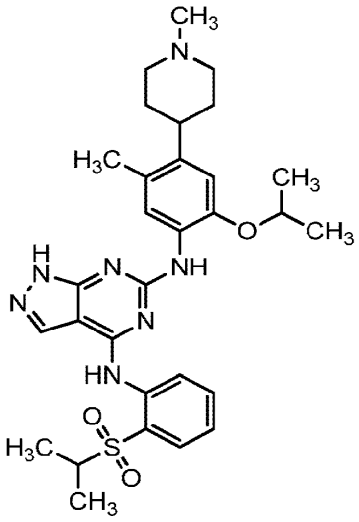
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A26		Human monoclonal antibody to PRLR	US 7,867,493
A27			WO 2010/026124 EP 2344474 US 2010/0056576 WO2008/106692

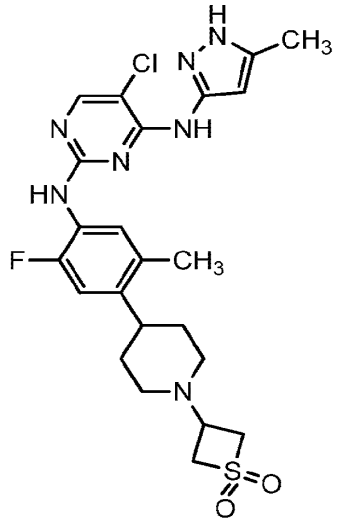
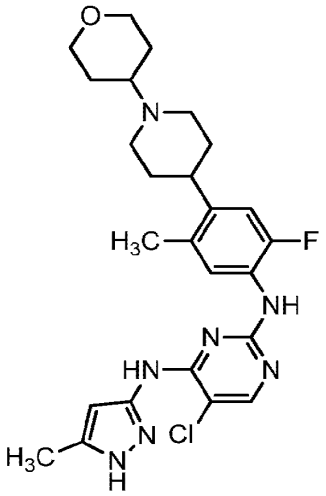
A28			WO 2010/101849
A29	Encorafenib		WO 2011/025927
A30			WO 2011/101409
A31		IHuman monoclonal antibody to IIER3	WO 2012/022814 EP 2606070

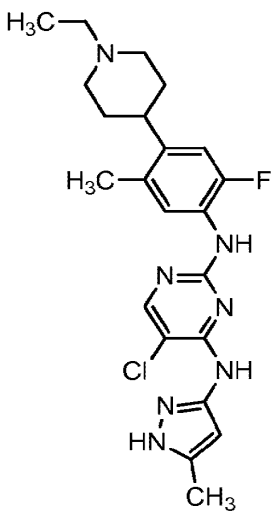
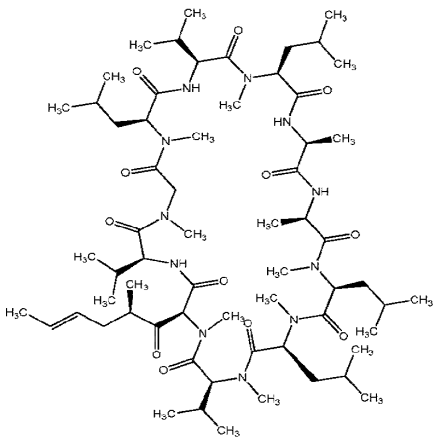
			US 8,735,551
A32		Antibody Drug Conjugate (ADC)	WO 2014/160160 Ab: 12425 (see Table 1, paragraph [00191]) Linker: SMCC (see paragraph [00117] Payload: DM1 (see paragraph [00111] See also Claim 29
A33		Monoclonal antibody or Fab to M-CSF	WO 2004/045532
A34	Binimetinib		WO 2003/077914
A35	Midostaurin		WO 2003/037347 EP 1441737 US 2012/252785

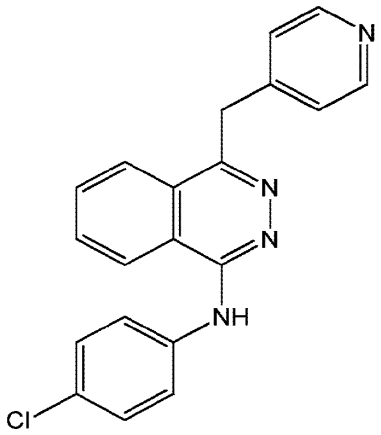
A36	Everolimus AFINITOR®		WO 2014/085318
A37			WO 2007/030377 US 7,482,367

A38	Pasireotide diaspartate SIGNIFOR®		WO2002/010192 US 7,473,761
A39	Dovitinib		WO 2009/115562 US 8,563,556
A40			WO 2013/184757

A41			WO 2006/122806
A42			WO 2008/073687 US 8,372,858

A43			WO 2010/002655 US 8,519,129
A44			WO 2010/002655 US 8,519,129

A45			WO 2010/002655
A46	Valspodar AMDRAY™		EP 296122

A47	Vatalanib succinate	 succinate	WO 98/35958
A48		IDH inhibitor	WO2014/141104
A49		BCR-ABL inhibitor	WO2013/171639 WO2013/171640 WO2013/171641 WO2013/171642
A50		cRAF inhibitor	WO2014/151616
A51		ERK1/2 ATP competitive inhibitor	PCT/US2014/062913

EXAMPLES

The Examples below are set forth to aid in the understanding of the inventions but are not intended to, and should not be construed to, limit its scope in any way.

Example 1: Humanization of Anti-PD-1 Antibody, BAP049

Murine anti-PD-1 monoclonal antibody BAP049 was humanized. The sequences and test samples of sixteen humanized BAP049 clones with unique variable region sequences were obtained. These clones were further analyzed for their biological functions (*e.g.*, antigen binding and ligand blocking), structural features, and transcient expression in CHO cells.

Example 1.1: Humanization Technology and Process

Humanization of BAP049 was performed using a combinatorial library of human germline variable region frameworks (FWs). The technology entails transferring the murine CDRs in frame to a library of human variable regions (VRs) that had been constructed by randomly combining human germ line FW1, FW2 and FW3 sequences. Only one FW4 sequence was used, which is WGQGTTVTVSS (SEQ ID NO: 169) for the heavy chain (HC) (Kabat human HC subgroup I, No. 21) and FGQGTKVEIK (SEQ ID NO: 208) for the light chain (LC) (Kabat human κ subgroup I, No. 5). The library of VR sequences was fused to human constant region (CR) sequences, human IgG4(S228P) of HC and human κ CR of LC, and the resulting library of whole IgG was expressed in CHO cells for screening. Screening was performed with tissue culture supernatants measuring binding avidity on antigen-expressing cells in a whole cell ELISA format or on FACS.

The humanization process was performed in a stepwise manner starting with the construction and expression of the appropriate chimeric mAb (murine VR, IgG4(S228P), human κ), which can serve as a comparator for the screening of the humanized clones. The constant region amino acid sequences for human IgG4(S228P) heavy chain and human kappa light chain are shown in Table 3.

Humanization of the VR of LC and HC were performed in two independent steps. The library of humanized LC (huLC) was paired with the chimeric HC (murine VR, IgG4(S228P)) and the resulting "half-humanized" mAbs were screened for binding activity by ELISA. The

huLC of clones with adequate binding activity (≥ binding of chimeric mAb) were selected. Analogously, the library of humanized HC (huHC) was paired with the chimeric LC (murine VR, human κ) and screened for binding activity by ELISA. The huHC of clones with appropriate binding activity (≥ binding of chimeric mAb) were selected.

The variable regions of the selected huLC and huHC were then sequenced to identify the huLC and huHC with unique sequences (some clones from the initial selection process may share the same LC or HC). The unique huLC and huHC were then randomly combined to form a small library of humanized mAbs (humAbs), which was expressed in CHO cells and screened on antigen- expressing cells in an ELISA and FACS format. Clones with binding activities that were equal or better than the binding of the chimeric comparator mAb are the final product of the humanization process.

Example 1.2: Sequence of Murine mAb BAP049

The LC and HC variable region sequences of murine anti-PD-1 mAb were determined. The sequences obtained from two independent analyses were identical and are shown in Figure 1.

Germline analysis was performed and part of the result is shown in Figure 2A as an amino acid sequence alignment. For the light chain, the V-gene is 98.65% identical to mIGKV8-19*01F (293/297 nts) and the J-gene is 97.30% identical to mIGKJ2*01F (36/37 nts). For the heavy chain, the V-gene is 92.83% identical to mIGHV1S22*01F (259/279 nts), the J-gene is 82.98% identical to mIGHJ3*01F (39/47 nts), and the D-gene is mIGHD2-14*01F. As shown in Figure 2B, the LC sequence of the murine mAb contains an unpaired Cys at position 102, which is in CDRL3 and arose through a point mutation in the murine J2 gene (tac → tgc; Y → C).

Example 1.3: Construction of Chimeric Antibody

Three variants of the chimeric antibody were prepared that either had a Cys, Tyr or Ser residue at position 102 of the LC sequence. The three chimeric antibodies, *i.e.,* BAP049-chi (Cys), BAP049-chi (Tyr), and BAP049-chi (Ser) (also known as BAP049-chi, BAP049-chi-Y, and BAP049-chi-S, respectively), were expressed in CHO cells and tested for their ability to compete with labeled murine antibody for binding to PD-1 expressing Jurkat cells. As shown in Figures 3A-3B, the three variants were indistinguishable in the competition experiment. The

results show that the three chimeric mAbs (Cys, Tyr, Ser) compete equally well with the binding of the labeled murine mAb BAP049. The slight difference between the chimeric mAb curves and the murine mAb curve is probably due to the different methods used for determining mAb concentrations. The concentration of the murine mAb was determined by OD280 measurement, whereas the chimeric mAb concentrations in supernatants were determined with an ELISA using an IgG4 standard. The germline residue Tyr was selected for humanized antibodies.

The amino acid sequences of the heavy and light chains for chimeric mAb BAP049-chi (Cys) are shown in Table 1. The nucleotide sequences of the heavy and light chains for chimeric mAb BAP049-chi (Cys) are shown in Table 1. In BAP049-chi (Tyr) and BAP049-chi (Ser), the unpaired Cys residue at position 102 of the LC were replaced with a Tyr or Ser residue.

Example 1.4: Humanized Antibody Clones

As shown in Figure 4, the process of humanization yielded sixteen clones with binding affinities comparable to that of the chimeric antibody. In addition to binding data, for each clone, the VR sequences were provided along with a sample of the mAb. The samples had been prepared by transient transfections of CHO cells and were concentrated tissue culture supernatants. The antibody concentrations in the solutions had been determined by an IgG4-specific ELISA.

As shown in Figure 5, the sixteen unique clones are combinations of four unique HC sequences and nine unique LC sequences. For the HC FW regions, the HC sequences are combinations of one of two different VHFW1, one of three different VHFW2, and one of two different VHFW3 sequences. For the LC FW regions, the LC sequences are combinations of one of five different VLFW1, one of three different VLFW2, and one of four different VLFW3 sequences. The amino acid and nucleotide sequences of the heavy and light chain variable domains for the humanized BAP049 clones are shown in Table 1. The amino acid and nucleotide sequences of the heavy and light chain CDRs of the humanized BAP049 clones are also shown in Table 1.

Figure 5 indicates that the samples varied in the concentration of the mAb, ranging from 7.9 μg/mL to 61.5 μg/mL. These numbers were representative of several transient expression experiments.

Example 1.5: Analysis of Humanized Clones

Example 1.5.1: Analysis of binding activity and binding specificity

The binding activity and specificity was measured in a competition binding assay using a constant concentration of Alexa 488-labeled murine mAb, serial dilutions of the test mAbs, and PD-1-expressing 300.19 cells. Incubations with the mAb mixtures having different concentration ratios of test mAb to labeled mAb was at 4 °C for 30 min. Bound labeled murine mAb was then quantified using a FACS machine. The experiment was performed twice. The results are shown in Figures 6A-6B.

Within the accuracy of the experiment, all humanized clones show similar activity for competing with binding of labeled murine mAb. The activity is also comparable to the activity of the parent murine mAb and chimeric mAb. MAbs were ranked relative to each other. For example, it can be a weaker competitor if in both experiments the curve of a certain clone is to the right of the chimeric mAb curve or it can be a better competitor if the curve of a certain clone is to the left of the chimeric mAb curve. Such a ranking system was used in Figure 7.

Example 1.5.2: Sequence analysis

Based on structural features, the sixteen humanized mAbs were divided into four groups and ranked from A to E. The results are shown in Figure 7.

Example 1.5.3: Selection of humanized clones

Figure 7 summarizes the data which was considered for the selection of humanized clones. Expression data (2nd column), the diversity in the composition of the variable regions (3rd column), relative rankings in binding studies (4th and 5th columns), and structural analysis (6th column), were considered.

Selected clones were further tested for their ability to block the binding of PD-L1 and PD-L2 to PD-1 and for enhancing T cell activity *in vitro* assays with human PBMC.

Example 1.5.4: Blocking of ligand binding

Murine anti-PD-1 mAb blocks the binding of the natural ligands PD-L1 and PD-L2 to PD-1 expressed on cells at low concentrations. Whether the humanized clones had preserved the blocking capacity of the parent murine mAb was tested in comparative experiments with murine

and chimeric antibodies.

The blocking capacity of the mAbs was evaluated in a competition binding assay using a constant concentration of PD-L1-huIgG1 Fc fusion protein or PD-L2-huIgG1 Fc fusion protein, serial dilutions of the mAbs to be tested, and PD-1-expressing 300.19 cells. Incubation was at 4 °C for 30 min. Bound ligand fusion proteins were detected with PE-conjugated $F(ab')_2$ fragment of goat anti-human IgG which doesn't recognize IgG4 mAbs (Southern Biotech 2043-09), and flow cytometry. The results are shown in Figures 8A-8B.

Within the accuracy of the experiments, the humanized clones, chimeric antibody and murine parent mAb demonstrated comparable blocking activity for both the PD-L1 and PD-L2 ligands.

Example 1.5.5: T-Cell epitope analysis

Humanized mAbs were analyzed for T cell epitopes using Epibase™. The algorithm analyzes each possible peptide (each 10-mer along the protein advancing by one amino acid) for binding to HLA class II. It estimates free energy of binding (ΔG_{bind}) for each peptide and calculates a putative K_D ($\Delta G_{bind} = RT \ln K_D$). Then peptides are labeled S, M, or N for strong, medium, and non-binders. Threshold values used for this classification are different for each allotype.

The data was normalized to a risk score. The overall "risk score" is the sum of all potential epitopes to all tested alleles, weighted by the affinities of the respective peptides but leaving out all potential epitopes in germ line sequences (lower value therefor is "better").

There are roughly three categories of mAbs, derived from a large set of mAbs of different composition as described below.

Risk score of around 500: fully human mAbs generated from humans, "humanized" mice, and phage libraries ("values below 500 are really good even for fully human antibodies"). Humanized mAbs specifically engineered (even the CDRs) to have a low score are typically in the 500-700 risk category.

Risk score around 900: typical CDR-grafted antibodies, which have fully murine CDRs with or without changes in the FW region; approved CDR-grafted mAbs are basically all in this category.

Risk score around 1500: chimeric mAbs.

The results for selected humanized BAP049 mAbs are:

Clone No.	Risk score
01	476
05	479
08	472
09	503
10	583
11	614

Selected humanized clones have low scores. Typically, values below 500 indicate low risk of immunogenicity even for fully human antibodies. For example, the human mAb, adalimumab (Humira®), has a score of 654, which is relatively high for human mAbs (at the upper end of the Gaussian curve) but low in comparison to a typical CDR-grafted mAb.

Summary and Conclusions

Murine anti-PD-1 monoclonal antibody, BAP049, was humanized. The technology entails the cloning of the murine CDRs in-frame into an ordered library of human germ line variable region frameworks, expressing the library of cloned variable regions as intact IgG4(S228P) humanized mAbs in CHO cells, and selecting clones that bind with comparable or higher affinity to the target as the parent mAb. Therefore, the murine CDRs were asked to select proper human germline framework sequences that preserve their conformations and thus the binding affinity and specificity of the parent murine mAb. The sequences and test samples of sixteen humanized mAbs with unique variable region sequences were obtained, which had passed a binding test with PD-1-transfected CHO cells. These clones were further analyzed for their biological functions (*e.g.*, antigen binding and ligand blocking), structural features, and transcient expression in CHO cells.

Example 2: Expression of Humanized Anti-PD-1 Antibody, BAP049

Five humanized clones descriebed in Example 1 were selected for evaluation of expression in Chinese Hamster Ovary (CHO) cells.

Single gene vectors (SGVs) were constructed using Lonza's GS Xceed vectors (IgG4proΔk for heavy chain and Kappa for light chain). The SGVs were amplified and transiently co-transfected into CHOK1SV GS-KO cells for expression at a volume of 2.8 L.

Expression cultures were harvested Day 6 post-transfection and clarified by centrifugation and sterile filtration. The clarified cell culture supernatant was purified using one-step Protein A chromatography. Product quality analysis in the form of SE-HPLC, SDS-PAGE, IEF, and LAL was carried out using purified material at a concentration of 1 mg/ml inicluding an antibody as a control sample.

Example 2.1: Vector Construction

The sequences of the light and heavy chain variable domain encoding regions were synthesised by GeneArt AG. Light chain variable domain encoding regions were sub-cloned into pXC-Kappa and heavy chain variable domain encoding regions into pXC-IgG4pro ΔK vectors respectively using the N-terminal restriction site Hind III and the C-terminal restriction sites BsiWI (light chain) and ApaI (heavy chain). Positive clones were screened by PCR amplification (primers 1053: GCTGACAGACTAACAGACTGTTCC (SEQ ID NO: 226) and 1072: CAAATGTGGTATGGCTGA (SEQ ID NO: 227)) and verified by restriction digest (using a double digest of EcoRI-HF and HindIII-HF) and nucleotide sequencing of the gene of interest.

Example 2.2: DNA Amplification

A single bacterial colony was picked into 15 ml Luria Bertani (LB) medium (LB Broth, Sigma-Aldrich, L7275) containing 50 μg/ml ampicillin and incubated at 37 °C overnight with shaking at 220 rpm. The resulting starter culture was used to inoculate 1 L Luria Bertani (LB) medium containing 50 μg/ml ampicillin and incubated at 37 °C overnight with shaking at 220 rpm. Vector DNA was isolated using the QIAGEN Plasmid Plus Gigaprep system (QIAGEN, 12991). In all instances, DNA concentration was measured using a Nanodrop 1000 spectrophotometer (Thermo-Scientific) and adjusted to 1 mg/ml with EB buffer (10 mM Tris-Cl, pH 8.5). DNA quality for the single gene vectors was assessed by measuring the absorbance ratio A260/A280. This was found to be between 1.88 and 1.90.

Example 2.3: Culture of CHOK1SV GS-KO Cells

CHOK1SV GS-KO cells were cultured in CD-CHO media (Invitrogen, 10743-029) supplemented with 6 mM glutamine (Invitrogen, 25030-123). Cells were incubated in a shaking

incubator at 36.5 °C, 5% CO_2, 85% humidity, 140 rpm. Cells were routinely sub-cultured every 3-4 days, seeding at 2 x 10^5 cells/ml and were propagated in order to have sufficient cells available for transfection. Cells were discarded by passage 20.

Example 2.4: Transient Transfections of CHOK1SV GS-KO Cells

Transient transfections were performed using CHOK1SV GS-KO cells which had been in culture a minimum two weeks. Cells were sub-cultured 24 h prior to transfection and cell viability was >99% at the time of transfection.

All transfections were carried out via electroporation using a Gene Pulse MXCell (Bio-Rad), a plate based system for electroporation. For each transfection, viable cells were resuspended in pre-warmed media to 2.86 x 10^7 cells/ml. 80 µg DNA (1:1 ratio of heavy and light chain SGVs) and 700 µl cell suspension were aliquotted into each cuvette/well. Cells were electroporated at 300 V, 1300 µF. Transfected cells were transferred to pre-warmed media in Erlenmeyer flasks and the cuvette/wells rinsed twice with pre-warmed media which was also transferred to the flasks. Transfected cell cultures were incubated in a shaking incubator at 36.5 °C, 5% CO_2, 85% humidity, 140 rpm for 6 days. Cell viability and viable cell concentrations were measured at the time of harvest using a Cedex HiRes automated cell counter (Roche).

Example 2.5: Protein A Affinity Chromatography

Cell culture supernatant was harvested and clarified by centrifugation at 2000 rpm for 10 min, then filtered through a 0.22 µm PES membrane filter. Clarified supernatant was purified using a pre-packed 5 ml HiTrap MabSelect SuRE column (GE Healthcare, 11-0034-94) on an AKTA purifier (10 ml/min). The column was equilibrated with 50 mM sodium phosphate, 125 mM sodium chloride, pH 7.0 (equilibration buffer) for 5 column volumes (CVs). After sample loading, the column was washed with 2 CVs of equilibration buffer followed by 3 CVs of 50 mM sodium phosphate, 1 M sodium chloride pH 7.0 and a repeat wash of 2 CVs of equilibration buffer. The Product was then eluted with 10 mM sodium formate, pH 3.5 over 5 CVs. Protein containing, eluted fractions were immediately pH adjusted to pH 7.2 and filtered through a 0.2 µm filter.

A single protein-containing peak was observed during the elution phase. This peak was shown to contain the mAb, when analyzed by SE-HPLC and SDS-PAGE. Recovered protein yield is shown in Table 5. The clones expressed transiently in a range from 32.4 to 43.0 mg/L.

Table 5. Summary of yield, titre, monomer content and endotoxin levels

Product	Yield* (mg)	Titre* (mg/L)	Monomer Content (%)	Endotoxin levels (EU/mg)
Clone A	107.5	38.38	93.94	0.04
Clone B	93.8	33.50	95.28	0.63
Clone C	90.7	32.38	97.83	0.04
Clone D	108.9	38.88	96.53	0.35
Clone E	120.4	43.00	97.73	0.14
*Post Protein A purification				

Example 2.6: SE-HPLC Analysis

Samples of Protein A purified antibodies were analyzed in duplicate by SE-HPLC on an Agilent 1200 series HPLC system, using a Zorbax GF-250 4 µm 9.4 mm ID x 250 mm column (Agilent). Aliquots of sample at a concentration of 1 mg/ml were filtered through a 0.2 µm filter prior to injection. 80 µl aliquots were injected respectively and run at 1 ml/min for 15 minutes. Soluble aggregate levels were analysed using Chemstation (Agilent) software.

Chromatography profiles with retention time showing the percentage of the overall detected peak areas were obtained for the tested antibodies and a control IgG4 antibody. The products show a single protein peak at approximately 8.65 to 8.72 min comparable to the human IgG4 antibody control (about 8.64 min) and consistent with a monomeric antibody. Small amounts (up to about 4-5%) of higher molecular weight impurities, consistent with soluble aggregates, were detected at retention times between about 7.43 and 8.08 min.

Example 2.7: SDS-PAGE Analysis

Reduced samples were prepared for analysis by mixing with NuPage 4x LDS sample buffer (Invitrogen, NP0007) and NuPage 10x sample reducing agent (Invitrogen, NP0009), and incubated at 70 °C, 10 min. For non-reduced samples, the reducing agent and heat incubation

were omitted. Samples were electrophoresed on 1.5 mm NuPage 4-12% Bis-Tris Novex pre-cast gels (Invitrogen, NP0335PK2) with NuPage MES SDS running buffer under denaturing conditions. 10 µl aliquots of SeeBlue Plus 2 pre-stained molecular weight standard (Invitrogen, LC5925) and a control IgG4 antibody at 1 mg/ml were included on the gel. 1 µl of each sample at 1 mg/ml were loaded onto the gel. Once electrophoresed, gels were stained with InstantBlue (TripleRed, ISB01L) for 30 min at room temperature. Images of the stained gels were analysed on a BioSpectrum Imaging System (UVP).

The analysis confirmed the presence of the antibody products and good levels of purity. Under non-reducing conditions, a predominant protein band close to 98 kDa was observed comparable with the control IgG4 antibody. The control IgG4 antibody and one tested clone display an additional fainter band corresponding to a heavy plus light chain half-antibody at approximately 70 kDa under non-reducing conditions. This is expected for the control antibody. Two bands were observed under reducing conditions consistent with the size of heavy (close to the position of the 49 kDa marker) and light chains (close to the position of the 28 kDa marker) and comparable with the bands found for the control IgG4 antibody.

Example 2.8: Iso-electric Focussing (IEF) Analysis

Non-reduced samples of Protein A purified antibody were electrophoresed as described below.

5 µg of Protein A purified samples were electrophoresed on a 1.0 mm Novex pH 3-10 gradient gel (Invitrogen, EC66552BOX) using manufacturers recommended running conditions. A 10 µl aliquot of IEF pH 3 – 10 markers (Invitrogen, 39212-01) was included on the gel. Once electrophoresed, gels were fixed with 10% TCA solution for 30 min and then stained with InstantBlue (TripleRed, ISB01L) over night at room temperature. Images of the stained gels were analysed on a BioSpectrum Imaging System (UVP).

As shown in Table 6, the tested clones show charge isoforms between pH 7.4 and 8.0 markers. The detected charge isoforms are slightly more basic than the theorectically calculated pIs for these antibodies which were predicted to be between 6.99 and 7.56. The general shift to more basic charge isoforms suggests the presence of post-translational modifications such as glycosylation on the molecules. Clone C and Clone E show comparable charge isoforms, which

is also consistent with the theorectically calculated pI being the same for both (6.99). The control IgG4 antibody behaved as expected.

Table 6. Charge isoforms as detected by Novex IEF analysis

Product	pI of predominant charge isoform*	Acidic charge isoforms*	Basic charge isoforms*
Clone A	7.6	2x; 7.5 to 7.55	7.7
Clone B	7.75	2x; 7.5 to 7.6	7.8
Clone C	7.5	2x; 7.4 to 7.5	7.55
Clone D	8.0	7.9	8.1
Clone E	7.5	2x; 7.4 to 7.5	7.55
*pI readings are estimated from the staining positions correlated against the IEF 3-10 marker.			

Example 2.9: Endotoxin Analysis

Endotoxin levels of purified proteins were measured at final concentrations (up to 3.44 mg/ml) using an Endosafe-PTS instrument, a cartridge based method based on the LAL assay (Charles River).

As shown in Table 5, the endotoxin content was found to range from 0.04 to 0.63 EU/mg.

Conclusion

GS single gene expression vectors for selected humanized anti-PD-1 mAbs were constructed and used to transiently transfect CHOK1SV GS-KO cells. 2.6 to 2.8 litres of expression culture were incubated under standard conditions for 6 days and the resulting cell culture supernatant purified using Protein A chromatography. Post-purification titres are indicated in Table 5 and were found to be ranging from 32.38 to 43.0 mg/L. The recovered yields range from 90.7 to 120.4 mg.

SDS-PAGE and SE-HPLC analysis indicated the presence of a small amount (up to 6.06%) of soluble aggregates present in the products being predominantly consistent with dimeric antibody for the mAb. The mAbs also showed higher molecular weight impurities at retention times consistent with that of trimeric antibodies.

Iso-electric focusing detected a number of charge isoforms for all mAbs. The mAbs showed isoforms generally more basic when based on theoretically calculated pI for these molecules indicating some level of post translation modification. The mAbs were found to be comparable to their theoretically calculated pI values.

The endotoxin levels for all samples were measured prior to provision of samples and found to be below 0.63 EU/mg.

Example 3: Characterization of Murine and Humanized Anti-PD-1 Antibodies

Example 3.1: Characterization of Murine Anti-PD-1 Antibody

The binding affinity of murine anti-PD-1 antibody BAP049 to PD-1 was investigated. The murine anti-PD-1 antibody binds to human PD-1-Ig fusion protein with a K_D of 0.04 nM as measured by ELISA. As shown by FACS analyses, the murine anti-PD-1 antibody binds to human PD-1 transfected Jurkat cells with a K_D of 0.06 nM, to cynomolgus T cells (*e.g.*, CD3/CD28 activated CD4 T cells) with a K_D of 0.4 nM, and to cells transfected with cynomolgus PD-1 with a K_D of 0.6 nM.

The blocking activity of murine anti-PD-1 antibody BAP049 was examined by competition binding assays. The murine anti-PD-1 antibody blocked PD-L1 binding to human PD-1-expressing 300.19 cells with an IC50 of 0.3 nM. It blocked PD-L2 binding to human PD-1-expressing 300.19 cells with an IC50 of 0.9 nM.

The effect of murine anti-PD-1 antibody BAP049 on interferon gamma (IFN-γ) expression was tested. The murine anti-PD-1 antibody resulted in 2.3±1.1 fold increase in IFN-γ expression on cells stimulated with anti-CD3 (0.1 μg/mL), 2.5±2.0 fold increase on cells stimulated with Staphylococcal enterotoxin B (SEB) (3 pg/mL), and 2.8±0.8 fold increase on cells stimulated with CMV peptides.

The murine anti-PD-1 antibody BAP049 was also found to increase proliferation of $CD8^+$ T cells activiated with CMV peptides as indicated by the percentages of $CD8^+$ cells that passed through at least certain number (*n*) of cell divisions (*e.g.*, *n*=2, 4, 6).

Example 3.2: Characterization of Humanized Anti-PD-1 Antibody

Binding affinity and specificity

The binding of an exemplary humanized anti-PD-1 antibody on human PD-1 protein was

measured using Biacore method. The results are: Ka = 2.78×10^{5} $M^{-1}s^{-1}$; Kd = 2.13×10^{-4} s^{-1}; K_D = 0.0827±0.005505 nM.

The binding of the same humanized anti-PD-1 antibody on human PD-1-expressing 300.19 cells was measured using FACS analysis. The result shows that the anti-PD-1 antibody (human IgG4) binds with high affinity to human PD-1 compared to a human IgG4 isotype control.

The exemplary humanized anti-PD-1 antibody was found to exhibit high affinity to cynomolgus PD-1 protein and cynomolgus PD-1-expressing 300.19 cells. As measured by Biacore method, the anti-PD-1 antibody binds to cynomolgus PD-1 with a K_D of 0.093±0.015 nM. The binding affinity to cynomolgus PD-1 is comparable to its binding affinity to human PD-1.

Additional binding analyses show that the exemplary humanized anti-PD-1 antibody is not cross-reactive with mouse PD-1 or cross-reactive with parental cell line.

Blocking of interactions between PD-1 and its ligands

The ability of the exemplary humanized anti-PD-1 antibody to block the interactions between PD-1 and both of its known ligands, PD-L1 and PD-L2 was examined. The results show that the anti-PD-1 antibody blocked the binding of PD-L1 and PD-L2 on human PD-1-expressing 300.19 cells compared to human IgG4 isotype control and no antibody control. The anti-PD-1 antibody blocked PD-L1 binding on the 300.19 cells with an IC50 of 0.94±0.15 nM. The same antibody blocked PD-L2 binding on the 300.19 cells with an IC50 of 1.3±0.25 nM.

Cellular activity

The ability of the exemplary humanized anti-PD-1 antibody to enhance the Staphylococcal enterotoxin B (SEB)-stimulated expression of IL-2 was tested in human whole blood *ex vivo* assay. Diluted human whole blood was incubated with the anti-PD-1 antibody in the presence or absence of SEB at 37°C for 48 hours prior to IL-2 measurement. The result shows that the anti-PD-1 antibody increased SEB-stimulated IL-2 expression by 2.28±0.32 fold compared to a human IgG4 isotype control (25 μg/ml SEB; n=5 donors).

Example 4: Patient selection based on PD-L1/CD8/IFN-γ status

For each of several types of cancer, samples from multiple patients were tested for PD-L1/CD8/IFN-γ status. Each sample was classified as: triple-negative for PD-L1/CD8/IFN-γ, single or double positive for these markers, or triple-positive for these markers. Figure 11 shows that in this experiment, within a population of patients, the following types of cancer are frequently triple-positive for PD-L1/CD8/IFN-γ: Lung cancer (squamous), lung cancer (adenocarcinoma), head and neck cancer, cervical cancer (squamous), stomach cancer, thyroid cancer, melanoma, and nasopharyngeal cancer. Patients having these types of cancer are good candidates for therapy with anti PD-1 antibodies and combination therapies as described herein. The likelihood of successful treatment can be further boosted by determining which patients are triple-positive for PD-L1/CD8/IFN-γ, and treating the triple-positive patients with anti PD-1 antibodies and combination therapies as described herein.

Figure 12 shows that within a population of patients, the following types of cancer are rarely triple positive for PD-L1/CD8/IFN-γ: ER+ breast cancer and pancreatic cancer. Notably, even in cancers that are generally not positive for for PD-L1/CD8/IFN-γ, one can increase the likelihood of successful treatment by determining which patients are triple-positive for PD-L1/CD8/IFN-γ, and treating the triple-positive patients with anti PD-1 antibodies and combination therapies as described herein.

Figure 13 shows the proportion of breast cancer patients that are triple positive for PD-L1/CD8/IFN-γ. Considering breast cancer in general, the proportion of triple-positives is somewhat low. However, when one focuses only on IM-TN breast cancer, it can be seen that a much larger percentage of patients is triple positive for PD-L1/CD8/IFN-γ. IM-TN breast cancer is particularly difficult to treat with conventional therapies. The discovery that IM-TN breast cancer is often triple-postive for PD-L1/CD8/IFN-γ opens up new avenues of therapy for this cancer with anti PD-1 antibodies and combination therapies as described herein.

Figure 14 shows the proportion of colon cancer patients that are triple positive for PD-L1/CD8/IFN-γ. Considering colon cancer in general, the proportion of triple-positive is somewhat low. However, when one focuses only on MSI-high (high microsatellite instability) breast cancer, it can be seen that a much larger percentage of patients is triple positive for PD-L1/CD8/IFN-γ. MSI levels can be assayed using, *e.g.*, commercially available PCR-based methods.

Gastric cancer samples were tested for levels of PD-L1/CD8/IFN-γ (data not shown). It was found that in MSI-high or EBV+ gastric cancers, about 49% were positive for PD-L1, and a high proportion of the PD-L1-positive cells were triple positive for PD-L1/CD8/IFN-γ. It was also found that a proportion of PD-L1-positive cells and PD-L1/CD8/IFN-γ positive cells were also positive for PIK3CA. This finding suggests that these cancers may be treated with a PD-1 antibody, optionally in combination with a PIK3 therapeutic.

MSI-high CRC samples were tested for a combination of markers (data not shown). It was found that in MSI-high CRC samples, a high proportion of the PD-L1/CD8/IFN-γ samples are also positive for LAG-3, PD-1 (also called PDCD1), RNF43, and BRAF. This finding suggests that these cancers may be treated with a PD-1 antibody, optionally in combination with a therapeutic that targets one or more of LAG-3, PDCD1, RNF43, and BRAF.

Squamous cell lung cancers were tested for a combination of markers (data not shown). It was found that in squamous cell lung cancer samples, a high proportion of the PD-L1/CD8/IFN-γ samples are also positive for LAG-3. This finding suggests that these cancers may be treated with a PD-1 antibody, optionally in combination with a therapeutic that targets LAG-3, *e.g.*, a LAG-3 antibody.

Papillary thyroid cancers were tested for a combination of markers including the BRAF V600E mutation (data not shown). It was found that a high proportion of thyroid cancer samples that are positive for PD-L1 are also positive for BRAF V600E. This finding suggests that these cancers may be treated with a PD-1 antibody, optionally in combination with a therapeutic that targets BRAF.

Example 5: Patient selection based on PD-L1 status

To enable broad examination of cancer indications for PD-1/PD-L1 based therapies, we evaluated PD-L1 expression at both the protein and mRNA level in human cancers including both lung and hepatic tumors.

PD-L1 protein expression was evaluated in a set of formalin-fixed paraffin-embedded non-small cell lung (NSCLC) adenocarcinoma (ACA), NSCLC squamous cell carcinoma (SCC), and hepatocellular carcinoma (HCC) tumors by immunohistochemistry (IHC). PD-L1 expression was scored semi-quantitatively by a manual histo-score (H-score) methodology based on staining intensity and percentage of positive tumor cells. In our IHC analysis, PD-L1

positivity (PD-L1+) was defined as an H-score $\geqslant$ 20. In parallel, PD-L1 mRNA expression data was examined from The Cancer Genome Atlas (TCGA) in these same indications (503 NSCLC ACA, 489 NSCLC SCC, and 191 HCC) and analyzed by comparing the expression in matched normal tissues from TCGA.

With RNAseq analysis, data was calculated as log2 (RPKM+0.1) after RSEM normalization, utilizing OmicSoft RNASeq pipelines across TCGA tumor indications. The expression of PD-L1 is elevated in NSCLC ACA and SCC, relative to that in HCC. By overlaying the distributions and comparing the expression levels across all indications in TCGA, we ranked overexpression profiles for PD-L1 and found the TCGA HCC cohort to have much reduced PD-L1 mRNA levels, with a median level of -0.8 compared to 1.3 for ACA and 1.5 for SCC, which amounts to more than a 2-fold change of median level expression. With RNAseq, our analysis defines 50% of NSCLC adenocarcinoma, 54% of NSCLC squamous cell carcinoma, and 6% of HCC as high expressers for PD-L1.

Tumor cell PD-L1 protein expression was measured in 45 lung adenocarcinoma (ACA) samples, 47 lung squamous cell carcinoma (SCC) samples, and 36 hepatocellular carcinoma (HCC) samples. 16/45 (35.6%) lung ACA, 21/47 (44.7%) lung SCC were PD-L1 positive. In contrast, PD-L1 positivity was seen in only 2/36 (5.6%) HCC samples.

In summary, with IHC and RNAseq analysis in large and independent human NSCLC and HCC sample sets, we have found PD-L1 expression to be more enriched in NSCLC than in HCC. Within NSCLC, there are comparable findings between adenocarcinoma and squamous cell carcinomas. Importantly, amongst the large number of samples (128 for IHC and 1183 for RNAseq) in the 3 indications, very good concordance is observed between protein- and mRNA-based analyses. Our finding thus establishes the basis for large scale mRNA-based data mining in TCGA for indications and patient segments that may be enriched for responses to PD-1/PD-L1-based immune therapies.

Example 6: Effects of Targeted Agents on PD-L1 Modulation

This example evaluates the effects of selected therapeutic agents (*e.g.*, a cMET inhibitor, a MEK inhibitor, a bRAF inhibitor, and an ALK inhibitor) on PD-L1 (CD274) modulation. Compound A17 can be prepared as disclosed in Example 21 of US Patent No. 8,420,645. The following compounds: Compound A18 (ruxolitinib phosphate), Compound A23 (ceritinib),

Compound A34 (Binimetinib), and Compound A29 (Encorafenib) are available from Novartis AG, Basel, Switzerland. Selected therapeutic agents were examined by real time PCR and flow cytometry on PD-L1 levels. Significant inhibition of PD-L1 by Compound A17, Compound A18, Compound A34, Compound A29, and Compound A23 on tumor cells was observed.

Compound A17 downregulation of PD-L1 protein in Non-Small Cell Lung Cancer cells

PD-L1 (CD274) expression was analyzed in cancer cell lines treated with Compound A17. Cells were obtained from ATCC and cultured *in vitro* following ATCC directions. The cell lines used were previously characterized by the Cancer Cell Line Encyclopedia Project (http://www.broadinstitute.org/ccle/home).

Cells plated in six-well culture plates were treated with the Compound A17 at different concentrations (10 nM, 100 nM, and 1000 nM) for 24, 48 and 72 hours. Equal amount of vehicle (DMSO) was used as a control. Cells were washed with PBS and then harvested using a cell scraper.

For each reaction, 0.5-1 x 10^6 cells were stained with 20μL of anti-human monoclonal PD-L1 – PE antibody, clone M1H1 (BD) for 30-60 minutes at 4°C. Cells were washed twice and data was acquired using a Canto II with FACSDiva software (BD Bioscience). Data analysis was performed using FlowJo software (Tree Star). Mean fluorescence intensity (MFI) was determined by gating on single cells. Unstained cells were used as a gating control.

In vitro treatment of EBC-1 cells (Non-Small Cell Lung Cancer (NSCLC) with cMET amplification) with Compound A17 led to significant downregulation of surface expression of PD-L1 as observed by flow cytometry (Figure 15). The results presented herein suggest that Compound A17 functions as a PD-L1/PD-1 inhibitor.

Compound A17, Compound A34, Compound A18, Compound A29, and Compound A23 downregulate PD-L1 mRNA

TaqMan RT PCR assays were developed to detect changes of expression levels of PD-L1 (CD274) in cell lines and xenograft tumors. mRNA was isolated from frozen cell pellets or tumor fragments using the Qiagen RNeasy Mini kit. Isolated RNA was frozen at -80°C. RNA quality was checked and RNA was quantified using a 2100 Agilent Bioanalyzer following the

protocol for the Agilent RNA 6000 Nano Kit. cDNA was prepared using a High Capacity RNA-to cDNA Kit (Applied Biosystems).

Real-time PCR reactions were carried out in 20µl total volume, including 10µl of Universal PCR master mix (Applied Biosystems), 1µl of human PD-L1 (CD274) probe/primer set (Applied Biosystems), and 8 µl of cDNA. Each sample was run in triplicate. The amount of cDNA produced from 25-50 ng of RNA in the reverse transcription reaction was used in each PCR reaction. Due to difference in mRNA levels between PD-L1 and GAPDH, the two real-time PCR reactions were done in separate tubes using same amount of cDNA. The real-time PCR reaction was run on the C1000 Thermal Cycle (BioRad) with the cycle program as follows: a 10 minute incubation at 95°C followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. After the reaction was complete, the PD-L1 average Ct was normalized relative to each Ct value from the GAPDH reference reaction. Each normalized logarithmic value was then converted into a linear value.

Inhibition of PD-L1 expression (mRNA) by Compound A17 was observed in a Hs.746.T tumor (gastric cancer cell with cMET amplification & mutation) xenograft (Figure 16). Inhibition of PD-L1 mRNA by Compound A23 was observed in H3122 (Non-Small Cell Lung Cancer (NSCLC) with ALK translocation) *in vitro* (Figure 17). Downregulation of PD-L1 mRNA by Compound A29, and Compound A34 was observed in tumor xenograft models bearing LOXIMV1 (BRAF mutant melanoma, Figure 18) and HEYA8 (KRAF mutant ovarian cancer, Figure 19) tumors, respectively. Downregulation of PD-L1 mRNA by Compound A18 was observed in tumor xenograft models bearing UKE-1 (Myeloproliferative Neoplasm (MPN) line with JAK2V617F mutation, Figure 20).

The results presented herein demonstrate a role of Compound A17, Compound A34, Compound A18, Compound A29, and Compound A23 in the regulation of immunecheckpoint molecules on cancer. The observed inhibition of PD-L1 expression by these agents suggests that these targeted agents may have immune-modulatory activities, in addition to their effects on cancer signaling. Thus, the results presented herein suggest that administration of targeted agents with inhibitors of immunecheckpoint inhibitors such as PD-1, PD-L1, LAG-3 and/or TIM-3 will achieve a more potent reversal of the immunecheckpoint-mediated immune suppression.

INCORPORATION BY REFERENCE

All publications, patents, and Accession numbers mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

EQUIVALENTS

While specific embodiments of the subject invention have been discussed, the above specification is illustrative and not restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification and the claims below. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

What is claimed is:

1. An isolated antibody molecule capable of binding to human Programmed Death-1 (PD-1), comprising:

(a) a heavy chain variable region (VH) comprising a VHCDR1 amino acid sequence of SEQ ID NO: 4, a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a light chain variable region (VL) comprising a VLCDR1 amino acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 33;

(b) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 1; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 32;

(c) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 224, a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 33; or

(d) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 224; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 32.

2. The antibody molecule of claim 1, comprising a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 4, a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 33.

3. The antibody molecule of claim 1, comprising a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 1; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino

acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 32.

4. The antibody molecule of claim 1, comprising a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 224, a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 33.

5. The antibody molecule of claim 1, comprising a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 224; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 32.

6. The antibody molecule of any of claims 1-5, wherein said antibody molecule is a humanized antibody molecule.

7. The antibody molecule of any of claims 1-6, wherein said antibody molecule is a monospecific antibody molecule.

8. The antibody molecule of any of claims 1-6, wherein said antibody molecule is a bispecific antibody molecule.

9. The antibody molecule of any of claims 1-8, which has a heavy chain variable region comprising at least one framework (FW) region comprising the amino acid sequence of any of SEQ ID NOs: 147, 151, 153, 157, 160, 162, 166, or 169, or an amino acid sequence at least 90% identical thereto, or having no more than two amino acid substitutions, insertions or deletions compared to the amino acid sequence of any of SEQ ID NOs: 147, 151, 153, 157, 160, 162, 166, or 169.

10. The antibody molecule of any of claims 1-9, which has a heavy chain variable region comprising at least one framework region comprising the amino acid sequence of any of SEQ ID NOs: 147, 151, 153, 157, 160, 162, 166, or 169.

11. The antibody molecule of any of claims 1-10, which has a heavy chain variable region comprising at least two, three, or four framework regions comprising the amino acid sequences of any of SEQ ID NOs: 147, 151, 153, 157, 160, 162, 166, or 169.

12. The antibody molecule of any of claims 1-11, which comprises a VHFW1 amino acid sequence of SEQ ID NO: 147 or 151, a VHFW2 amino acid sequence of SEQ ID NO: 153, 157, or 160, and a VHFW3 amino acid sequence of SEQ ID NO: 162 or 166, and, optionally, further comprising a VHFW4 amino acid sequence of SEQ ID NO: 169.

13. The antibody molecule of any of claims 1-12, which has a light chain variable region comprising at least one framework region comprising the amino acid sequence of any of SEQ ID NOs: 174, 177, 181, 183, 185, 187, 191, 194, 196, 200, 202, 205, or 208, or an amino acid sequence at least 90% identical thereto, or having no more than two amino acid substitutions, insertions or deletions compared to the amino acid sequence of any of 174, 177, 181, 183, 185, 187, 191, 194, 196, 200, 202, 205, or 208.

14. The antibody molecule of any of claims 1-13, which has a light chain variable region comprising at least one framework region comprising the amino acid sequence of any of SEQ ID NOs: 174, 177, 181, 183, 185, 187, 191, 194, 196, 200, 202, 205, or 208.

15. The antibody molecule of any of claims 1-14, which has a light chain variable region comprising at least two, three, or four framework regions comprising the amino acid sequences of any of SEQ ID NOs: 174, 177, 181, 183, 185, 187, 191, 194, 196, 200, 202, 205, or 208.

16. The antibody molecule of any of claims 1-15, which comprises a VLFW1 amino acid sequence of SEQ ID NO: 174, 177, 181, 183, or 185, a VLFW2 amino acid sequence of

SEQ ID NO: 187, 191, or 194, and a VLFW3 amino acid sequence of SEQ ID NO: 196, 200, 202, or 205, and, optionally, further comprising a VLFW4 amino acid sequence of SEQ ID NO: 208.

17. The antibody molecule of any of claims 1-16, which comprises a heavy chain variable domain comprising an amino acid sequence at least 85% identical to any of SEQ ID NOs: 38, 50, 82, or 86.

18. The antibody molecule of any of claims 1-17, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38, 50, 82, or 86.

19. The antibody molecule of any of claims 1-18, which comprises a light chain variable domain comprising an amino acid sequence at least 85% identical to any of SEQ ID NOs: 42, 46, 54, 58, 62, 66, 70, 74, or 78.

20. The antibody molecule of any of claims 1-19, which comprises a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 42, 46, 54, 58, 62, 66, 70, 74, or 78.

21. The antibody molecule of any one of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38.

22. The antibody molecule of any of claims 1-21, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 or SEQ ID NO: 91.

23. The antibody molecule of any one of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 50.

24. The antibody molecule of any of claims 1-20 and 23, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 52 or SEQ ID NO: 102.

25. The antibody molecule of any one of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 82.

26. The antibody molecule of any of claims 1-20 and 25, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 84.

27. The antibody molecule of any one of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 86.

28. The antibody molecule of any of claims 1-20 and 27, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 88.

29. The antibody molecule of any of claims 1-28, which comprises a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 42.

30. The antibody molecule of any of claims 1-29, which comprises a light chain comprising the amino acid sequence of SEQ ID NO: 44.

31. The antibody molecule of any of claims 1-28, which comprises a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 46.

32. The antibody molecule of any of claims 1-28 and 31, which comprises a light chain comprising the amino acid sequence of SEQ ID NO: 48.

33. The antibody molecule of any of claims 1-28, which comprises a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 54.

34. The antibody molecule of any of claims 1-28 and 33, which comprises a light chain comprising the amino acid sequence of SEQ ID NO: 56.

35. The antibody molecule of any of claims 1-28, which comprises a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 58.

36. The antibody molecule of any of claims 1-28 and 35, which comprises a light chain comprising the amino acid sequence of SEQ ID NO: 60.

37. The antibody molecule of any of claims 1-28, which comprises a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 62.

38. The antibody molecule of any of claims 1-28 and 37, which comprises a light chain comprising the amino acid sequence of SEQ ID NO: 64.

39. The antibody molecule of any of claims 1-28, which comprises a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 66.

40. The antibody molecule of any of claims 1-28 and 39, which comprises a light chain comprising the amino acid sequence of SEQ ID NO: 68.

41. The antibody molecule of any of claims 1-28, which comprises a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 70.

42. The antibody molecule of any of claims 1-28 and 41, which comprises a light chain comprising the amino acid sequence of SEQ ID NO: 72.

43. The antibody molecule of any of claims 1-28, which comprises a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 74.

44. The antibody molecule of any of claims 1-28 and 43, which comprises a light chain comprising the amino acid sequence of SEQ ID NO: 76.

45. The antibody molecule of any of claims 1-28, which comprises a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 78.

46. The antibody molecule of any of claims 1-28, which comprises a light chain comprising the amino acid sequence of SEQ ID NO: 80.

47. The antibody molecule of claim 45, which comprises a light chain comprising the amino acid sequence of SEQ ID NO: 80.

48. The antibody molecule of any of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 42.

49. The antibody molecule of any of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 66.

50. The antibody molecule of any of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 70.

51. The antibody molecule of any of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 50 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 70.

52. The antibody molecule of any of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 46.

53. The antibody molecule of any of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 50 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 46.

54. The antibody molecule of any of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 50 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 54.

55. The antibody molecule of any of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 54.

56. The antibody molecule of any of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 58.

57. The antibody molecule of any of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 62.

58. The antibody molecule of any of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 50 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 66.

59. The antibody molecule of any of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 74.

60. The antibody molecule of any of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 78.

61. The antibody molecule of any of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 82 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 70.

62. The antibody molecule of any of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 82 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 66.

63. The antibody molecule of any of claims 1-20, which comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 86 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 66.

64. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 91 and a light chain comprising the amino acid sequence of SEQ ID NO: 44.

65. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 91 and a light chain comprising the amino acid sequence of SEQ ID NO: 56.

66. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 91 and a light chain comprising the amino acid sequence of SEQ ID NO: 68.

67. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 91 and a light chain comprising the amino acid sequence of SEQ ID NO: 72.

68. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 102 and a light chain comprising the amino acid sequence of SEQ ID NO: 72.

69. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 44.

70. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 48.

71. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 52 and a light chain comprising the amino acid sequence of SEQ ID NO: 48.

72. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 52 and a light chain comprising the amino acid sequence of SEQ ID NO: 56.

73. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 56.

74. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 60.

75. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 64.

76. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 52 and a light chain comprising the amino acid sequence of SEQ ID NO: 68.

77. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 68.

78. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 52 and a light chain comprising the amino acid sequence of SEQ ID NO: 72.

79. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 72.

80. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 76.

81. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 80.

82. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 84 and a light chain comprising the amino acid sequence of SEQ ID NO: 72.

83. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 84 and a light chain comprising the amino acid sequence of SEQ ID NO: 68.

84. The antibody molecule of any of claims 1-20, which comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 88 and a light chain comprising the amino acid sequence of SEQ ID NO: 68.

85. The antibody molecule of any of claims 1-84, which is a Fab, F(ab')2, Fv, or a single chain Fv fragment (scFv).

86. The antibody molecule of any of claims 1-84, which comprises a heavy chain constant region selected from IgG1, IgG2, IgG3, and IgG4.

87. The antibody molecule of claim 86, which comprises a light chain constant region chosen from the light chain constant regions of kappa or lambda.

88. The antibody molecule of claim 86 or 87, which comprises a human IgG4 heavy chain constant region with a mutation at position 228 according to EU numbering or position 108 of SEQ ID NO: 212 or 214 and a kappa light chain constant region.

89. The antibody molecule of claim 86 or 87, which comprises a human IgG4 heavy chain constant region with a Serine to Proline mutation at position 228 according to EU numbering or position 108 of SEQ ID NO: 212 or 214 and a kappa light chain constant region.

90. The antibody molecule of claim 86 or 87, which comprises a human IgG1 heavy chain constant region with an Asparagine to Alanine mutation at position 297 according to EU numbering or position 180 of SEQ ID NO: 216 and a kappa light chain constant region.

91. The antibody molecule of claim 86 or 87, which comprises a human IgG1 heavy chain constant region with an Aspartate to Alanine mutation at position 265 according to EU

numbering or position 148 of SEQ ID NO: 217 and Proline to Alanine mutation at position 329 according to EU numbering or position 212 of SEQ ID NO: 217, and a kappa light chain constant region.

92. The antibody molecule of claim 86 or 87, which comprises a human IgG1 heavy chain constant region with a Leucine to Alanine mutation at position 234 according to EU numbering or position 117 of SEQ ID NO: 218 and Leucine to Alanine mutation at position 235 according to EU numbering or position 118 of SEQ ID NO: 218, and a kappa light chain constant region.

93. The antibody molecule of any of claims 1-92, which is capable of binding to human PD-1 with a dissociation constant (K_D) of less than about 0.2 nM.

94. The antibody molecule of any of claims 1-93, which binds an extracellular Ig-like domain of PD-1.

95. The antibody molecule of any of claims 1-94, which is capable of reducing binding of PD-1 to PD-L1, PD-L2, or both, or a cell that expresses PD-L1, PD-L2, or both.

96. The antibody molecule of any of claims 1-95, which is capable of enhancing an antigen-specific T cell response.

97. The antibody molecule of any of claims 1-96, wherein said antibody molecule has a first binding specificity for PD-1 and a second binding specificity for TIM-3, LAG-3, CEACAM-1, CEACAM-5, PD-L1 or PD-L2.

98. The antibody molecule of any of claims 1-97, wherein said antibody molecule comprises an antigen binding fragment of an antibody, *e.g.*, a half antibody or antigen binding fragment of a half antibody.

99. A pharmaceutical composition comprising the isolated antibody molecule of any of claims 1-98 and a pharmaceutically acceptable carrier, excipient or stabilizer.

100. An isolated nucleic acid encoding the antibody heavy or light chain variable region of the antibody molecule of any of claims 1-98.

101. An isolated nucleic acid encoding heavy chain CDRs 1-3, wherein said nucleic acid comprises a nucleotide sequence of SEQ ID NO: 108-112, 223, 122-126, 133-137, or 144-146.

102. An isolated nucleic acid encoding light chain CDRs 1-3, wherein said nucleic acid comprises a nucleotide sequence of SEQ ID NO: 113-120, 127-132, or 138-143.

103. The nucleic acid of claim 101, further comprising a nucleotide sequence encoding a heavy chain variable domain, wherein said nucleotide sequence is at least 85% identical to any of SEQ ID NO: 39, 51, 83, 87, 90, 95, or 101.

104. The nucleic acid of claim 101, further comprising a nucleotide sequence encoding a heavy chain variable domain, wherein said nucleotide sequence comprises any of SEQ ID NO: 39, 51, 83, 87, 90, 95, or 101.

105. The nucleic acid of claim 103, further comprising a nucleotide sequence encoding a heavy chain, wherein said nucleotide sequence is at least 85% identical to any of SEQ ID NO: 41, 53, 85, 89, 92, 96, or 103.

106. The nucleic acid of claim 104, further comprising a nucleotide sequence encoding a heavy chain, wherein said nucleotide sequence comprises any of SEQ ID NO: 41, 53, 85, 89, 92, 96, or 103.

107. The nucleic acid of claim 102, which further comprises a nucleotide sequence encoding a light chain variable domain, wherein said nucleotide sequence is at least 85% identical to any of SEQ ID NO: 43, 47, 55, 59, 63, 67, 71, 75, 79, 93, 97, 99, 104, or 106.

108. The nucleic acid of claim 102, which further comprises a nucleotide sequence encoding a light chain variable domain, wherein said nucleotide sequence comprises any of SEQ ID NO: 43, 47, 55, 59, 63, 67, 71, 75, 79, 93, 97, 99, 104, or 106.

109. The nucleic acid of claim 107, further comprising a nucleotide sequence encoding a light chain, wherein said nucleotide sequence is at least 85% identical to any of SEQ ID NO: 45, 49, 57, 61, 65, 69, 73, 77, 81, 94, 98, 100, 105 or 107.

110. The nucleic acid of claim 108, further comprising a nucleotide sequence encoding a light chain, wherein said nucleotide sequence comprises any of SEQ ID NO: 45, 49, 57, 61, 65, 69, 73, 77, 81, 94, 98, 100, 105 or 107.

111. An expression vector comprising the nucleic acid of any of claims 100-110.

112. A host cell comprising the nucleic acid of any of claims 100-110.

113. A method of producing an antibody molecule or fragment thereof, comprising culturing the host cell of claim 112 under conditions suitable for gene expression.

114. A method of stimulating an immune response in a subject, comprising administering to a subject in need thereof an isolated antibody molecule of any of claims 1-98, or a pharmaceutical composition of claim 99, in an amount effective to stimulate the immune response.

115. A method of treating a cancer, comprising administering to a subject in need thereof an isolated antibody molecule of any of claims 1-98, or a pharmaceutical composition of claim 99, in an amount effective to treat the cancer.

116. The method of claim 115, wherein the cancer is chosen from a lung cancer, a squamous cell lung cancer, a melanoma, a renal cancer, a liver cancer, a myeloma, a prostate cancer, a breast cancer, an ER+ breast cancer, an IM-TN breast cancer, a colorectal cancer, a colorectal cancer with high microsattelite instability, an EBV+ gastric cancer, a pancreatic cancer, a thyroid cancer, a hematological cancer, a non-Hogdkin's lymphoma, or a leukemia, or a metastatic lesion of the cancer.

117. The method of claim 115, wherein the cancer is chosen from a non-small cell lung cancer (NSCLC), a NSCLC adenocarcinoma, a NSCLC squamous cell carcinoma, a hepatocellular carcinoma, an advanced melanoma, a metastatic renal cell carcinoma, or a multiple myeloma.

118. The method of any of claims 114-117, wherein the antibody molecule is administered in combination with a second therapeutic agent or procedure.

119. The method of claim 118, wherein the second therapeutic agent or procedure is chosen from one or more of chemotherapy, a targeted anti-cancer therapy, an oncolytic drug, a cytotoxic agent, an immune-based therapy, a cytokine, surgical procedure, a radiation procedure, an activator of a costimulatory molecule, an inhibitor of an inhibitory molecule, a vaccine, or a cellular immunotherapy.

120. The method of claim 118, wherein the antibody molecule is administered in combination with an agonist of a costimulatory molecule chosen from one or more of OX40, CD2, CD27, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD30, CD40, BAFFR, HVEM, CD7, LIGHT, NKG2C, SLAMF7, NKp80, CD160, B7-H3 or CD83 ligand.

121. The method of claim 118, wherein the antibody molecule is administered in combination with an inhibitor of an immune checkpoint molecule chosen from one or more of

PD-L1, PD-L2, CTLA-4, TIM-3, LAG-3, CEACAM-1, CEACAM-5, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 or TGFR.

122. A method of treating an infectious disease, comprising administering to a subject in need thereof an isolated antibody molecule of any of claims 1-98, or a pharmaceutical composition of claim 99, in an amount effective to treat the infectious disease.

123. A method of detecting PD-1 in a biological sample, comprising (i) contacting the sample or the subject (and optionally, a reference sample or subject) with an isolated antibody molecule of any of claims 1-98 under conditions that allow interaction of the antibody molecule and the polypeptide to occur, and (ii) detecting formation of a complex between the antibody molecule and the sample or the subject (and optionally, the reference sample or subject).

124. An antibody molecule of any of claims 1-98, or a pharmaceutical composition of claim 99, for use in treating a cancer or an infectious disease in a subject.

125. Use of an antibody molecule of any of claims 1-98, or a pharmaceutical composition of claim 99, in the manufacture of a medicament for treating a cancer or an infectious disease in a subject.

Heavy Chain (murine IgG1)

```
      FWH1                          CDRH1    FWH2           CDRH2
QVQLQQSGSE LVRPGASVKL SCKAS GYTFT TYWMHWVRQR PGQGLEWIGN IYPGTGGSNF DEKFKNRTSL
QVQLQQPGSE LVRPGASVKL SCKAS GYTFT TYWMHWVRQR PGQGLEWIGN IYPGTGGSNF DEKFKNRTSL

       FWH3                     CDRH3        FWH4
TVDTSSTTAY MHLASLTSED SAVYYCTR WT TGTGAYWGQG TLVTVSA
TVDTSSTTAY MHLASLTSED SAVYYCTR WT TGTGAYWGQG TLVTVSAAKT TPPSVYPLAP GSAA
```

Light Chain (murine κ)

```
      FWL1                      CDRL1              FWL2       CDRL2
DIVMTQSPSS LTVTAGEKVT MSCKS SQSLL DSGNQKNFLT WYQQKPGQPP KLLIF WAS TR ESGVPDRFTG
DIVMTQSPSS LTVTAGEKVT MSCKS SQSLL DSGNQKNFLT WYQQKPGQPP KLLIF WAS TR ESGVPDRFTG

    FWL3                     CDRL3     FWL4
SGSVTDFTLT ISSVQAEDLA VYYCQN DYSY PCTFGGGTKL EIK
SGSVTDFTLT ISSVQAEDLA VYYCQN DYSY PCTFGGGTKL EIKRAD
```

FIGURE 1

Heavy Chain

```
GL       QVQLQQPGSE LVRPGASVKL SCKASGYTFT SYWMHWVKQR HGQGLEWIGN IYPGSGSTNY
Mu mAb   ------S--- ---------- ---------- T------R-- P--------- ----T-GS-F

GL       DEKFKSKGTL TVDTSSSTAY MHLSSLTSED SAVYYCTR
Mu mAb   -----NRTS- ------T--- ---A------ --------WT TGTGAYWGQG TLVTVSA
```

Light Chain

```
GL       DIVMTQSPSS LTVTAGEKVT MSCKSSQSLL NSGNQKNYLT WYQQKPGQPP KLLIYWASTR
Mu mAb   ---------- ---------- ---------- D------F-- ---------- ----F-----

GL       ESGVPDRFTG SGSGTDFTLT ISSVQAEDLA VYYCQNDYSY P
Mu mAb   ---------- ---V------ ---------- ---------- -CTFGGGTKL EIK
```

FIGURE 2A

```
mAb        C   T   F   G   G   G   T   K   L   E   I   K
mAb    g tgc acg ttc gga ggg ggg acc aag ctg gaa ata aaa
J2     - -a- --- --- --- --- --- --- --- --- --- --- ---c
J2         Y
```

FIGURE 2B

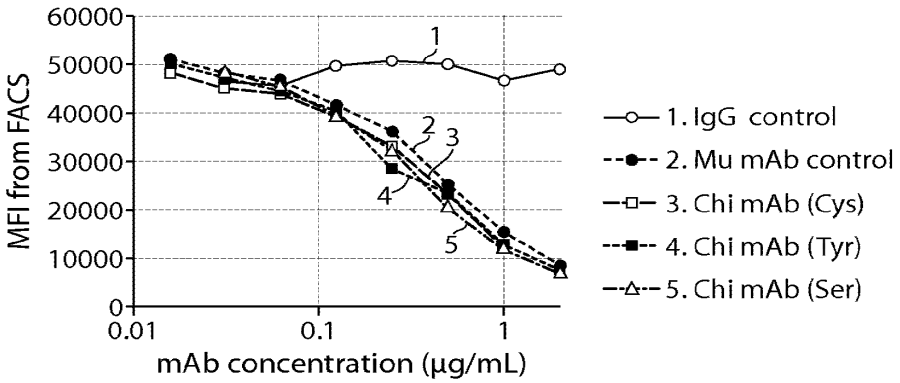


FIGURE 3A

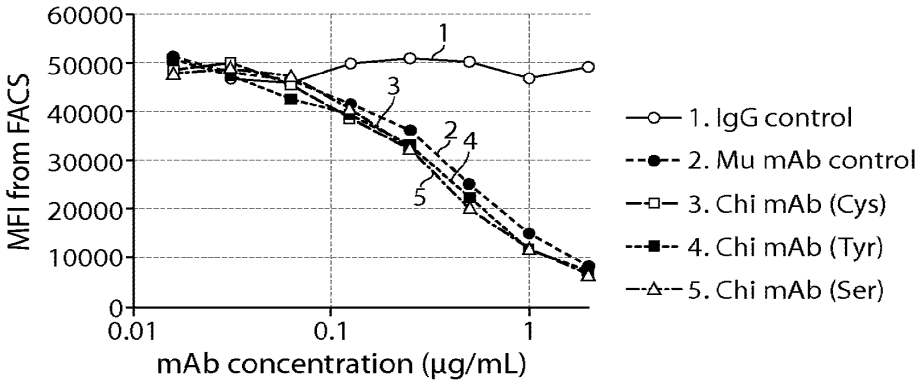


FIGURE 3B

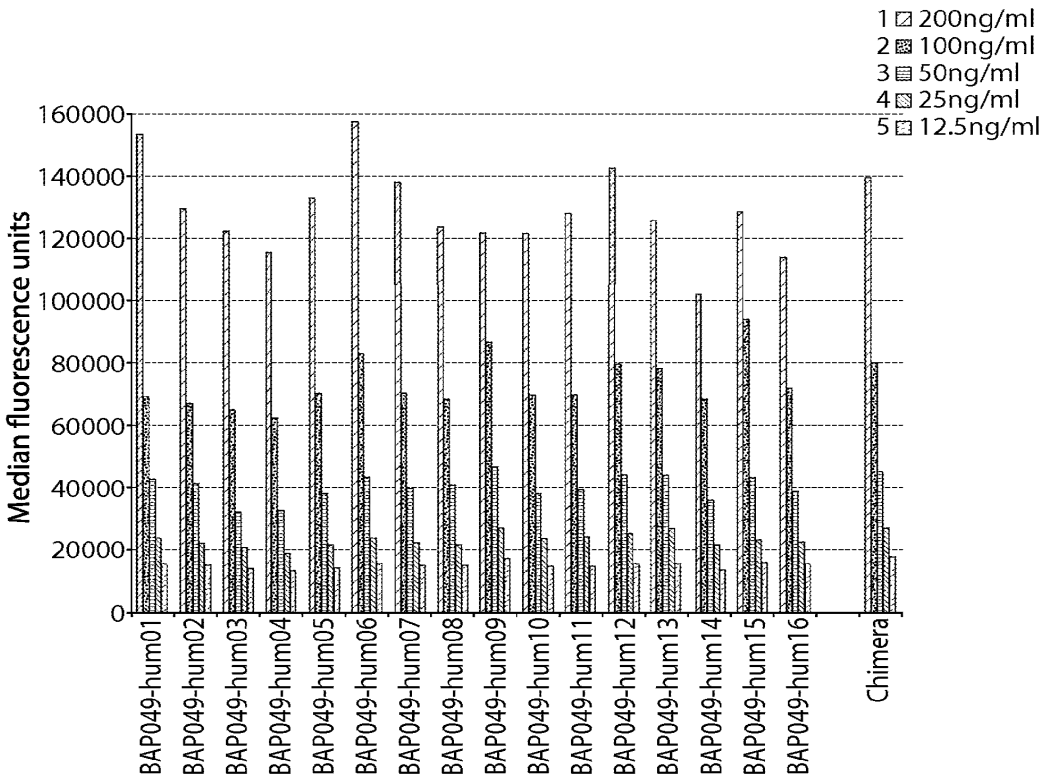


FIGURE 4

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Clone No.	Concentration μg/mL	Sequence					
		HC			LC		
		FW1	FW2	FW3	FW1	FW2	FW3
		4 unique HC			9 unique LC		
1	23.3	a	a	a	b	a	c
2	45.5	a	a	a	e	a	b
3	58.4	a	b	b	e	a	b
4	52.9	a	b	b	b	b	d
5	30	a	a	a	b	b	d
6	7.9	a	a	a	c	a	a
7	24.9	a	a	a	b	b	a
8	32.8	a	b	b	a	a	a
9	16.3	a	a	a	a	a	a
10	61.5	a	b	b	b	a	a
11	31.4	a	a	a	b	a	a
12	34.8	a	a	a	e	c	a
13	8.6	a	a	a	d	b	a
14	48.4	b	b	b	b	a	a
15	20.7	b	b	b	a	a	a
16	32.8	a	c	b	a	a	a

FIGURE 5

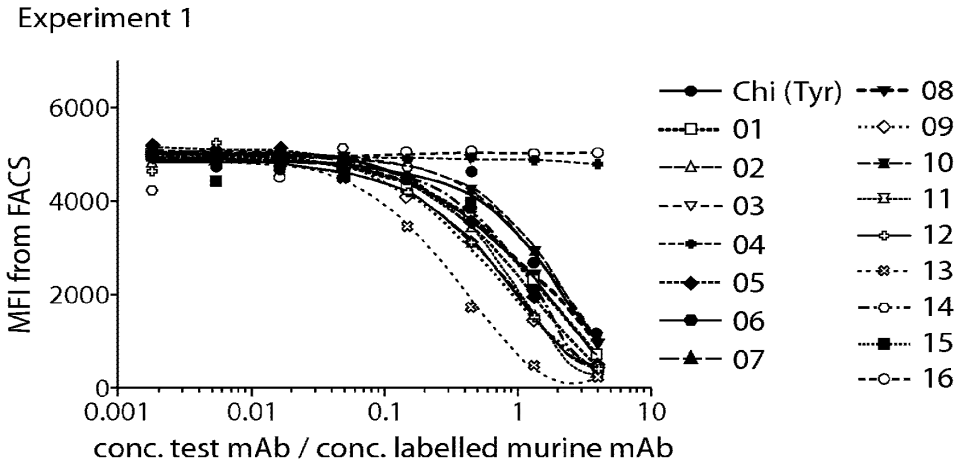


FIGURE 6A

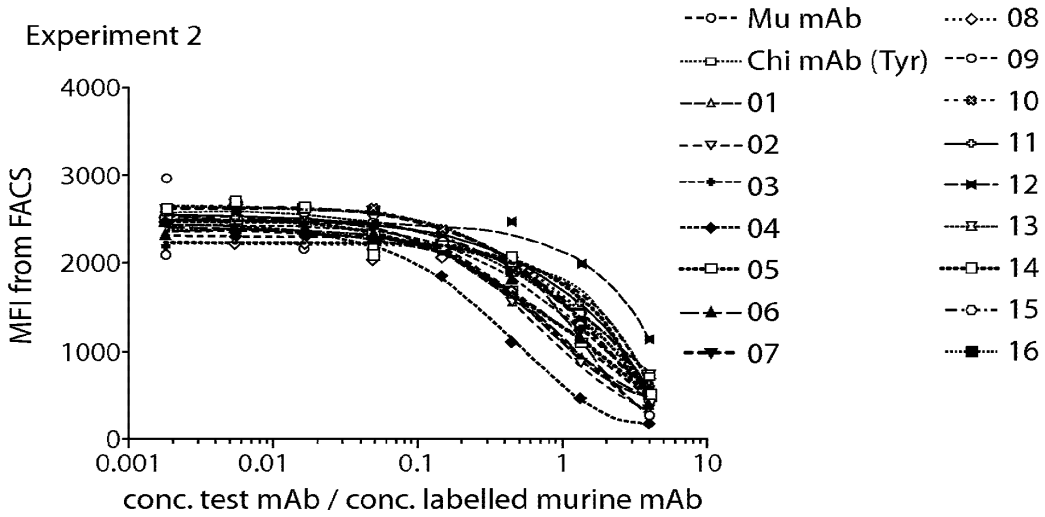


FIGURE 6B

Clone No.	Conc. μg/mL	Sequence						Ranking	Competition Binding		Ranking
		HC			LC			FACS data	1st exp.	2nd exp.*	
		FW1	FW2	FW3	FW1	FW2	FW3				
Chimeric	20.6	4 unique HC			9 unique LC						
1	23.3	a	a	a	b	a	c	**2**	**7**	**2**	**A**
2	45.5	a	a	a	e	a	b	**6**	**3**	**2**	**D**
3	58.4	a	b	b	e	a	b	**7**	**8**	**14**	**E**
4	52.9	a	b	b	b	b	d	**14**	**15**	**15**	**B**
5	30	a	a	a	b	b	d	**5**	**5**		**A**
6	7.9	a	a	a	c	a	a	**1**	**7**	**3**	**D**
7	24.9	a	a	a	b	b	a	**4**	**7**		**D**
8	32.8	a	b	b	a	a	a	**7**	**7**	**4**	**C**
9	16.3	a	a	a	a	a	a	**7**	**2**	**4**	**B**
10	61.5	a	b	b	b	a	a	**7**	**6**		**C**
11	31.4	a	a	a	b	a	a	**6**	**4**		**B**
12	34.8	a	a	a	e	c	a	**3**	**8**	**16**	**D**
13	8.6	a	a	a	d	b	a	**6**	**1**	**1**	**D**
14	48.4	b	b	b	b	a	a	**16**	**7**	**15**	**C**
15	20.7	b	b	b	a	a	a	**6**	**7**	**15**	**C**
16	32.8	a	c	b	a	a	a	**15**	**16**	**15**	**C**

*empty boxes means worse than 4

FIGURE 7

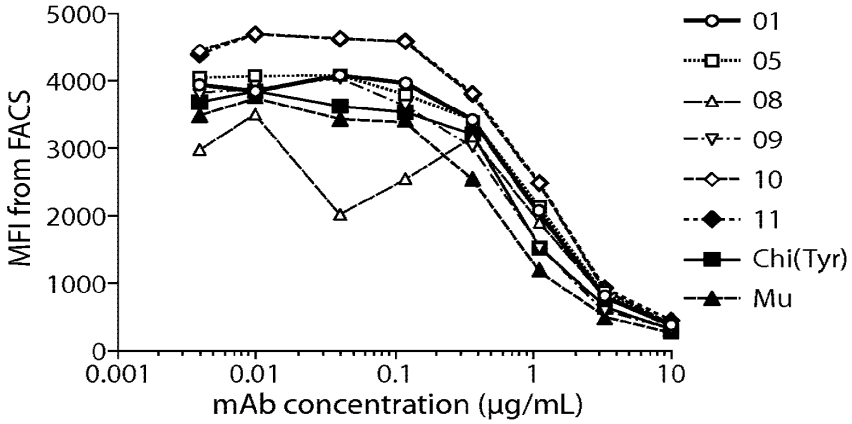


FIGURE 8A

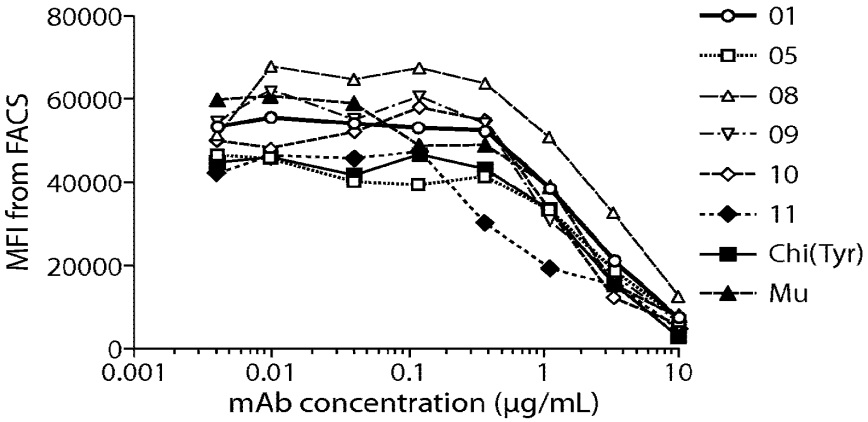


FIGURE 8B

```
                        10        20        30        40        50        60
                   ....|....|....|....|....|....|....|....|....|....|....|
BAP049-chi-HC      QVQLQQSGSELVRPGASVKLSCKASGYTFTTYWMHWVRQRPGQGLEWIGNIYPGTGGSNF
BAP049-hum01-HC    EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMHWVRQATGQGLEWMGNIYPGTGGSNF
BAP049-hum02-HC    EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMHWVRQATGQGLEWMGNIYPGTGGSNF
BAP049-hum05-HC    EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMHWVRQATGQGLEWMGNIYPGTGGSNF
BAP049-hum06-HC    EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMHWVRQATGQGLEWMGNIYPGTGGSNF
BAP049-hum07-HC    EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMHWVRQATGQGLEWMGNIYPGTGGSNF
BAP049-hum09-HC    EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMHWVRQATGQGLEWMGNIYPGTGGSNF
BAP049-hum11-HC    EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMHWVRQATGQGLEWMGNIYPGTGGSNF
BAP049-hum12-HC    EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMHWVRQATGQGLEWMGNIYPGTGGSNF
BAP049-hum13-HC    EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMHWVRQATGQGLEWMGNIYPGTGGSNF
BAP049-hum03-HC    EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMHWIRQSPSRGLEWLGNIYPGTGGSNF
BAP049-hum04-HC    EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMHWIRQSPSRGLEWLGNIYPGTGGSNF
BAP049-hum08-HC    EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMHWIRQSPSRGLEWLGNIYPGTGGSNF
BAP049-hum10-HC    EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMHWIRQSPSRGLEWLGNIYPGTGGSNF
BAP049-hum14-HC    QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWMHWIRQSPSRGLEWLGNIYPGTGGSNF
BAP049-hum15-HC    QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWMHWIRQSPSRGLEWLGNIYPGTGGSNF
BAP049-hum16-HC    EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMHWVRQAPGQGLEWMGNIYPGTGGSNF

                        70        80        90       100       110
                   ....|....|....|....|....|....|....|....|....|....|..
BAP049-chi-HC      DEKFKNRTSLTVDTSSTTAYMHLASLTSEDSAVYYCTRWTTGTGAYWGQGTTVTVSS
BAP049-hum01-HC    DEKFKNRVTITADKSTSTAYMELSSLRSEDTAVYYCTRWTTGTGAYWGQGTTVTVSS
BAP049-hum02-HC    DEKFKNRVTITADKSTSTAYMELSSLRSEDTAVYYCTRWTTGTGAYWGQGTTVTVSS
BAP049-hum05-HC    DEKFKNRVTITADKSTSTAYMELSSLRSEDTAVYYCTRWTTGTGAYWGQGTTVTVSS
BAP049-hum06-HC    DEKFKNRVTITADKSTSTAYMELSSLRSEDTAVYYCTRWTTGTGAYWGQGTTVTVSS
BAP049-hum07-HC    DEKFKNRVTITADKSTSTAYMELSSLRSEDTAVYYCTRWTTGTGAYWGQGTTVTVSS
BAP049-hum09-HC    DEKFKNRVTITADKSTSTAYMELSSLRSEDTAVYYCTRWTTGTGAYWGQGTTVTVSS
BAP049-hum11-HC    DEKFKNRVTITADKSTSTAYMELSSLRSEDTAVYYCTRWTTGTGAYWGQGTTVTVSS
BAP049-hum12-HC    DEKFKNRVTITADKSTSTAYMELSSLRSEDTAVYYCTRWTTGTGAYWGQGTTVTVSS
BAP049-hum13-HC    DEKFKNRVTITADKSTSTAYMELSSLRSEDTAVYYCTRWTTGTGAYWGQGTTVTVSS
BAP049-hum03-HC    DEKFKNRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRWTTGTGAYWGQGTTVTVSS
BAP049-hum04-HC    DEKFKNRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRWTTGTGAYWGQGTTVTVSS
BAP049-hum08-HC    DEKFKNRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRWTTGTGAYWGQGTTVTVSS
BAP049-hum10-HC    DEKFKNRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRWTTGTGAYWGQGTTVTVSS
BAP049-hum14-HC    DEKFKNRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRWTTGTGAYWGQGTTVTVSS
BAP049-hum15-HC    DEKFKNRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRWTTGTGAYWGQGTTVTVSS
BAP049-hum16-HC    DEKFKNRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRWTTGTGAYWGQGTTVTVSS
```

FIGURE 9A

```
                        10        20        30        40        50        60
                  ....|....|....|....|....|....|....|....|....|....|....|....|
BAP049-chi-HC     QVQLQQSGSELVRPGASVKLSCKASGYTFTTYWMHWVRQRPGQGLEWIGNIYPGTGGSNF
BAP049-hum01-HC   E...V...A.VKK..E.LRI...G..............AT......M............
BAP049-hum02-HC   E...V...A.VKK..E.LRI...G..............AT......M............
BAP049-hum05-HC   E...V...A.VKK..E.LRI...G..............AT......M............
BAP049-hum06-HC   E...V...A.VKK..E.LRI...G..............AT......M............
BAP049-hum07-HC   E...V...A.VKK..E.LRI...G..............AT......M............
BAP049-hum09-HC   E...V...A.VKK..E.LRI...G..............AT......M............
BAP049-hum11-HC   E...V...A.VKK..E.LRI...G..............AT......M............
BAP049-hum12-HC   E...V...A.VKK..E.LRI...G..............AT......M............
BAP049-hum13-HC   E...V...A.VKK..E.LRI...G..............AT......M............
BAP049-hum03-HC   E...V...A.VKK..E.LRI...G............I..S.SR....L............
BAP049-hum04-HC   E...V...A.VKK..E.LRI...G............I..S.SR....L............
BAP049-hum08-HC   E...V...A.VKK..E.LRI...G............I..S.SR....L............
BAP049-hum10-HC   E...V...A.VKK..E.LRI...G............I..S.SR....L............
BAP049-hum14-HC   ....V...A.VKK......V................I..S.SR....L............
BAP049-hum15-HC   ....V...A.VKK......V................I..S.SR....L............
BAP049-hum16-HC   E...V...A.VKK..E.LRI...G..............A.......M............

                        70        80        90       100       110
                  ....|....|....|....|....|....|....|....|....|....|....|..
BAP049-chi-HC     DEKFKNRTSLTVDTSSTTAYMHLASLTSEDSAVYYCTRWTTGTGAYWGQGTTVTVSS
BAP049-hum01-HC   .......VTI.A.K.TS....E.S..R...T.........................
BAP049-hum02-HC   .......VTI.A.K.TS....E.S..R...T.........................
BAP049-hum05-HC   .......VTI.A.K.TS....E.S..R...T.........................
BAP049-hum06-HC   .......VTI.A.K.TS....E.S..R...T.........................
BAP049-hum07-HC   .......VTI.A.K.TS....E.S..R...T.........................
BAP049-hum09-HC   .......VTI.A.K.TS....E.S..R...T.........................
BAP049-hum11-HC   .......VTI.A.K.TS....E.S..R...T.........................
BAP049-hum12-HC   .......VTI.A.K.TS....E.S..R...T.........................
BAP049-hum13-HC   .......VTI.A.K.TS....E.S..R...T.........................
BAP049-hum03-HC   .......FTISR.N.KN.L.LQMN..RA..T.........................
BAP049-hum04-HC   .......FTISR.N.KN.L.LQMN..RA..T.........................
BAP049-hum08-HC   .......FTISR.N.KN.L.LQMN..RA..T.........................
BAP049-hum10-HC   .......FTISR.N.KN.L.LQMN..RA..T.........................
BAP049-hum14-HC   .......FTISR.N.KN.L.LQMN..RA..T.........................
BAP049-hum15-HC   .......FTISR.N.KN.L.LQMN..RA..T.........................
BAP049-hum16-HC   .......FTISR.N.KN.L.LQMN..RA..T.........................
```

FIGURE 9B

```
                        10        20        30        40        50        60
                    ....|....|....|....|....|....|....|....|....|....|....|
BAP049-chi-LC       DIVMTQSPSSLTVTAGEKVTMSCKSSQSLLDSGNQKNFLTWYQQKPGQPPKLLIFWASTR
BAP049-hum08-LC     EIVLTQSPDFQSVTPKEKVTITCKSSQSLLDSGNQKNFLTWYQQKPGQAPRLLIYWASTR
BAP049-hum09-LC     EIVLTQSPDFQSVTPKEKVTITCKSSQSLLDSGNQKNFLTWYQQKPGQAPRLLIYWASTR
BAP049-hum15-LC     EIVLTQSPDFQSVTPKEKVTITCKSSQSLLDSGNQKNFLTWYQQKPGQAPRLLIYWASTR
BAP049-hum16-LC     EIVLTQSPDFQSVTPKEKVTITCKSSQSLLDSGNQKNFLTWYQQKPGQAPRLLIYWASTR
BAP049-hum10-LC     EIVLTQSPATLSLSPGERATLSCKSSQSLLDSGNQKNFLTWYQQKPGQAPRLLIYWASTR
BAP049-hum11-LC     EIVLTQSPATLSLSPGERATLSCKSSQSLLDSGNQKNFLTWYQQKPGQAPRLLIYWASTR
BAP049-hum14-LC     EIVLTQSPATLSLSPGERATLSCKSSQSLLDSGNQKNFLTWYQQKPGQAPRLLIYWASTR
BAP049-hum06-LC     DIVMTQTPLSLPVTPGEPASISCKSSQSLLDSGNQKNFLTWYQQKPGQAPRLLIYWASTR
BAP049-hum07-LC     EIVLTQSPATLSLSPGERATLSCKSSQSLLDSGNQKNFLTWYQQKPGKAPKLLIYWASTR
BAP049-hum13-LC     DVVMTQSPLSLPVTLGQPASISCKSSQSLLDSGNQKNFLTWYQQKPGKAPKLLIYWASTR
BAP049-hum12-LC     DIQMTQSPSSLSASVGDRVTITCKSSQSLLDSGNQKNFLTWYLQKPGQSPQLLIYWASTR
BAP049-hum02-LC     DIQMTQSPSSLSASVGDRVTITCKSSQSLLDSGNQKNFLTWYQQKPGQAPRLLIYWASTR
BAP049-hum03-LC     DIQMTQSPSSLSASVGDRVTITCKSSQSLLDSGNQKNFLTWYQQKPGQAPRLLIYWASTR
BAP049-hum01-LC     EIVLTQSPATLSLSPGERATLSCKSSQSLLDSGNQKNFLTWYQQKPGQAPRLLIYWASTR
BAP049-hum04-LC     EIVLTQSPATLSLSPGERATLSCKSSQSLLDSGNQKNFLTWYQQKPGKAPKLLIYWASTR
BAP049-hum05-LC     EIVLTQSPATLSLSPGERATLSCKSSQSLLDSGNQKNFLTWYQQKPGKAPKLLIYWASTR

                        70        80        90        100       110
                    ....|....|....|....|....|....|....|....|....|...
BAP049-chi-LC       ESGVPDRFTGSGSVTDFTLTISSVQAEDLAVYYCQNDYSYPCTFGQGTKVEIK
BAP049-hum08-LC     ESGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQNDYSYPYTFGQGTKVEIK
BAP049-hum09-LC     ESGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQNDYSYPYTFGQGTKVEIK
BAP049-hum15-LC     ESGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQNDYSYPYTFGQGTKVEIK
BAP049-hum16-LC     ESGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQNDYSYPYTFGQGTKVEIK
BAP049-hum10-LC     ESGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQNDYSYPYTFGQGTKVEIK
BAP049-hum11-LC     ESGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQNDYSYPYTFGQGTKVEIK
BAP049-hum14-LC     ESGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQNDYSYPYTFGQGTKVEIK
BAP049-hum06-LC     ESGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQNDYSYPYTFGQGTKVEIK
BAP049-hum07-LC     ESGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQNDYSYPYTFGQGTKVEIK
BAP049-hum13-LC     ESGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQNDYSYPYTFGQGTKVEIK
BAP049-hum12-LC     ESGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQNDYSYPYTFGQGTKVEIK
BAP049-hum02-LC     ESGIPPRFSGSGYGTDFTLTINNIESEDAAYYFCQNDYSYPYTFGQGTKVEIK
BAP049-hum03-LC     ESGIPPRFSGSGYGTDFTLTINNIESEDAAYYFCQNDYSYPYTFGQGTKVEIK
BAP049-hum01-LC     ESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQNDYSYPYTFGQGTKVEIK
BAP049-hum04-LC     ESGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQNDYSYPYTFGQGTKVEIK
BAP049-hum05-LC     ESGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQNDYSYPYTFGQGTKVEIK
```

FIGURE 10A

```
                        10        20        30        40        50        60
                   ....|....|....|....|....|....|....|....|....|....|....|
BAP049-chi-LC      DIVMTQSPSSLTVTAGEKVTMSCKSSQSLLDSGNQKNFLTWYQQKPGQPPKLLIFWASTR
BAP049-hum08-LC    E..L....DFQS..PK....IT.........................A.R...Y.....
BAP049-hum09-LC    E..L....DFQS..PK....IT.........................A.R...Y.....
BAP049-hum15-LC    E..L....DFQS..PK....IT.........................A.R...Y.....
BAP049-hum16-LC    E..L....DFQS..PK....IT.........................A.R...Y.....
BAP049-hum10-LC    E..L....AT.SLSP..RA.L..........................A.R...Y.....
BAP049-hum11-LC    E..L....AT.SLSP..RA.L..........................A.R...Y.....
BAP049-hum14-LC    E..L....AT.SLSP..RA.L..........................A.R...Y.....
BAP049-hum06-LC    ......T.L..P..P..PASI..........................A.R...Y.....
BAP049-hum07-LC    E..L....AT.SLSP..RA.L..........................KA.....Y.....
BAP049-hum13-LC    .V......L..P..L.QPASI..........................KA.....Y.....
BAP049-hum12-LC    ..Q........SASV.DR..IT.....................L.....S.Q...Y.....
BAP049-hum02-LC    ..Q........SASV.DR..IT.........................A.R...Y.....
BAP049-hum03-LC    ..Q........SASV.DR..IT.........................A.R...Y.....
BAP049-hum01-LC    E..L....AT.SLSP..RA.L..........................A.R...Y.....
BAP049-hum04-LC    E..L....AT.SLSP..RA.L..........................KA.....Y.....
BAP049-hum05-LC    E..L....AT.SLSP..RA.L..........................KA.....Y.....

                        70        80        90        100       110
                   ....|....|....|....|....|....|....|....|....|...
BAP049-chi-LC      ESGVPDRFTGSGSVTDFTLTISSVQAEDLAVYYCQNDYSYPCTFGQGTKVEIK
BAP049-hum08-LC    .....S..S....G....F....LE...A.T..........Y..........
BAP049-hum09-LC    .....S..S....G....F....LE...A.T..........Y..........
BAP049-hum15-LC    .....S..S....G....F....LE...A.T..........Y..........
BAP049-hum16-LC    .....S..S....G....F....LE...A.T..........Y..........
BAP049-hum10-LC    .....S..S....G....F....LE...A.T..........Y..........
BAP049-hum11-LC    .....S..S....G....F....LE...A.T..........Y..........
BAP049-hum14-LC    .....S..S....G....F....LE...A.T..........Y..........
BAP049-hum06-LC    .....S..S....G....F....LE...A.T..........Y..........
BAP049-hum07-LC    .....S..S....G....F....LE...A.T..........Y..........
BAP049-hum13-LC    .....S..S....G....F....LE...A.T..........Y..........
BAP049-hum12-LC    .....S..S....G....F....LE...A.T..........Y..........
BAP049-hum02-LC    ...I.P..S...YG.......NNIES..A.Y.F........Y..........
BAP049-hum03-LC    ...I.P..S...YG.......NNIES..A.Y.F........Y..........
BAP049-hum01-LC    .....S..S....G.E.......L.PD.F.T..........Y..........
BAP049-hum04-LC    .....S..S....G....F....L.P..I.T..........Y..........
BAP049-hum05-LC    .....S..S....G....F....L.P..I.T..........Y..........
```

FIGURE 10B

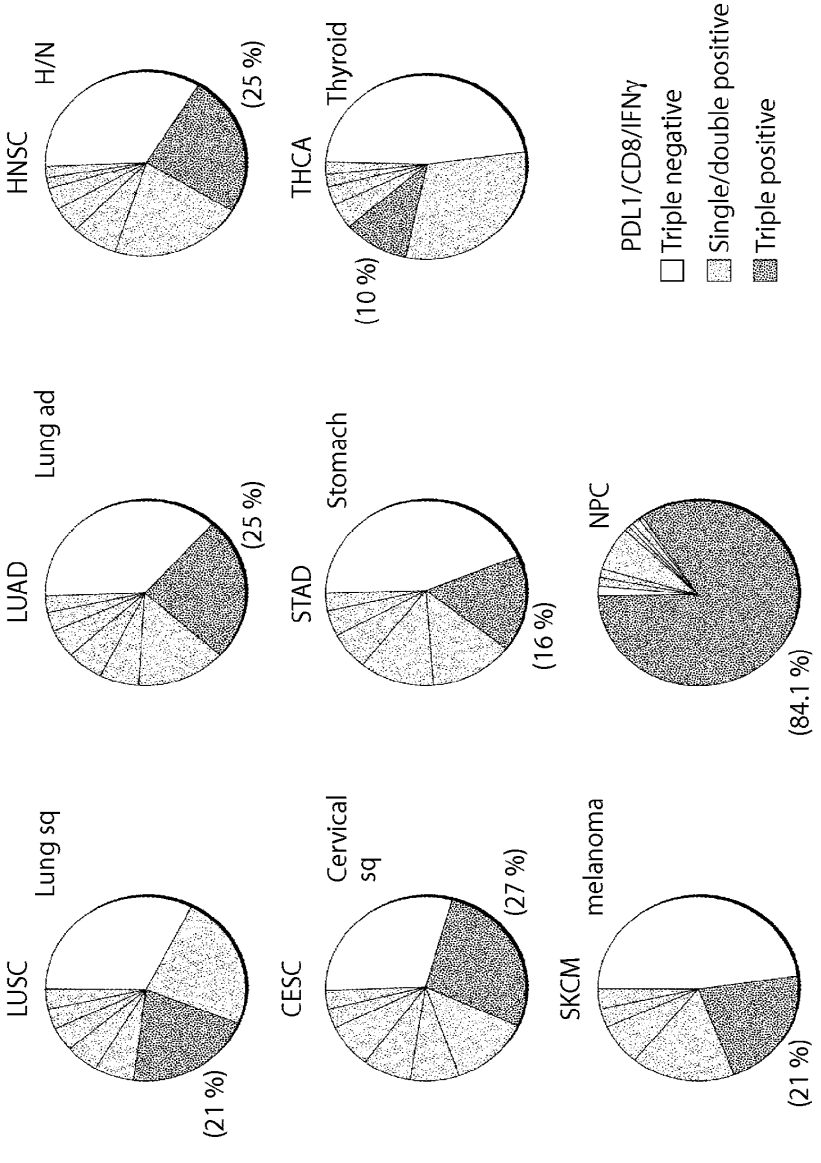

HNSC
H/N
(25 %)
THCA
Thyroid
(10 %)
PDL1/CD8/IFNγ
Triple negative
Single/double positive
Triple positive
LUAD
Lung ad
(25 %)
STAD
Stomach
(16 %)
NPC
(84.1 %)
LUSC
Lung sq
(21 %)
CESC
Cervical sq
(27 %)
SKCM
melanoma
(21 %)

FIGURE 11

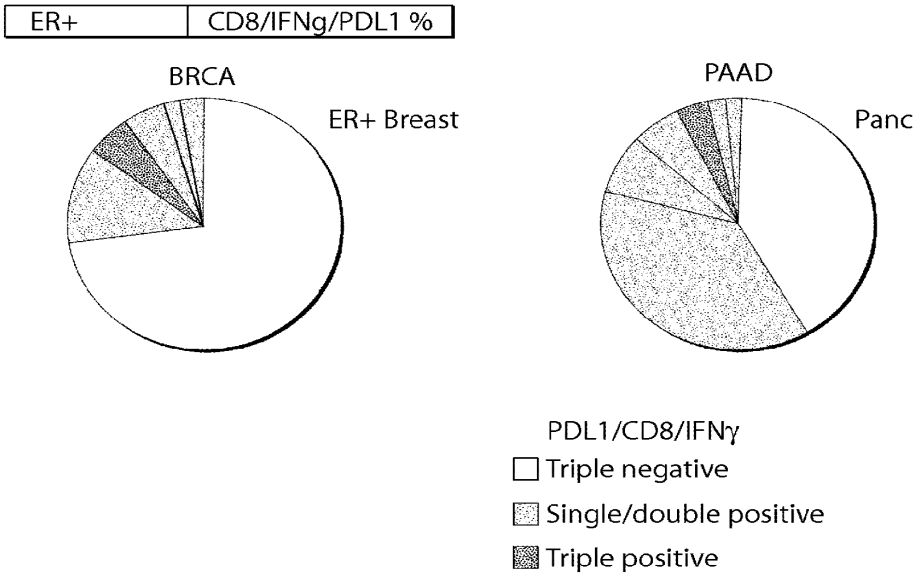

ER+
CD8/IFNg/PDL1 %
BRCA
ER+ Breast
PAAD
Panc
PDL1/CD8/IFNγ
Triple negative
Single/double positive
Triple positive

FIGURE 12

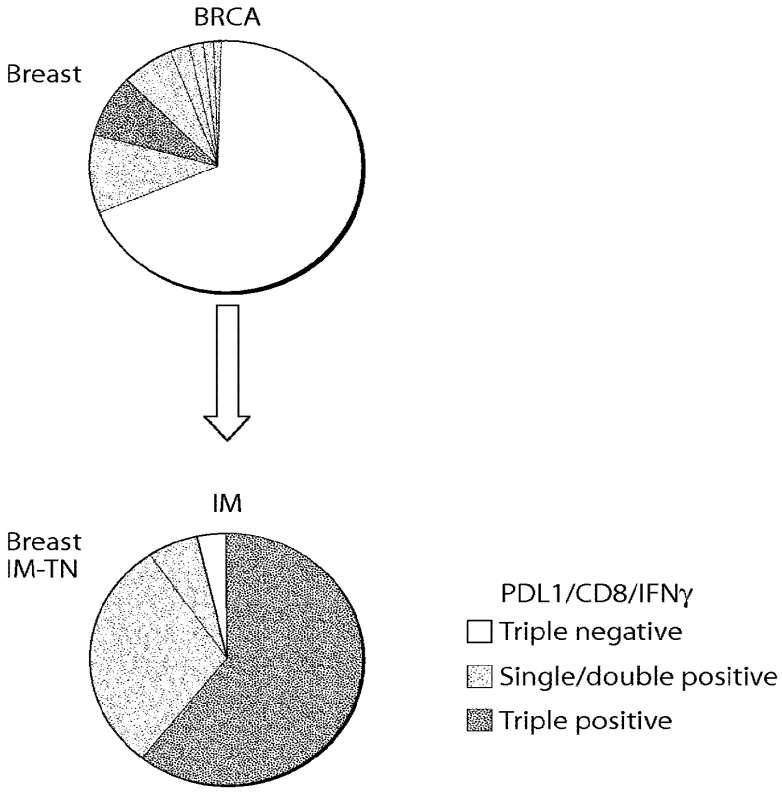

BRCA
Breast
IM
Breast
IM-TN
PDL1/CD8/IFNγ
Triple negative
Single/double positive
Triple positive

FIGURE 13

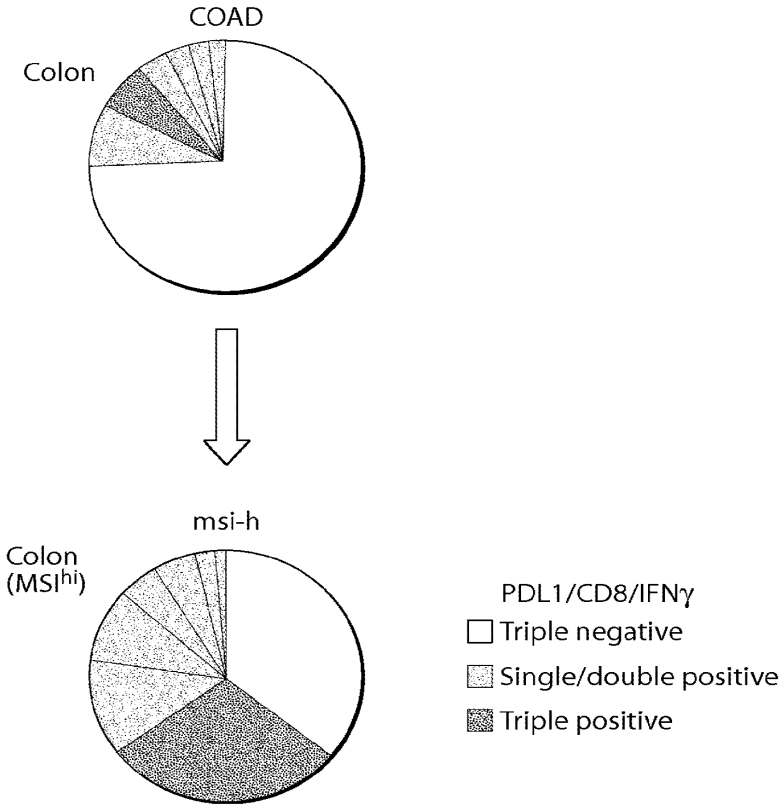

COAD
Colon
msi-h
Colon
(MSI^hi)
PDL1/CD8/IFNγ
Triple negative
Single/double positive
Triple positive

FIGURE 14

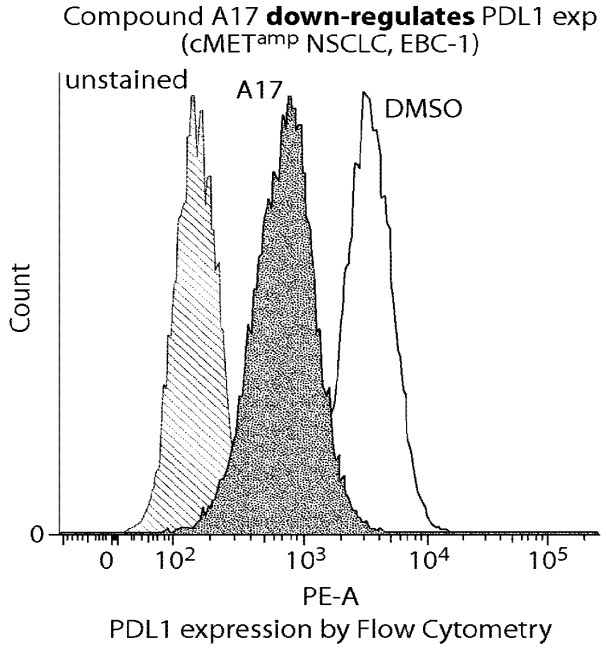

Compound A17 down-regulates PDL1 exp
(cMET amp NSCLC, EBC-1)
unstained
A17
DMSO
Count
0
0
10^2
10^3
10^4
10^5
PE-A
PDL1 expression by Flow Cytometry

FIGURE 15

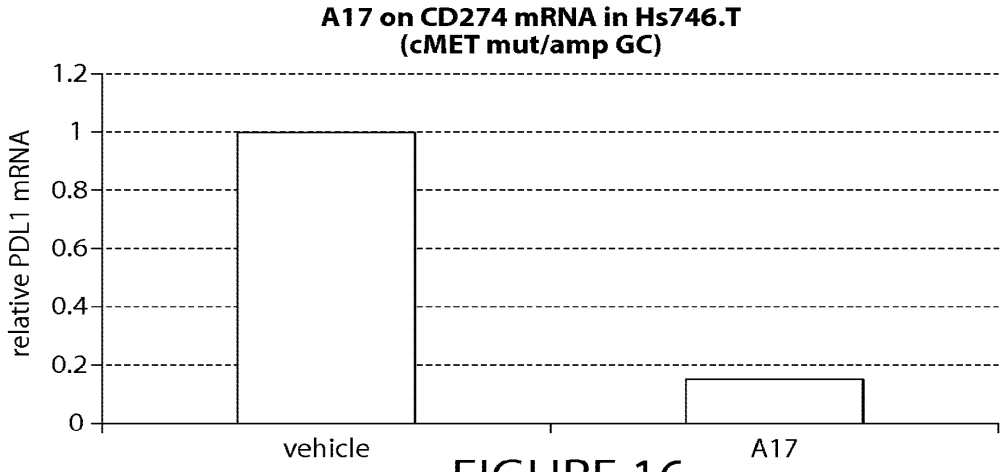


FIGURE 16

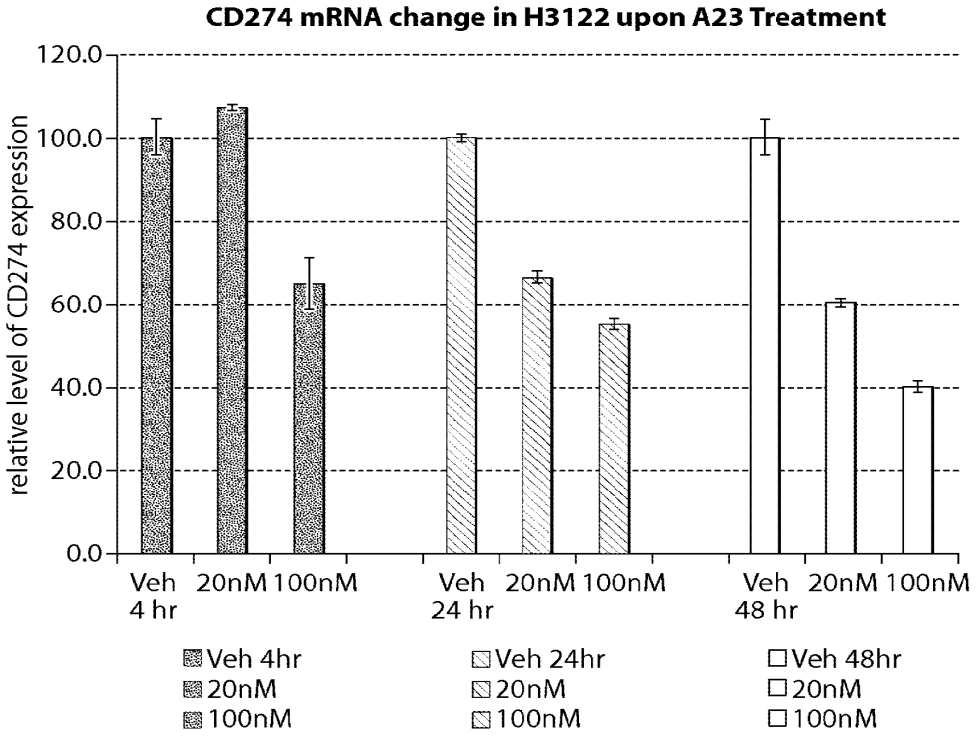


FIGURE 17

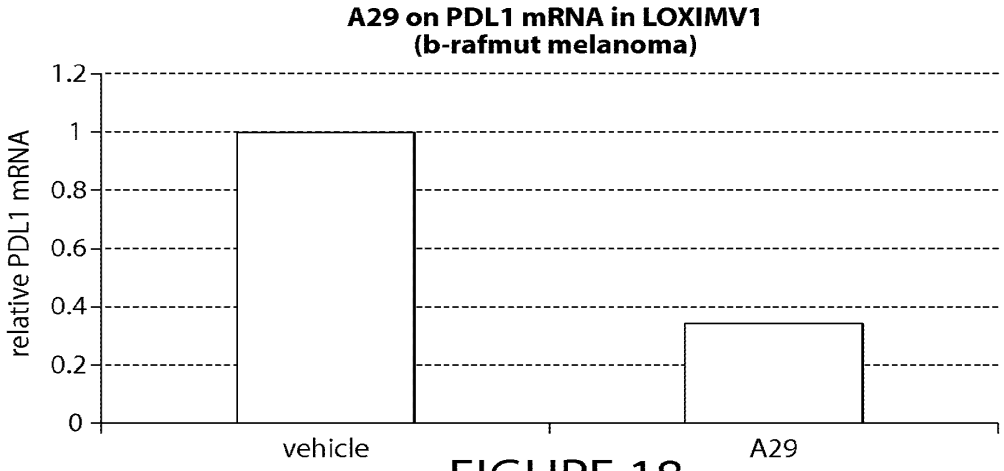


FIGURE 18

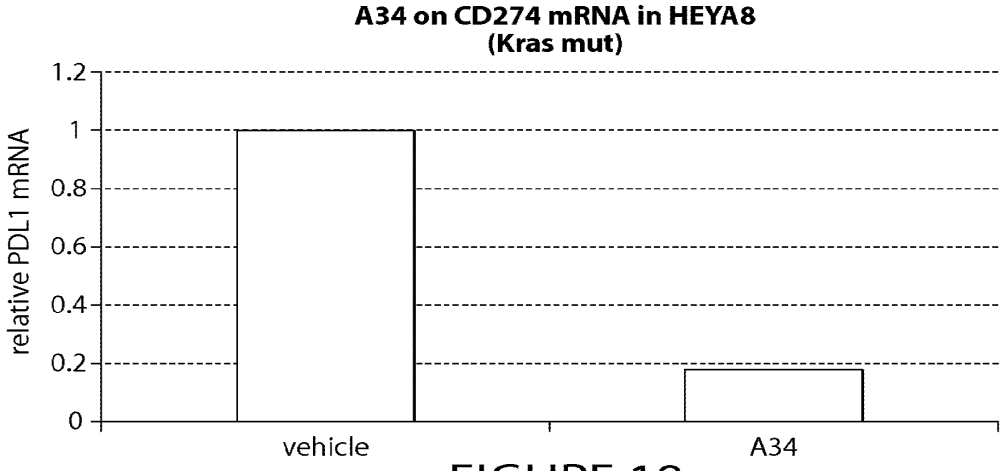


FIGURE 19

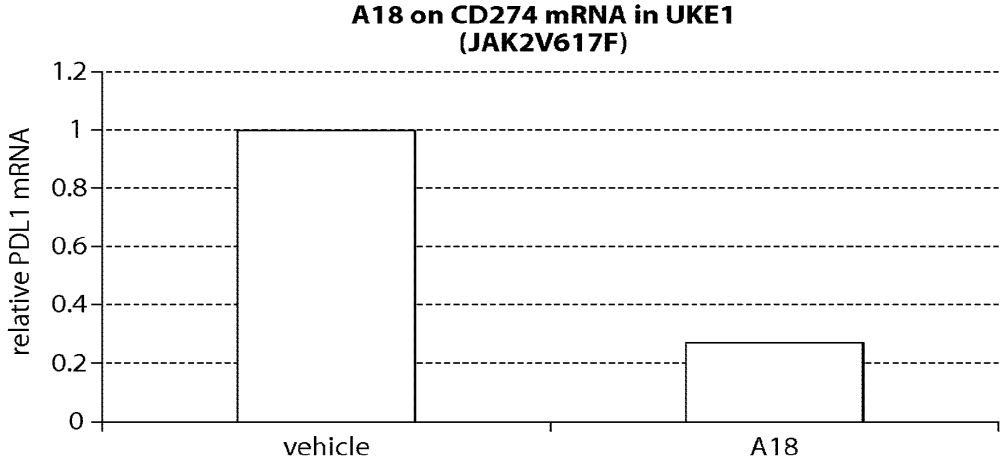

A18 on CD274 mRNA in UKE1
(JAK2V617F)
relative PDL1 mRNA
1.2
1
0.8
0.6
0.4
0.2
0
vehicle
A18

FIGURE 20

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/012754

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, Sequence Search, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/121168 A1 (ONO PHARMACEUTICAL CO [JP]; MEDAREX INC [US]; KORMAN ALAN J; SRINIVASA) 16 November 2006 (2006-11-16) cited in the application claim 1	1-125
X	WO 2009/114335 A2 (MERCK & CO INC [US]; FINNEFROCK ADAM C [US]; FU TONG-MING [US]; FREED) 17 September 2009 (2009-09-17) cited in the application claim 18; figure 2	1-125

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 April 2015	20/05/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Lonnoy, Olivier

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OMID HAMID ET AL: "Safety and Tumor Responses with Lambrolizumab (Anti-PD-1) in Melanoma", NEW ENGLAND JOURNAL OF MEDICINE, vol. 369, no. 2, 11 July 2013 (2013-07-11) , pages 134-144, XP055182016, ISSN: 0028-4793, DOI: 10.1056/NEJMoa1305133 cited in the application page 135, column 1, paragraph 3 - column 2, paragraph 1	1-125
X	WO 2009/101611 A1 (CURETECH LTD [IL]; ROTEM-YEHUDAR RINAT [IL]; RODIONOV GALINA [IL]) 20 August 2009 (2009-08-20) cited in the application example 8	1-125
X	WO 2004/056875 A1 (WYETH CORP [US]; CAMBRIDGE ANTIBODY TECH [GB]; COLLINS MARY [US]; WOOD) 8 July 2004 (2004-07-08) cited in the application claim 15	1-125
X	WO 2008/083174 A2 (UNIV EMORY [US]; DANA FARBER CANCER INST INC [US]; HARVARD COLLEGE [US) 10 July 2008 (2008-07-10) claim 18	1-125
X	JOHN M. KIRKWOOD ET AL: "Immunotherapy of cancer in 2012", CA: A CANCER JOURNAL FOR CLINICIANS, vol. 62, no. 5, 1 September 2012 (2012-09-01), pages 309-335, XP055059122, ISSN: 0007-9235, DOI: 10.3322/caac.20132 page 320, column 1, paragraph 2 - column 2, paragraph 1	1-125
X	WO 2010/036959 A2 (DANA FARBER CANCER INST INC [US]; UNIV EMORY [US]; FREEMAN GORDON [US]) 1 April 2010 (2010-04-01) figure 14	1-125