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(54) Title: ENGINEERED PD-1 VARIANTS

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hPD-1     WNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQ

hPD-1     DCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEV

hPD-1     PTAHPSPSP (SEQ ID NO:1)
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Fig. 1

(57) Abstract: Isolated polypeptides comprising engineered mutant PD-1 polypeptide are provided, as are fusion polypeptides comprising the mutant and methods of use thereof. Bispecific PD-L1 and PD-L2 binding mutant PD-1 polypeptides are provided. PD-L2-specific binding mutant PD-1 polypeptides are also provided.

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ENGINEERED PD-1 VARIANTS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Provisional Application No. 62/337,388, filed May 17, 2016, the contents of which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

[0002] Throughout this application various publications are referred to. The disclosures of these publications, and of all patents, patent application publications and books referred to herein, are hereby incorporated by reference in their entirety into the subject application to more fully describe the art to which the subject invention pertains.

[0003] In recent years, T cell costimulatory pathways have been identified as versatile novel targets for immunotherapeutic strategies. The CD28:B7 family of T cell costimulatory molecules includes CD28 and ICOS as positive co-receptors, and CTLA4 and PD-1 as co-inhibitors, which tightly regulate all T cell activation processes.

[0004] Enhancing T cell activation by blockade of the PD-L/PD-1 inhibitory pathway has enormous potential for the treatment of infectious diseases and malignant tumors. Recent studies have shown that enhancing T cell activation by blocking PD-1 could be beneficial in chronic viral infections, as well as other infections in which this costimulatory pathway is involved. Host responses to pathogens such as fungi, protozoa, worms and bacteria have been shown to be regulated by PD-1, and therefore could be improved by manipulating the PD-1 pathway.

[0005] Although targeting costimulatory pathways is a relatively recent approach, there are a number of antibodies approved for clinical use and myriad others in development for clinical trials. One such FDA-approved drug is Yervoy (Ipilimumab), which is a monoclonal antibody directed against the co-inhibitory receptor CTLA-4. Yervoy has been shown to be effective in increasing survival of metastatic melanoma patients (10 months median survival for the antibody treated group versus 6.4 months for the control group, Hodi FS et al, N Engl J Med 2010). Yervoy acts through inducing activation of T cells by blocking CTLA-4, causing significant immune stimulation, including anti-tumor immune responses. Due to the central role of CTLA-4 in all immune responses (central and

peripheral), Yervoy can cause significant side effects associated with an overly active immune response, e.g. autoimmune symptoms can develop and in some cases these can be lethal. Notably, the absence of CTLA-4 in mice caused by genetic deletion is lethal, highlighting the importance of this molecular "brake" on the general immune response. Furthermore, the FDA recently granted accelerated approval for Keytruda (pembrolizumab) which is a monoclonal antibody directed against PD-1 to treat patients with advanced (metastatic) non-small cell lung cancer (NSCLC) whose disease has progressed after other treatments failed, and for tumors that express a PD-1 ligand protein called PD-L1. Keytruda is also approved for use with a companion diagnostic. Many other PD-1 and PD-L1 targeting antibodies are under clinical and preclinical development.

[0006] The present invention addresses the need for improved targeting of costimulatory pathways by manipulating the PD-1 pathway and provides high affinity PD-1-based immune stimulatory agents.

SUMMARY OF THE INVENTION

[0007] This invention provides an isolated polypeptide comprising a mutant PD-1 polypeptide having the sequence (i) SEQ ID NO:2, or (ii) SEQ ID NO:3, or (iii) having a sequence at least 95% identical to SEQ ID NO:2 or SEQ ID NO:3 with the proviso that the sequence at least 95% identical to SEQ ID NO:2 or SEQ ID NO:3 is not SEQ ID NO:1.

[0008] This invention provides an isolated polypeptide comprising a mutant PD-1 polypeptide comprising SEQ ID NO:2 or SEQ ID NO:3, wherein the mutant PD-1 polypeptide is a mutant by having a mutation relative to NCBI Reference Sequence NP_005009.2 or to SEQ ID NO:1.

[0009] This invention also provides a fusion polypeptide comprising a mutant PD-1 polypeptide (or engineered PD-1) described herein, fused to an immunoglobulin domain polypeptide.

[0010] This invention also provides a homo-oligomer comprising the isolated polypeptide comprising a mutant PD-1 polypeptide described herein, or comprising the fusion polypeptide. In a preferred embodiment, the homo-oligomer comprises two of the isolated polypeptides, or two of the fusion polypeptides.

[0011] This invention also provides a composition comprising the isolated mutant PD-1 polypeptide in monovalent form or oligomeric form. This invention also provides a

composition comprising the fusion polypeptide comprising the mutant PD-1 in monovalent form or oligomeric form.

[0012] Also provided is a method of stimulating T cell activation in a subject comprising administering to the subject the isolated mutant PD-1 polypeptide described herein, or the composition or homo-oligomer comprising the isolated mutant PD-1 polypeptide described herein, in an amount sufficient to stimulate T cell activation in a subject.

[0013] Also provided is a method of stimulating T cell activation in a subject comprising administering to the subject the isolated fusion polypeptide comprising the mutant PD-1described herein, or the composition or homo-oligomer comprising the isolated fusion polypeptide comprising the mutant PD-1 described herein, in an amount sufficient to stimulate T cell activation in a subject.

[0014] Also provided is a method of treating a tumor, or treating an infection, in a subject comprising administering to the subject the isolated mutant PD-1 polypeptide described herein, or the composition or homo-oligomer comprising the the isolated mutant PD-1 polypeptide described herein, in an amount sufficient to stimulate T cell activation, treat a tumor, or treat an infection, respectively, in a subject.

[0015] Also provided is a method of treating a tumor, or treating an infection, in a subject comprising administering to the subject the isolated fusion polypeptide comprising the mutant PD-1described herein, or the composition or homo-oligomer comprising the isolated fusion polypeptide comprising the mutant PD-1described herein, in an amount sufficient to stimulate T cell activation, treat a tumor, or treat an infection, respectively, in a subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] Fig. 1: Human PD-1 (SEQ ID NO:1) engineered variants design. The designed library allows limited variation at the underlined residues.

[0017] Fig. 2: Bispecific ePD-1s (bind both PD-L1 and PD-L2). Monoclonal phage ELISAs of hits from the PD-L1/PD-L2 toggle selection.

[0018] Fig. 3: Bispecific ePD-1s (bind both PD-L1 and PD-L2). Monoclonal phage ELISAs of hits from the PD-L1/PD-L2 toggle selection.

[0019] Fig. 4: PD-L2-specific ePD-1s. Monoclonal phage ELISAs of hits from the PD-L1/PD-L2 toggle selection.

[0020] Fig. 5: PD-L2-specific ePD-1s. Monoclonal phage ELISAs of hits from the PD-L1/PD-L2 toggle selection and control WT PD1 data.

[0021] Fig. 6: Bispecific (PD-L1 and PD-L2) engineered mutant PD-1 sequences. (Sequences split into two sections for visual display due to length of sequences). hPD-1 is SEQ ID NO:1; Bi-1 is SEQ ID NO:4; Bi-2 is SEQ ID NO:5; Bi-3 is SEQ ID NO:6; Bi-4 is SEQ ID NO:7; Bi-5 is SEQ ID NO:8; Bi-6 is SEQ ID NO:9; Bi-7 is SEQ ID NO:10; Bi-8 is SEQ ID NO:11; Bi-9 is SEQ ID NO:12; Bi-10 is SEQ ID NO:13; Bi-11 is SEQ ID NO:14; Bi-12 is SEQ ID NO:15; and Bi-13 is SEQ ID NO:16.

[0022] Fig. 7: PD-L2-specific engineered mutant PD-1 sequences. (Sequences split into two sections for visual display due to length of sequences). hPD-1 is SEQ ID NO:1; L2-1 is SEQ ID NO:17; L2-2 is SEQ ID NO:18; L2-3 is SEQ ID NO:19; L2-4 is SEQ ID NO:20; L2-5 is SEQ ID NO:21; L2-6 is SEQ ID NO:22; L2-7 is SEQ ID NO:23; L2-8 is SEQ ID NO:24; L2-9 is SEQ ID NO:25; L2-10 is SEQ ID NO:26; L2-11 is SEQ ID NO:27; L2-12 is SEQ ID NO:28; L2-13 is SEQ ID NO:29; L2-14 is SEQ ID NO:30; L2-15 is SEQ ID NO:31; L2-16 is SEQ ID NO:32; L2-17 is SEQ ID NO:33; L2-18 is SEQ ID NO:34; L2-19 is SEQ ID NO:35; L2-20 is SEQ ID NO:36; and L2-21 is SEQ ID NO:37.

DETAILED DESCRIPTION OF THE INVENTION

[0023] An isolated mutant PD-1 polypeptide is provided comprising (i) SEQ ID NO:2, or (ii) SEQ ID NO:3, or (ii) having a sequence 95% or greater identical to SEQ ID NO:2 or SEQ ID NO:3 with the proviso that the mutant PD-1 polypeptide does not comprise SEQ ID NO:1.

[0024] An engineered PD-1 polypeptide is provided comprising (i) SEQ ID NO:2, or (ii) SEQ ID NO:3, or (ii) having a sequence 95% or greater identical to SEQ ID NO:2 or SEQ ID NO:3 with the proviso that the engineered PD-1 polypeptide does not comprise SEQ ID NO:1.

[0025] In an embodiment, the isolated mutant PD-1 polypeptide comprises SEQ ID NO:2. In an embodiment the isolated mutant PD-1 polypeptide comprises SEQ ID NO:3. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at either the N-terminal, the C-terminal or,

independently, at both N and C terminals. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at either the N-terminal, the C-terminal or, independently, at both N and C terminals.

[0026] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 1 additional amino acid at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 1 additional amino acid at one terminal thereof and 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0027] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 2 additional amino acids at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 2 additional amino acids at one terminal thereof and 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0028] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 3 additional amino acids at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 3 additional amino acids at one terminal thereof and 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0029] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 4 additional amino acids at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 4 additional amino acids at one terminal thereof and 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0030] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 5 additional amino acids at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 5 additional amino acids at one terminal thereof and 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0031] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 6 additional amino acids at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 6 additional amino acids at one terminal thereof and 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0032] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 7 additional amino acids at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 7 additional amino acids at one terminal thereof and 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0033] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 8 additional amino acids at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 8 additional amino acids at one terminal thereof and 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0034] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 9 additional amino acids at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 9 additional amino acids at one terminal thereof and 1,

2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0035] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 10 additional amino acids at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:2 with only 10 additional amino acids at one terminal thereof and 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0036] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 1 additional amino acid at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 1 additional amino acid at one terminal thereof and 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0037] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 2 additional amino acids at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 2 additional amino acids at one terminal thereof and 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0038] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 3 additional amino acids at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 3 additional amino acids at one terminal thereof and 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0039] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 4 additional amino acids at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 4 additional amino acids at one terminal thereof and 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0040] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 5 additional amino acids at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 5 additional amino acids at one terminal thereof and 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0041] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 6 additional amino acids at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 6 additional amino acids at one terminal thereof and 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0042] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 7 additional amino acids at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 7 additional amino acids at one terminal thereof and 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0043] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 8 additional amino acids at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 8 additional amino acids at one terminal thereof and 1,

2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0044] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 9 additional amino acids at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 9 additional amino acids at one terminal thereof and 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0045] In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 10 additional amino acids at one terminal thereof and no additional amino acid at the other terminal thereof. In an embodiment, the isolated mutant PD-1 polypeptide consists of SEQ ID NO:3 with only 10 additional amino acids at one terminal thereof and 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at the other terminal thereof. In an embodiment, the one terminal thereof is the N-terminal. In an embodiment, the one terminal thereof is the C-terminal.

[0046] A fusion polypeptide is provided comprising a mutant PD-1 polypeptide comprising (i) SEQ ID NO:2, or (ii) SEQ ID NO:3 or (ii) having a sequence 95% or greater identical to SEQ ID NO:2 or SEQ ID NO:3, with the proviso that the mutant PD-1 polypeptide does not comprise SEQ ID NO:1, fused to a second polypeptide that does not naturally occur contiguous with (i), (ii), or (iii).

[0047] A fusion polypeptide is provided comprising a mutant PD-1 polypeptide comprising (i) SEQ ID NO:2, or (ii) SEQ ID NO:3, or (ii) having a sequence 95% or greater identical to SEQ ID NO:2 or SEQ ID NO:3, with the proviso that the mutant PD-1 polypeptide does not comprise SEQ ID NO:1, fused to an immunoglobulin domain polypeptide.

[0048] In an embodiment, the mutant PD-1 polypeptide is fused (bonded) to the immunoglobulin domain polypeptide by a peptide bond between a terminal amino acid of the mutant PD-1 polypeptide and a terminal amino acid of the immunoglobulin domain polypeptide. In an embodiment, the mutant polypeptide is fused to the immunoglobulin domain polypeptide by a linker molecule. In an embodiment, the linker molecule is a

peptide. In an embodiment, the peptide linker permits flexibility. In an embodiment, the linker is rigid. In an embodiment the linker is cleavable. Non-limiting examples of flexible linkers within the scope of the invention are Gn, and GGGGS, and (GGGGS)n where n = 2, 3, 4 or 5. Non-limiting examples of rigid linkers within the scope of the invention are (EAAAK)n, (XP)n where X = any amino acid. Non-limiting examples of cleavable linkers within the scope of the invention include disulfide links and protease cleavable linkers. In a preferred embodiment, the linker is a peptide linker.

[0049] In an embodiment, the immunoglobulin domain polypeptide comprises an immunoglobulin IgG1 Fc domain. In an embodiment, the immunoglobulin IgG1 Fc domain is human. In an embodiment, the immunoglobulin IgG1 Fc domain has the sequence of a human immunoglobulin IgG1 Fc domain. In an embodiment, the immunoglobulin IgG1 Fc domain is recombinant. In an embodiment, the immunoglobulin IgG1 Fc domain is not produced by a human body. Human igG1 Fc domain sequences and encoding nucleic acids are widely available in the art. For example, expressed from a DNA sequence encoding the human IgG1-Fc region (AAC82527.1). For example, recombinant human immunoglobulin IgG1 Fc domain expressed from a DNA sequence encoding the human IgG1-Fc region (AAC82527.1) containing residues 99 to 330, including a 103 Cys/Ser mutation (ThermoFisher Scientific®, Waltham, MA, USA).

[0050] In an embodiment of the mutant polypeptide, the polypeptide is in monovalent form. In an embodiment of the fusion polypeptide, the fusion polypeptide is in monovalent form.

[0051] A homo-oligomer comprising a mutant polypeptide as described herein is also provided.

[0052] A homo-oligomer comprising a fusion polypeptide as described herein is also provided.

[0053] In an embodiment, the homo-oligomer comprises two mutant polypeptides of the same type as described herein. In an embodiment, the homo-oligomer comprises two mutant polypeptides of different types, each as described herein. In an embodiment, the homo-oligomer comprises two fusion polypeptides of the same type as described herein. In an embodiment, the homo-oligomer comprises two fusion polypeptides of different types, each as described herein.

[0054] Also provided is a composition comprising the mutant polypeptide as described herein.

[0055] Also provided is a composition comprising the fusion polypeptide as described herein.

[0056] Also provided is a composition comprising the homo-oligomer as described herein.

[0057] In an embodiment of the compositions, the composition comprises a pharmaceutically acceptable carrier.

[0058] Also provided is a method for stimulating T cell activation, treating a tumor, or treating an infection in a subject comprising administering to the subject the mutant polypeptide described herein or the mutant polypeptide-containing composition described herein, in an amount sufficient to stimulate T cell activation, treat a tumor, or treat an infection, respectively, in a subject. In an embodiment, the mutant polypeptide is administered in monovalent form. In an embodiment, the mutant polypeptide is administered as a homo-oligomer. In an embodiment, the method is for treating a tumor. In an embodiment, the method is for treating an infection. In an embodiment, the method is for stimulating T cell activation.

[0059] Also provided is a method for stimulating T cell activation, treating a tumor, or treating an infection in a subject comprising administering to the subject the fusion polypeptide described herein or the fusion polypeptide-containing composition described herein, in an amount sufficient to stimulate T cell activation, treat a tumor, or treat an infection, respectively, in a subject. In an embodiment, the fusion polypeptide is administered in monovalent form. In an embodiment, the fusion polypeptide is administered as a homo-oligomer. In an embodiment, the fusion polypeptide is a mutant polypeptide fused to an immunoglobulin domain polypeptide. In an embodiment, the mutant PD-1 polypeptide is fused to the immunoglobulin domain polypeptide by a peptide bond between a terminal amino acid of the mutant PD-1 polypeptide and a terminal amino acid of the immunoglobulin domain polypeptide. In an embodiment, the immunoglobulin domain polypeptide comprises an immunoglobulin IgG1 Fc domain. In an embodiment, the immunoglobulin IgG1 Fc domain is human.

[0060] In an embodiment of the methods, the T cell activation comprises cytokine secretion.

[0061] In an embodiment of the methods, the method is for treating a tumor.

[0062] In an embodiment of the methods, the polypeptide, or the fusion peptide, respectively, comprises SEQ ID NO:3. In an embodiment of the methods, the method is for treating a tumor and the tumor is a PD-L2-expressing tumor.

[0063] In an embodiment of the methods, the polypeptide, or the fusion peptide, respectively, comprises SEQ ID NO:2. In an embodiment of the methods, the method is for treating a tumor and the tumor is a PD-L1-expressing tumor or is a PD-L1-expressing and PD-L2-expressing tumor.

[0064] In an embodiment of the methods, the method is for treating an infection.

[0065] Also provided is an isolated nucleic acid encoding a mutant PD-1 polypeptide as described herein. Also provided is an isolated nucleic acid encoding a fusion polypeptide comprising the mutant PD-1 polypeptide as described herein. In an embodiment, the nucleic acid has been labeled with a synthetic marker. In an embodiment, the nucleic acid is a recombinant nucleic acid. In an embodiment, the nucleic acid comprises cDNA.

[0066] An isolated cell is provided containing a vector comprising an isolated nucleic acid as described herein. In an embodiment, the cell is a mammalian cell. In an embodiment, the cell is not in a human. In an embodiment, the cell is derived from a mammalian cell.

[0067] This invention provides an isolated polypeptide having the following sequence:
WNPPTFSPALLVVTEGDNATFTCSFSNTSEX_1FX_2LNWYRX_3SX_4SNQTDKLAAFPEDRSQPG QDCRFRVTQLPNGRDFHMSVVRARRNDSGTYX_5CAAI$X_6$$X_7$$X_8PX_9$$X_{10}QX_{11}$KESLRAELRVTE RRAEVPTAHPSPSP (SEQ ID NO:2)

Wherein:

X1= S or A

X2 = V or I

X3 = L or M

X4 = P or A

X5 = L or I

X6 = A or S

X7 = L or I

X8 = A or S

X9 = R or K

X10 = A or S

X11 = I or V.

[0068] In an embodiment, this polypeptide binds to PD-L1 and to PD-L2. In an embodiment, the polypeptide having SEQ ID NO:2 does not comprise SEQ ID NO:1. In an embodiment, the polypeptide having SEQ ID NO:2 does not comprise SEQ ID NO:3.

[0069] This invention provides an isolated polypeptide having the following sequence:

WNPPTFSPALLVVTEGDNATFTCSFSNTSEX_{12}FX_{13}LNWYR$X_{14}X_{15}X_{16}X_{17}X_{18}$QTDKLAAFPED RSQ$X_{19}$GQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYX_{20}CAA$X_{21}X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}QX_{28}$ KESLRAELRVTERRAEVPTAHPSPSP (SEQ ID NO:3)

Wherein:

X12= S or A

X13 = V or I

X14 = L or M

X15 = S or A

X16 = P or A

X17 = S or A

X18 = N or D

X19 = P r S

X20 = L or I

X21 = I or V

X22 = A or S

X23 = L or I

X24 = A or S

X25 = P or A

X26 = R or K

X27 = A or S

X28 = I or V.

[0070] In an embodiment, this polypeptide binds to PD-L2. In an embodiment, this polypeptide binds to PD-L2 but not to PD-L1. In an embodiment, the polypeptide having SEQ ID NO:3 does not comprise SEQ ID NO:1. In an embodiment, the polypeptide having SEQ ID NO:3 does not comprise SEQ ID NO:2.

[0071] In an embodiment, the polypeptide of the invention as described herein is not naturally occurring.

[0072] In an embodiment, the polypeptide is in monovalent form. In an embodiment, the mutant polypeptide is soluble. In an embodiment, the mutant polypeptide does not comprise a transmembrane domain. In an embodiment, the mutant polypeptide does not comprise a intracellular domain. In an embodiment, the mutant polypeptide further comprises a sequence having the same sequence as a PD-1 transmembrane domain. In an embodiment, the mutant polypeptide does not further comprise a sequence having the same sequence as a PD-1 transmembrane domain. In an embodiment, the mutant polypeptide further comprises a sequence having the same sequence as a PD-1 intracellular domain. In an embodiment, the mutant polypeptide does not further comprise a sequence having the same sequence as a PD-1 intracellular domain.

[0073] In an embodiment, the mutant PD-1 polypeptide comprises consecutive amino acid residues (i) having the sequence set forth in SEQ ID NO:2, or (ii) having a sequence 95% or greater identical to SEQ ID NO:2, but wherein in (ii) the polypeptide has the following residues at the same positions as set forth in SEQ ID NO:2:

X1= S or A

X2 = V or I

X3 = L or M

X4 = P or A

X5 = L or I

X6 = A or S

X7 = L or I

X8 = A or S

X9 = R or K

X10 = A or S

X11 = I or V.

[0074] In an embodiment, the mutant PD-1 polypeptide comprises consecutive amino acid residues having a sequence 96% or greater identical to SEQ ID NO:2. In an embodiment, the mutant PD-1 polypeptide comprises consecutive amino acid residues having a sequence 97% or greater identical to SEQ ID NO:2. In an embodiment, the mutant PD-1 polypeptide comprises consecutive amino acid residues having a sequence 98% or

greater identical to SEQ ID NO:2. In an embodiment, the mutant PD-1 polypeptide comprises consecutive amino acid residues having a sequence 99% or greater identical to SEQ ID NO:2. In an embodiment the mutant PD-1 polypeptide does not comprise SEQ ID NO:1.

[0075] In an embodiment, the mutant PD-1 polypeptide comprises consecutive amino acid residues (i) having the sequence set forth in SEQ ID NO:3, or (ii) having a sequence 95% or greater identical to SEQ ID NO:3, but wherein in (ii) the polypeptide has the following residues at the same positions as set forth in SEQ ID NO:3:

X12= S or A

X13 = V or I

X14 = L or M

X15 = S or A

X16 = P or A

X17 = S or A

X18 = N or D

X19 = P r S

X20 = L or I

X21 = I or V

X22 = A or S

X23 = L or I

X24 = A or S

X25 = P or A

X26 = R or K

X27 = A or S

X28 = I or V.

[0076] In an embodiment, the mutant PD-1 polypeptide comprises consecutive amino acid residues having a sequence 96% or greater identical to SEQ ID NO:3. In an embodiment, the mutant PD-1 polypeptide comprises consecutive amino acid residues having a sequence 97% or greater identical to SEQ ID NO:3. In an embodiment, the mutant PD-1 polypeptide comprises consecutive amino acid residues having a sequence 98% or greater identical to SEQ ID NO:3. In an embodiment, the mutant PD-1 polypeptide comprises consecutive amino acid residues having a sequence 99% or greater identical to

SEQ ID NO:3. In an embodiment the mutant PD-1 polypeptide does not comprise SEQ ID NO:1.

[0077] Substitution variants of the mutant PD-1, as provided by the invention, have at least one amino acid residue in the polypeptide removed and a different residue inserted in its place (except for the conserved residues X1 through X11 of SEQ ID NO:2; and except for the conserved residues X12 through X28 of SEQ ID NO:3). In an embodiment, the substitution is a conservative substitution. Conservative substitutions are shown in Table 1 under the heading of "conservative substitutions." In an embodiment, the substitution is an exemplary substitution as listed in Table 1. In an embodiment, the PD-1 mutant contains one of 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO:2 or SEQ ID NO:3:

[0078] Table 1: Amino Acid Substitutions

Original Residue	Conservative Substitutions	Exemplary Substitutions
Ala (A)	Val	Val; Leu; Ile
Arg (R)	Lys	Lys; Gln; Asn
Asn (N)	Gln	Gln; His; Asp, Lys; Arg
Asp (D)	Glu	Glu; Asn
Cys (C)	Ser	Ser; Ala
Gln (Q)	Asn	Asn; Glu
Glu (E)	Asp	Asp; Gln
Gly (G)	Ala	Ala
His (H)	Arg	Asn; Gln; Lys; Arg
Ile (I)	Leu	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Ile	Norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg	Arg; Gln; Asn
Met (M)	Leu	Leu; Phe; Ile
Phe (F)	Tyr	Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr; Phe
Tyr (Y)	Phe	Trp; Phe; Thr; Ser
Val (V)	Leu	Ile; Leu; Met; Phe; Ala; Norleucine

[0079] This invention also provides a fusion polypeptide comprising the isolated mutant PD-1 polypeptide described herein, fused to an immunoglobulin domain polypeptide. In an embodiment, the mutant PD-1 polypeptide is fused to the immunoglobulin domain polypeptide by a peptide bond between a terminal amino acid of the mutant PD-1 polypeptide and a terminal amino acid of the immunoglobulin domain polypeptide. In an embodiment, the mutant PD-1 polypeptide is fused to the immunoglobulin domain polypeptide by a linker peptide between a terminal amino acid of the mutant PD-1 polypeptide and a terminal amino acid of the immunoglobulin domain polypeptide. In an embodiment, the immunoglobulin domain polypeptide comprises an immunoglobulin IgG Fc domain. In an embodiment, the immunoglobulin domain polypeptide comprises an immunoglobulin IgM Fc domain. In an embodiment, the immunoglobulin domain polypeptide comprises an immunoglobulin IgG1 Fc domain. In an embodiment, the immunoglobulin IgG or IgM Fc domain is human. In an embodiment, the immunoglobulin IgG1 Fc domain is human. In an embodiment, the fusion polypeptide is in monovalent form. In an embodiment, the fusion polypeptide comprises SEQ ID NO:2 as disclosed herein.

[0080] This invention also provides a homo-oligomer comprising the isolated mutant PD-1 polypeptide, or comprising the fusion polypeptide. In a preferred embodiment, the homo-oligomer comprises two of the isolated polypeptides, or two of the fusion polypeptides.

[0081] This invention also provides a composition comprising the isolated polypeptide in monovalent form or oligomeric form. This invention also provides a composition comprising the fusion polypeptide in monovalent form or oligomeric form. In an embodiment, the compositions comprise a pharmaceutically acceptable carrier. In an embodiment, the pharmaceutically acceptable carrier comprises a single type of molecule. In an embodiment, the pharmaceutically acceptable carrier comprises a mixture of molecules.

[0082] Using Fc-fusion polypeptides are widely known in the art. See, for example, Czajkowsky DM et al., EMBO Mol Med. 2012 Oct; 4(10): 1015–1028, hereby incorporated by reference.

[0083] Also provided is a method of stimulating T cell activation in a subject comprising administering to the subject the isolated mutant PD-1 polypeptide described herein, or the composition or homo-oligomer comprising the isolated mutant PD-1 polypeptide described herein, in an amount sufficient to stimulate T cell activation in a subject. In an embodiment, the subject has a tumor. In an embodiment, the subject has an infection.

[0084] Also provided is a method of treating a tumor in a subject comprising administering to the subject the isolated fusion polypeptide comprising the mutant PD-1 described herein, or the composition or homo-oligomer comprising the isolated fusion polypeptide comprising the mutant PD-1 described herein, in an amount sufficient to treat a tumor in a subject.

[0085] In an embodiment, the tumor is on of a breast, lung, colon, ovarian, melanoma, bladder, liver, salivary, stomach, gliomas, thyroid, thymus, epithelial, head, or neck tumor. Each of these tumors is also provided herein as an independent, individual embodiment. In an embodiment, the tumor is a hematological malignancy. In an embodiment, the tumor is a lymphoma. In an embodiment, the tumor is a myeloma. In an embodiment, the tumor is a multiple myeloma. In an embodiment, the tumor is a PD-L2 expressing tumor. In an embodiment, the PD-L2 expressing tumor is a pancreatic tumor. In an embodiment, the PD-L2 expressing tumor is a esophageal tumor.

[0086] Also provided is a method of treating an infection in a subject comprising administering to the subject the isolated fusion polypeptide described herein, or the composition or homo-oligomer comprising the isolated fusion polypeptide described herein, in an amount sufficient to treat an infection in a subject.

[0087] In an embodiment, the infection is a viral infection. In a further embodiment, the virus is a HIV, HCV, HBV or HTLV. In an embodiment, the infection is a bacterial, fungal, protozoal or parasitic infection. In embodiments, the infection is caused by *Helicobacter pylori*, the fungus *Histoplasma capsulatum*, the parasite *Taenia crassiceps* or *Schistosoma mansoni*, or the protozoa *Leishmania mexicana*. Each of these infections is also provided herein as an independent, individual embodiment.

[0088] In an embodiment, the composition is a pharmaceutical composition. In an embodiment, the pharmaceutical composition comprises a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" or "pharmaceutical acceptable

excipient" includes any material which, when combined with an active ingredient, allows the ingredient to retain biological activity and is non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Preferred diluents for aerosol or parenteral administration are phosphate buffered saline (PBS) or normal (0.9%) saline. Compositions comprising such carriers are formulated by well known conventional methods (see, for example, Remington's Pharmaceutical Sciences, 18th edition, A. Gennaro, ed., Mack Publishing Co., Easton, PA, 1990; and Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing, 2000).

[0089] Also provided is an isolated nucleic acid encoding an isolated mutant PD-1 polypeptide described herein. Also provided is an isolated nucleic acid encoding a fusion polypeptide comprising the mutant PD-1described herein. In an embodiment the encoded fusion protein comprises a human Fc sequence. In an embodiment, the nucleic acid is a recombinant nucleic acid. In an embodiment, the nucleic acid is an RNA. In an embodiment, the nucleic acid is a DNA. In an embodiment, the nucleic acid comprises cDNA.

[0090] An isolated cell containing a vector comprising a nucleic acid encoding an isolated mutant PD-1 polypeptide described herein is also provided. In an embodiment, the cell is used for production of the mutant PD-1 polypeptide.

[0091] As described herein, a mutant PD-1 polypeptide is not a naturally occurring mutant PD-1 polypeptide.

[0092] Also provided is an isolated mutant PD-1 polypeptide, as described hereinabove, or a fusion polypeptide comprising the polypeptide, as described hereinabove, fused to an immunoglobulin domain polypeptide, for treating an infection in a subject, or for treating a tumor in a subject, or for stimulating T-cell activation in a subject. In an embodiment, the mutant PD-1 polypeptide is for treating an infection in a subject. Exemplary infections are described hereinabove. In an embodiment, the mutant PD-1 polypeptide is for treating a tumor in a subject. Exemplary tumors are described hereinabove. In an embodiment, the mutant PD-1 polypeptide is for stimulating T-cell activation in a subject.

[0093] In a preferred embodiment of the methods, the subject is a human.

[0094] Also provided is a composition comprising a dendritic cell, loaded with any of the isolated mutant polypeptides or fusion proteins described herein. In an embodiment, the dendritic cell is mammalian. In an embodiment, it is derived from a human. In an embodiment, it is not derived from a human.

[0095] In an embodiment, the isolated mutant PD-1 polypeptide as described herein is capable of preferentially binding to a human PD-L2 over a PD-L1. In an embodiment, the isolated mutant PD-1 polypeptide as described herein specifically binds to a human PD-L2, with binding to PD-L1 undetectable. In an embodiment, the isolated mutant PD-1 polypeptide as described herein is capable of preferentially binding to a human PD-L1 over a PD-L2.

[0096] In an embodiment of the inventions set forth herein comprising SEQ ID NO:2, the sequence is any one of SEQ ID NOS:4-16. In an embodiment, the sequence is SEQ ID NO:4. In an embodiment, the sequence is SEQ ID NO:5. In an embodiment, the sequence is SEQ ID NO:6. In an embodiment, the sequence is SEQ ID NO:7. In an embodiment, the sequence is SEQ ID NO:8. In an embodiment, the sequence is SEQ ID NO:9. In an embodiment, the sequence is SEQ ID NO:10. In an embodiment, the sequence is SEQ ID NO:11. In an embodiment, the sequence is SEQ ID NO:12. In an embodiment, the sequence is SEQ ID NO:13. In an embodiment, the sequence is SEQ ID NO:14. In an embodiment, the sequence is SEQ ID NO:15. In an embodiment, the sequence is SEQ ID NO:16.

[0097] In an embodiment of the inventions set forth herein comprising SEQ ID NO:3, the sequence is any one of SEQ ID NOS:17-37. In an embodiment, the sequence is SEQ ID NO:17. In an embodiment, the sequence is SEQ ID NO:18. In an embodiment, the sequence is SEQ ID NO:19. In an embodiment, the sequence is SEQ ID NO:20. In an embodiment, the sequence is SEQ ID NO:21. In an embodiment, the sequence is SEQ ID NO:22. In an embodiment, the sequence is SEQ ID NO:23. In an embodiment, the sequence is SEQ ID NO:24. In an embodiment, the sequence is SEQ ID NO:25. In an embodiment, the sequence is SEQ ID NO:26. In an embodiment, the sequence is SEQ ID NO:27. In an embodiment, the sequence is SEQ ID NO:28. In an embodiment, the sequence is SEQ ID NO:29. In an embodiment, the sequence is SEQ ID NO:30. In an embodiment, the sequence is SEQ ID NO:31. In an embodiment, the sequence is SEQ ID NO:32. In an embodiment, the sequence is SEQ ID NO:33. In an embodiment, the sequence is SEQ ID NO:34. In an embodiment,

the sequence is SEQ ID NO:35. In an embodiment, the sequence is SEQ ID NO:36. In an embodiment, the sequence is SEQ ID NO:37.

[0098] All combinations of the various elements described herein are within the scope of the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0099] This invention will be better understood from the Experimental Details, which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims that follow thereafter.

EXPERIMENTAL DETAILS

Introduction

[00100] In recent years, the targeting of T cell costimulatory pathways has been demonstrated to represent powerful and effective strategies for immunotherapy. The best characterized costimulatory pathways include those associated with members of the CD28:B7 family, such as CD28 and ICOS as positive co-receptors, and CTLA4 and PD-1 as co-inhibitors. Herein, structure-guided approaches have been used to develop a novel protein reagent by engineering the human PD-1 sequence.

Results

[00101] Phage display was used to engineer the wild type human PD-1 IgV domain with two objectives in mind: (i) to identify engineered PD-1 IgV variants (ePD-1s) that bind both PD-L1 and PD-L2 with high affinity; (ii) to identify ePD-1s that preferentially bind to PD-L2 with enhanced specificity. Previously, yeast display have been used to engineer high affinity PD-L1-specific PD-1 variants for therapeutic and diagnostic/imaging application: Proc Natl Acad Sci U S A. 2015 Nov 24;112(47):E6506-14. Herein it was speculated that the natural "bispecific" recognition tendencies already inherent in PD-1 for binding to both PD-L1 and PD-L2 might be enhanced simultaneously to create higher affinity variants that are still bispecific for both ligands. Furthermore, although PD-L1 is more widely expressed than PD-L2, there are several cancers in which PD-L2 is dominantly expressed thus high-affinity PD-L2-specific $(ePD\text{-}1)_2$-Fc molecules (fusion polypeptides) could be used as specific therapeutics or diagnostics in those cases.

[00102] The human PD-1 IgV domain was expressed on M13 bacteriophage as a fusion to the pIII coat protein. Using the murine PD-1/PD-L1 and PD-1/PD-L2 crystal structures, and previous mutagenesis results as our guide, a homolog scanning library in which selected residues (elaborated in Fig. 1) were allowed to vary among their WT residue identify or homologous residues. The variations may allow "optimization" of minor contact or supporting conformations to enhance or change specificity. This library was subjected to two different selection schemes: (i) "Bi" a toggle selection between PD-L1 and PD-L2; (ii) "L2" a selection only against PD-L2. A number of hits were been characterized by phage ELISA and were sequenced. A number of cross-reactive clones that bind both PD-L1 and PD-L2 at low phage titer were been identified (Fig. 2, 3). The affinity of these relative to WT PD-1 is assessed. Furthermore, several PD-L2-specific ePD-1s were identified, in which there is no binding to PD-L1 observed (Fig. 4, 5). The ePD-1s were expressed and purified (gel data not shown).

[00103] Equilibrium dissociation constants (K_ds) can be determined by non-linear fitting of SPR data using, for example, immobilized PD-1 Ligands (e.g. PD-L1 and/or PD-L2) and soluble wild-type or mutant PD-1. K_d values can be expressed in, for example, μM concentration, followed by the standard errors of the fittings. Human monocyte-derived dendritic cells expressing PD-L1 and PD-L2 are one exemplary target. K_d values of 10 μM down to 1 pM are of most use. Preferred K_d values are 1 μM or below.

[00104] Dimeric soluble Fc chimera of the mutant PD-1 polypeptides can be further engineered, for example, by fusing a mutant PD-1 polypeptide to the Fc part of human IgG1.

Discussion

[00105] Blocking the PD-1/PD-L pathways using the mutant PD-1 polypeptides or mutant PD-1 Ig fusion proteins disclosed herein would result in an enhanced immune response, similar to that of the CTLA-4 blockade caused by Yervoy; however, due to differences in the roles of the CTLA-4/B7 and the PD-1/PD-L pathways, less severe side effects would be seen with PD-1 blockade. Due to the expression patterns of PD-1 and its ligands on both peripheral and immune cells (unlike CTLA-4 and its ligands which are expressed on immune cells only), the paradigm is that PD-1/PD-L pathway predominantly regulates peripheral immune responses at the tissue level (with a lesser role in central

immunity than CTLA-4), consistent with its major role in peripheral tolerance. Yervoy (anti-CTLA-4) targets predominantly interactions between T cells and APCs, and is thus expected to cause systemic immune stimulation accompanied by adverse effects. In contrast, due to the large number of studies reporting upregulation of PD-Ls on infected cells and tumor cells, the high-affinity mutant PD-1 Ig fusion protein would preferentially bind to these target cells and act primarily on T cells (effectors) that are specifically recruited to these sites, as opposed to the systemic immune activation elicited by an antibody that binds to and activates all T cells.

[00106] An additional advantage is conferred by the mutant PD-L2 specific polypeptides disclosed herein. These can be used, alone or, for example, as fusion protein constructs, to treat PD-L2 expressing tumors with less off-target effects than agents that bind both PD-L1 and PD-L2.

[00107] Specific therapeutic targets for the agents disclosed herein are numerous. Both chronic infections and malignant tumors affect increasing numbers of patients not just in the US but worldwide. Chronic infections such as HIV, and HCV affect 33 million and 170 million people worldwide, respectively, as well as HBV infection which affects about 2 billion people worldwide (the most common infectious disease today). Acute infections in which the PD-1 pathway is involved, such as histoplasmosis, are also prevalent (50 million people affected in North America), as are rabies and RSV infections. Malignancies constitute another major area that could be targeted for treatment using the agents of invention. The incidence of tumors continues to increase worldwide; currently about 12.7 million new cases are reported each year. The incidence of cancer cases is highest for Australia/New Zealand, North America and Europe, where treatment is also more available or sought-after. Since several tumor cell types upregulate ligands of inhibitory receptors, such as PD-L1 and PD-L2, to promote immune evasion, administration of the agents disclosed herein is expected to be beneficial in such states. Natural immunological clearance of tumors is rare, due to the immunosuppressive environment generated by the developing tumor. As part of this immunosuppressive mechanism, many tumors upregulate ligands of inhibitory receptors such as PD-L1 and PD-L2. PD-L1 has been shown to be upregulated on a variety of solid tumors, such as breast, lung, colon, ovarian, melanoma, bladder, liver, salivary, stomach, gliomas, thyroid, thymic, epithelial, head, and neck (Keir ME et al, *Annu Rev Immunol* 2008). Both PD-Ligands have been shown to be upregulated

in hematologic malignancies such as lymphomas and multiple myeloma. In addition, PD-1 is upregulated on tumor infiltrating lymphocytes, which is also expected to contribute to tumor immunosuppression. In addition, HA PD-1 Ig can be beneficial to treat tumors that do not express PD-Ls (although less efficient, compared to PD-L-expressing tumors), by causing immune activation at the level of initial antigen presentation to T cells (an analogous mechanism to that of CTLA4 blockade).

[00108] Since the mutant PD-1 ligands can bind the PD-Ligands with high affinity, they will prevent inhibitory signals into tumor infiltrating T cells through the endogenous PD-1 receptor. Removal of these inhibitory signals has dramatic effects on anti-tumor immune responses (e.g. as observed with Yervoy, a CTLA-4-specific monoclonal antibody, approved by FDA for the treatment of metastatic melanoma), the fusion polypeptide can be used in the malignancies listed above to activate the tumor-specific T cell response and induce tumor regression. In addition, the PD-L2-specific mutant ligands can be used to treat PD-L2-expressing disease states. For example, esophageal and pancreatic cancers have both been found to express PD-L2.

References

Hodi FS et al., *N Engl J Med* (2010), 363:711-723.

Keir ME et al, *Annu Rev Immunol* (2008), 26:677–704

Czajkowsky DM et al., *EMBO Mol Med.* 2012 Oct; 4(10): 1015–1028

What is claimed is:

1. An isolated mutant PD-1 polypeptide comprising (i) SEQ ID NO:2, or (ii) SEQ ID NO:3 or (ii) having a sequence 95% or greater identical to SEQ ID NO:2 or SEQ ID NO:3 with the proviso that the mutant PD-1 polypeptide does not comprise SEQ ID NO:1.

2. The polypeptide of Claim 1, comprising SEQ ID NO:2.

3. The polypeptide of Claim 1, comprising SEQ ID NO:3.

4. A fusion polypeptide comprising the polypeptide of Claim 1, 2 or 3, fused to an immunoglobulin domain polypeptide.

5. The fusion polypeptide of Claim 4, wherein the mutant PD-1 polypeptide is fused to the immunoglobulin domain polypeptide by a peptide bond between a terminal amino acid of the mutant PD-1 polypeptide and a terminal amino acid of the immunoglobulin domain polypeptide.

6. The fusion polypeptide of Claim 4 or 5, wherein the immunoglobulin domain polypeptide comprises an immunoglobulin IgG1 Fc domain.

7. The fusion polypeptide of Claim 6, wherein the immunoglobulin IgG1 Fc domain is human.

8. The polypeptide of Claim 1, 2 or 3, or the fusion polypeptide of any of Claims 4-7, in monovalent form.

9. A homo-oligomer comprising the polypeptide of Claim 1, 2 or 3, or the fusion polypeptide of any of Claims 4-7.

10. The homo-oligomer of Claim 9, comprising two polypeptides of Claim 1, 2 or 3, or two fusion polypeptides of any of Claims 4-7.

11. A composition comprising the polypeptide of Claim 1, 2, 3 or 8.

12. A composition comprising the fusion polypeptide of Claim 4, 5, 6, or 7.

13. A composition comprising the homo-oligomer of Claim 9 or 10.

14. The composition of Claim 11, 12 or 13, comprising a pharmaceutically acceptable carrier.

15. A method for stimulating T cell activation, treating a tumor, or treating an infection in a subject comprising administering to the subject the polypeptide of Claims 1, 2, 3, or 8-10, or the polypeptide-containing composition of Claim 11, 13 or 14, in an amount sufficient to stimulate T cell activation, treat a tumor, or treat an infection, respectively, in a subject.

16. A method for stimulating T cell activation, treating a tumor, or treating an infection in a subject comprising administering to the subject the fusion peptide of any of Claims 4 to 10, or the fusion peptide-containing composition of Claim 12 or 14, in an amount sufficient to stimulate T cell activation, treat a tumor, or treat an infection, respectively, in a subject.

17. The method of Claim 15 or 16, wherein the T cell activation comprises cytokine secretion.

18. The method of Claim 15 or 16, wherein the method is for treating a tumor.

19. The method of Claim 15 or 16 wherein the polypeptide, or the fusion peptide, respectively, comprises SEQ ID NO:3.

20. The method of Claim 19, wherein the method is for treating a tumor and the tumor is a PD-L2-expressing tumor.

21. The method of Claim 15 or 16 wherein the polypeptide, or the fusion peptide, respectively, comprises SEQ ID NO:2.

22. The method of Claim 21, wherein the method is for treating a tumor and the tumor is a PD-L1-expressing tumor or a PD-L1- and PD-L2-expressing tumor.

23. The method of Claim 15 or 16, wherein the method is for treating an infection.

24. An isolated nucleic acid encoding the isolated mutant PD-1 polypeptide of Claim 1, 2 or 3.

25. An isolated nucleic acid encoding the fusion polypeptide of any of Claims 4, 5, 6 or 7.

26. The nucleic acid of Claim 24 or 25, which is a recombinant nucleic acid.

27. The nucleic acid of Claim 24, 25 or 26, which comprises cDNA.

28. An isolated cell containing a vector comprising a nucleic acid of any of Claims 24 - 27.

29. A method of reducing an amount of PD-L2 on a cell in a subject available for binding to a PD-1 receptor in a body of a subject, the method comprising administering to the subject an amount of the polypeptide of Claims 1, 2, 3, or 8-10, or the polypeptide-containing composition of Claim 11, 13 or 14, effective to reduce the amount of PD-L2 on a cell available for binding to a PD-1 receptor.

30. A method of reducing an amount of PD-L2 on a cell in a subject available for binding to a PD-1 receptor in a body of a subject, the method comprising administering to the subject an amount of the fusion peptide of any of Claims 4 to 10, or the fusion peptide-containing composition of Claim 12 or 14, effective to reduce the amount of PD-L2 on a cell available for binding to a PD-1 receptor.

31. A method of reducing an amount of PD-L1 on a cell in a subject available for binding to a PD-1 receptor in a body of a subject, the method comprising administering to the subject an amount of the polypeptide of Claims 1, 2, 3, or 8-10, or the polypeptide-containing composition of Claim 11, 13 or 14, effective to reduce the amount of PD-L1 on a cell available for binding to a PD-1 receptor.

32. A method of reducing an amount of PD-L1 on a cell in a subject available for binding to a PD-1 receptor in a body of a subject, the method comprising administering to the subject an amount of the fusion peptide of any of Claims 4 to 10, or the fusion peptide-containing composition of Claim 12 or 14, effective to reduce the amount of PD-L1 on a cell available for binding to a PD-1 receptor.

33. The method of any of Claims 29-32, wherein the PD-1 receptor is on a T-cell.

34. The method of any of Claims 15-22 or 29-32, wherein the subject is a human.

hPD-1 WNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQ

hPD-1 DCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEV

hPD-1 PTAHPSPSP (SEQ ID NO:1)

Fig. 1

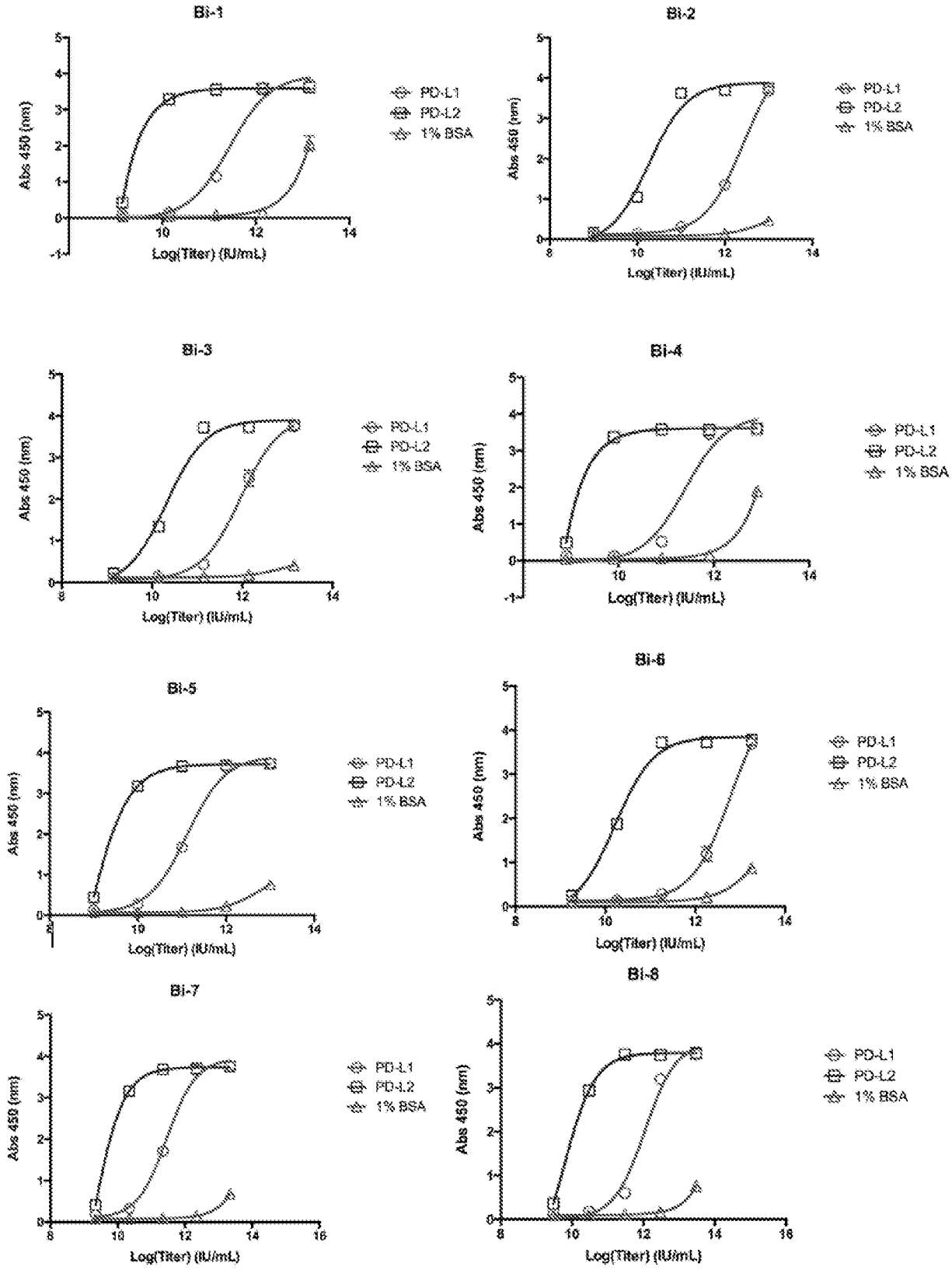

Bi-1
Abs 450 (nm)
Log(Titer) (IU/mL)
PD-L1
PD-L2
1% BSA
Bi-2
Abs 450 (nm)
Log(Titer) (IU/mL)
PD-L1
PD-L2
1% BSA
Bi-3
Abs 450 (nm)
Log(Titer) (IU/mL)
PD-L1
PD-L2
1% BSA
Bi-4
Abs 450 (nm)
Log(Titer) (IU/mL)
PD-L1
PD-L2
1% BSA
Bi-5
Abs 450 (nm)
Log(Titer) (IU/mL)
PD-L1
PD-L2
1% BSA
Bi-6
Abs 450 (nm)
Log(Titer) (IU/mL)
PD-L1
PD-L2
1% BSA
Bi-7
Abs 450 (nm)
Log(Titer) (IU/mL)
PD-L1
PD-L2
1% BSA
Bi-8
Abs 450 (nm)
Log(Titer) (IU/mL)
PD-L1
PD-L2
1% BSA

Fig. 2

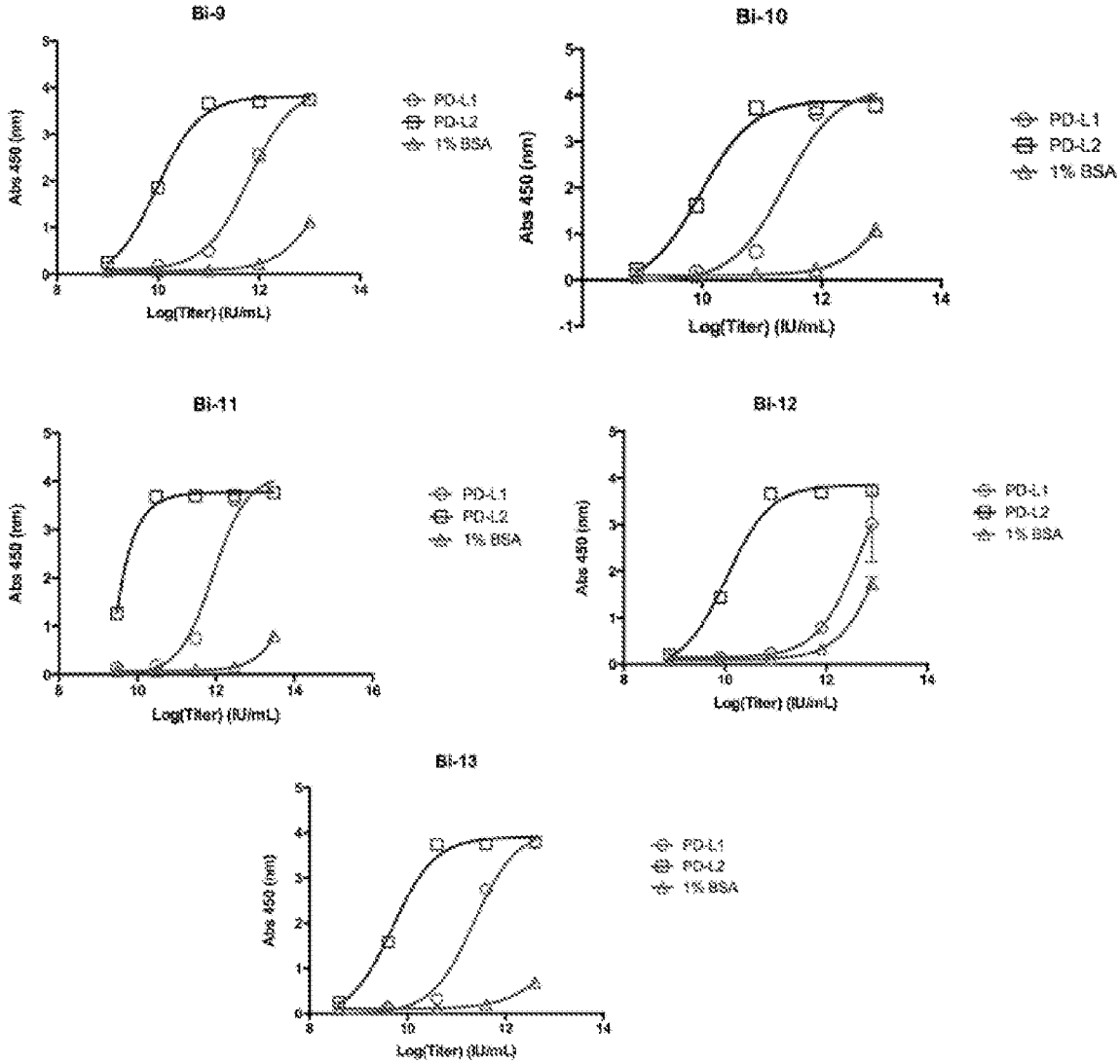

Bi-9
Abs 450 (nm)
Log(Titer) (IU/mL)
PD-L1
PD-L2
1% BSA
Bi-10
Abs 450 (nm)
Log(Titer) (IU/mL)
PD-L1
PD-L2
1% BSA
Bi-11
Abs 450 (nm)
Log(Titer) (IU/mL)
PD-L1
PD-L2
1% BSA
Bi-12
Abs 450 (nm)
Log(Titer) (IU/mL)
PD-L1
PD-L2
1% BSA
Bi-13
Abs 450 (nm)
Log(Titer) (IU/mL)
PD-L1
PD-L2
1% BSA

Fig. 3

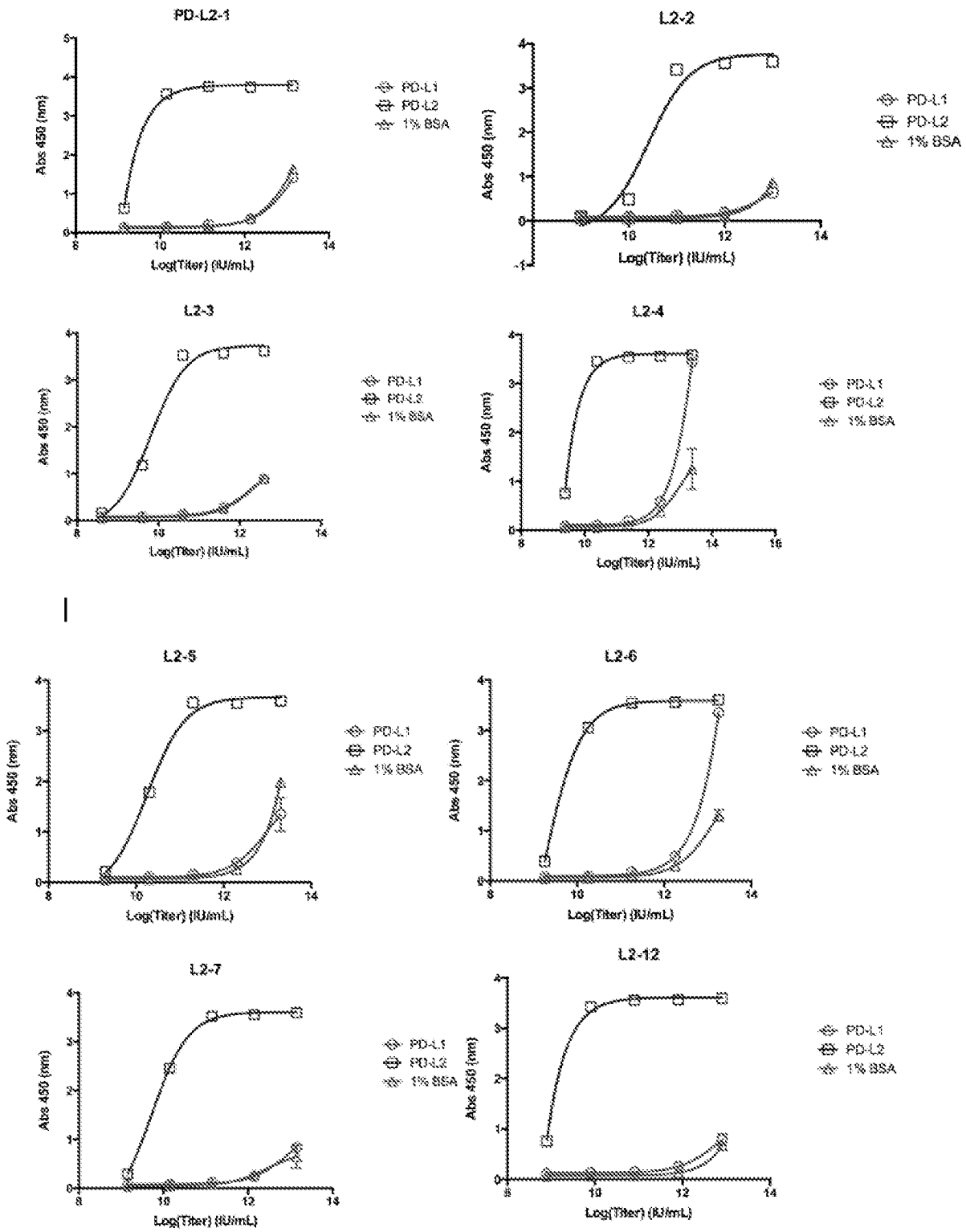

PD-L2-1
L2-2
L2-3
L2-4
L2-5
L2-6
L2-7
L2-12
Abs 450 (nm)
Log(Titer) (IU/mL)
PD-L1
PD-L2
1% BSA

Fig. 4

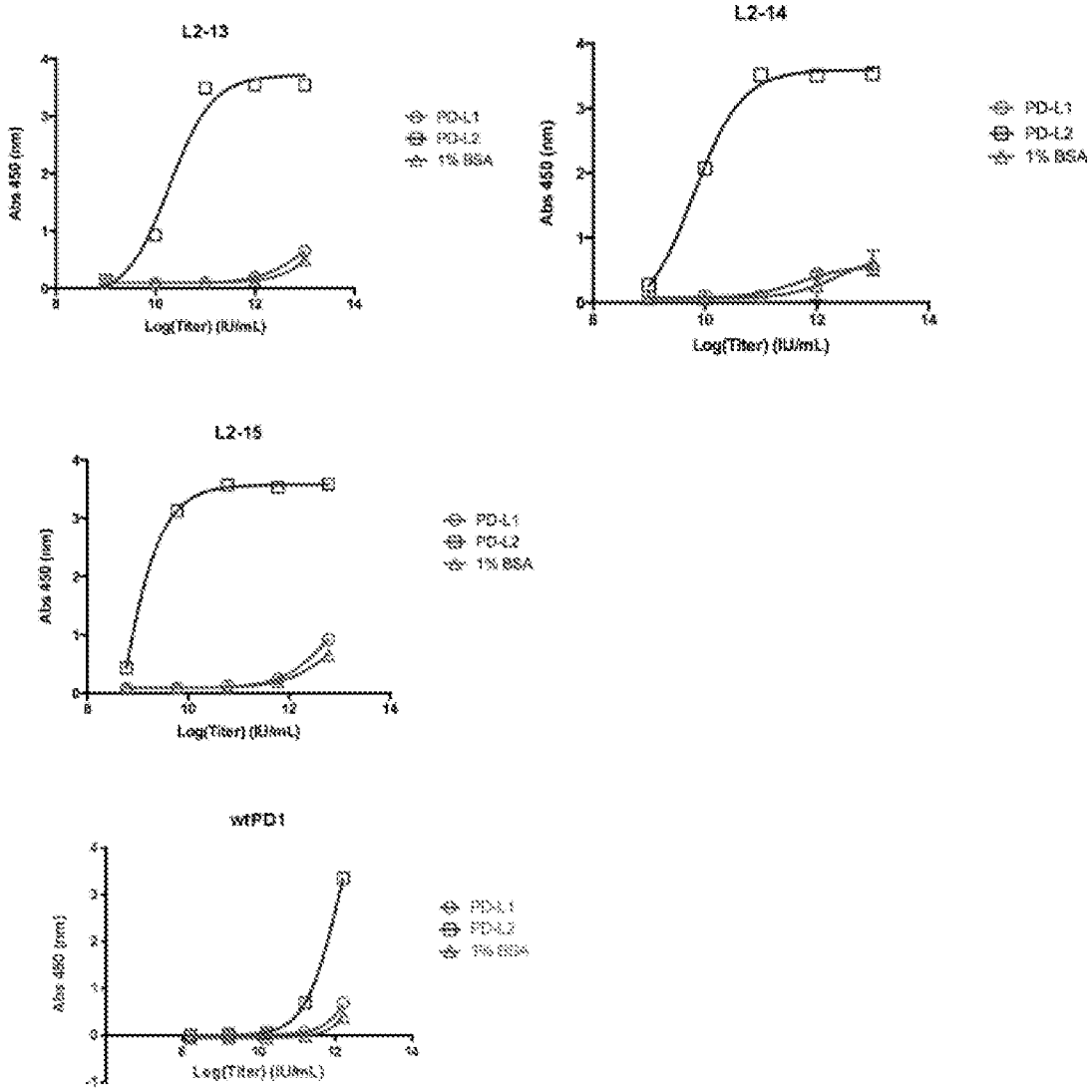

L2-13
Abs 450 (nm)
Log(Titer) (IU/mL)
PD-L1
PD-L2
1% BSA
L2-14
Abs 450 (nm)
Log(Titer) (IU/mL)
PD-L1
PD-L2
1% BSA
L2-15
Abs 450 (nm)
Log(Titer) (IU/mL)
PD-L1
PD-L2
1% BSA
wtPD1
Abs 450 (nm)
Log(Titer) (IU/mL)
PD-L1
PD-L2
1% BSA

Fig. 5

Bispecific PD-1 sequences:

```
hPD-1:WNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFR
Bi-1:                                  S V N Y LSPSNQT K    P     PG
Bi-2:                                  S I N Y LSPSNQT K    P     PG
Bi-3:                                  S V N Y LSPSNQT K    P     PG
Bi-4:                                  S I N Y LSPSNQT K    P     PG
Bi-5:                                  S V N Y LSPSNQT K    P     PG

Bi-6:                                  A I N Y LSPSNQT K    P     PG
Bi-7:                                  S I N Y LSASNQT K    P     PG
Bi-8:                                  A I N Y LSPSNQT K    P     PG
Bi-9:                                  A V N Y MSPSNQT K    P     PG
Bi-10:                                 S V N Y LSPSNQT K    P     PG
Bi-11:                                 S I N Y LSPSNQT K    P     PG
Bi-12:                                 S I N Y MSPSNQT K    P     PG
Bi-13:                                 S V N Y MSASNQT K    P     PG


hPD-1: VTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSP
Bi-1:                            L A IALSPRA I E
Bi-2:                            L A IALSPRS I E
Bi-3:                            L A IALSPKA I E
Bi-4:                            I A IALSPRA I E
Bi-5:                            L A ISLSPRA I E
Bi-6:                            I A ISIAPRA I E
Bi-7:                            L A ISLSPKA I E
Bi-8:                            I A ISLSPRA I E
Bi-9:                            I A ISLSPKA I E
Bi-10:                           I A IALSPRA I E
Bi-11:                           I A ISLSPKA V E
Bi-12:                           I A ISLAPKS I E
Bi-13:                           I A ISLSPRA I E
```

Fig. 6

PD-L2 specific sequences:

```
hPD-1: WNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFR
 L2-1:                                S I N Y LSPSNQT K    P     PG
 L2-2:                                S I N Y MAPSNQT K    P     PG
 L2-3:                                A V N Y MSPSNQT K    P     PG
 L2-4:                                S I N Y LSPSNQT K    P     PG
 L2-5:                                S I N Y LSPSDQT K    P     PG
 L2-6:                                S I N Y MSASNQT K    P     PG
 L2-7:                                S I N Y LSPSNQT K    P     PG
 L2-8:                                A I N Y MSASNQT K    P     PG

 L2-9:                                S V N Y LSASNQT K    P     PG
L2-10:                                S I N Y LSAANQT K    P     PG
L2-11:                                S I N Y MSPSNQT K    P     PG
L2-12:                                A I N Y LSPSNQT K    P     PG
L2-13:                                A I N Y MSPSDQT K    P     PG
L2-14:                                S I N Y MSPANQT K    P     PG
L2-15:                                S I N Y LSPANQT K    P     PG
L2-16:                                S I N Y MSASDQT K    P     SG

L2-17:                                A I N Y LSPSNQT K    P     PG
L2-18:                                S I N Y MSASNQT K    P     PG
L2-19:                                S I N Y LSASNQT K    P     PG
L2-20:                                A I N Y LSASNQT K    P     PG
L2-21:                                S I N Y LSASNQT K    P     PG


hPD-1: VTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSP
 L2-1:                             I A IALSAKS V E
 L2-2:                             L A VALSPRA I E
 L2-3:                             L A IAISAKS V E
 L2-4:                             L A ISLSPRS V E
 L2-5:                             L A VSISPRS V E
 L2-6:                             L A ISLAPKA V E
 L2-7:                             L A ISLSPKS V E
 L2-8:                             L A VSLSAKS V E
 L2-9:                             L A ISISAKS V E
L2-10:                             L A VSLSPKA V E
L2-11:                             L A VAIAPRS V E
L2-12:                             L A  SLSPRA V E
L2-13:                             L A ISLSAKS I E
L2-14:                             L A VAIAPRA V E
L2-15:                             L A VSLAAKA V E
L2-16:                             L A IALSAKA V E
L2-17:                             L A ISLSAKS V E
L2-18:                             L A VSLSPKA I E
L2-19:                             I A ISLSARS V E
L2-20:                             L A   IA KA V E
L2-21:                             L A IAISARS V E
```

Fig. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/031681

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/17; C07K 14/47; C07K 14/705 (2017.01)
CPC - A61K 38/1761; C07K 14/4747; C07K 14/70596; C07K 2319/30 (2017.02)

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)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/134.1; 435/328; 514/18.9; 514/19.3 (keyword delimited)

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 8,735,553 B1 (LI et al) 27 May 2014 (27.05.2014) entire document	1, 4, 5, 24
Y	FORNI et al. "A 175 million year history of T cell regulatory molecules reveals widespread selection, with adaptive evolution of disease alleles," Immunity, 27 June 2013 (27.06.2013), Vol. 38, No. 6, Pgs. 1129-1141. entire document	1, 4, 5, 24
A	US 2004/0033497 A1 (ALARCON-RIQUELME et al) 19 February 2004 (19.02.2004) entire document	1-5, 24
A	US 2015/0366174 A1 (REGENERON PHARMACEUTICALS INC) 24 December 2015 (24.12.2015) entire document	1-5, 24
A	WO 2011/109789 A2 (THE JOHNS HOPKINS UNIVERSITY et al) 09 September 2011 (09.09.2011) entire document	1-5, 24

☐ 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
30 July 2017	16 AUG 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/031681

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.: 6-23, 25-34
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:

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

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

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

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

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/031681

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs:1-3 were searched.