



(51) International Patent Classification:

G01N 33/569 (2006.01) G01N 33/68 (2006.01)
G01N 33/574 (2006.01)

(21) International Application Number:

PCT/US2018/029897

(22) International Filing Date:

27 April 2018 (27.04.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/491,479 28 April 2017 (28.04.2017) US

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(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, DJ, DK, DM, DO,

DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, 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))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: METHODS OF TREATMENT WITH CD80 EXTRACELLULAR DOMAIN POLYPEPTIDES

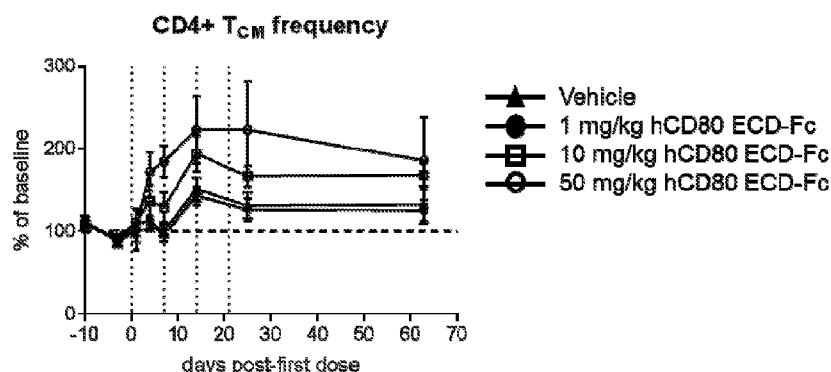


Fig. 1

(57) Abstract: This application relates to use of CD80 (B7-1) extracellular domain (ECD) polypeptides and CD80-ECD fusion molecules and their use in methods of increasing the number of central memory T cells or for treatment for cancer or for use in cancer vaccine compositions.

METHODS OF TREATMENT WITH CD80 EXTRACELLULAR DOMAIN POLYPEPTIDES

SEQUENCE LISTING

[0001] 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 of the sequence listing, created on April 26, 2018, is named 3986.007PC01_SequenceListing_ST25.txt and is 60,296 bytes in size.

FIELD

[0002] This application relates to CD80 (B7-1) extracellular domain (ECD) polypeptides and CD80-ECD fusion molecules and their uses in increasing central memory T cells and in methods of treatment, such as methods of treating cancer.

BACKGROUND

[0003] CD80, also known as B7-1, is one of the B7 family of membrane-bound proteins involved in immune regulation by delivering costimulatory or coinhibitory responses through their ligand binding activities. Other members of the B7 family of proteins include CD86 (B7-2), inducible costimulator ligand (ICOS-L), programmed death-1 ligand (PD-L1; B7-H1), programmed death-2 ligand (PD-L2; B7-H2), B7-H3, and B7-H4. CD80 is a transmembrane protein expressed on the surface of T cells, B cells, dendritic cells and monocytes, and binds to the receptors CD28, CTLA4 (CD152), and PD-L1. (See, e.g., M. Collins et al., *Genome Biol.* 6:223 doi:10.1186/bg-2005-6-6-223 (2005).) CD80 and CD86 and their receptors CTLA4 and CD28 operate as a costimulatory-coinhibitory system, for example, to control T cell activation, expansion, differentiation, and survival. CD80 and CD86 interaction with CD28 results in costimulatory signals that lead, for example, to activation of T cell responses. CD80, in turn, stimulates upregulation of CTLA4, which, upon binding to CD80, acts to suppress the T cell response previously triggered by CD80/CD28 interactions. This feedback loop allows for fine control of immune responses. (See, e.g., R. Peach et al., *J. Biol. Chem.* 270(36): 21181-87 (1995).)

[0004] CD80 has also been shown to interact with another B7 family member, PD-L1 with similar affinity to CD28, whereas CD86 does not interact with PD-L1. (*See, e.g., P. Greaves & J.G. Gribbon, Blood* 121(5): 734-44 (2013).) PD-L1 is one of two ligands for the programmed death-1 (PD-1) protein, which is also involved in T cell regulation. Specifically, expression of PD-1 on T cells may be induced after T cells have been activated, and binding of PD-1 to PD-L1 downregulates T cell activity by promoting T cell inactivation. (*See, e.g., S. Ostrand-Rosenberg, J. Immunol.* 193: 3835-41 (2014).) Many tumor cells express PD-L1 on their surface, potentially leading to PD-1/PD-L1 interactions and the inhibition of T cell responses against the tumor. This observation has led to the development of inhibitors of the PD-1/PD-L1 interaction as cancer therapeutics designed to stimulate natural immune responses against tumors in patients. (*See Id.*) Binding of CD80 to PD-L1 may serve as an alternative mechanism to block the PD-1/PD-L1 interaction and prevent inhibition of T cell responses at the site of a tumor. (*See Id.* at 3839.) At the same time, however, increased levels of CD80 might also be available to bind to CD28 and to induce CTLA4, thus either inducing or inhibiting T cell responses. Some soluble forms of CD80 may also function to block CTLA4 activation by blocking endogenous CD80 activity. (*See Id.*)

[0005] The present inventors have shown that administration of a CD80 extracellular domain (ECD) fusion polypeptide to cynomolgus monkeys induced expansion and proliferation of CD4⁺ and CD8⁺ central memory T cells in a dose dependent manner. Memory T cells are a subset of T cells that are directed to particular antigens due to previous encounters with those antigens. The presence of memory T cells may allow for a stronger and swifter immune response to the antigen if it is re-encountered in future. Central memory T cells (T_{cm}) are a subset of memory T cells that may retain some stem-cell-like properties. (*See Y.D. Mahnke, Eur. J. Immunol.* 43: 2797-2809 (2013).) Particular subsets of T_{cm} have been shown to be active against certain cancers in murine studies, for example, mounting an active tumor antigen recall response when mice are re-presented with previously encountered tumor antigens. (*See Klebanoff et al., PNAS* 102(27): 9571-76 (2005). Clinical studies on ipilimumab, an anti-CTLA4 antibody, have also shown alterations in T cell populations in patients who are responsive to the antibody treatment. (J. Felix et al., *Oncoimmunology* 5(7): e1136045 (2016); J.S. Weber, *J. Immunother.* 35(1): 89-97 (2012)).

SUMMARY

- [0006]** Provided herein are methods of determining the activity of a CD80 extracellular domain (ECD) fusion molecule in a subject comprising determining the frequency of central memory T cells and/or detecting the proliferation of central memory T cells in a sample obtained from the subject after administration of the CD80 ECD fusion molecule to the subject, wherein the CD80 ECD fusion molecule comprises a human CD80 ECD polypeptide and a human IgG1 Fc domain.
- [0007]** Also provided herein are methods of detecting central memory T cell frequency or proliferation in a subject comprising determining the frequency of central memory T cells and/or detecting the proliferation of central memory T cells in a sample obtained from the subject after administration of the CD80 ECD fusion molecule to the subject, wherein the CD80 ECD fusion molecule comprises a human CD80 ECD polypeptide and a human IgG1 Fc domain.
- [0008]** Also provided herein are methods of determining the activity of a CD80 extracellular domain (ECD) fusion molecule in a subject comprising (a) obtaining a sample from the subject after a CD80 ECD fusion molecule has been administered to the subject and (b) determining the frequency of central memory T cells and/or detecting the proliferation of central memory T cells in the sample, wherein the CD80 ECD fusion molecule comprises a human CD80 ECD polypeptide and a human IgG1 Fc domain.
- [0009]** Also provided herein are methods of detecting central memory T cell frequency or proliferation in a subject comprising (a) obtaining a sample from the subject after a CD80 ECD fusion molecule has been administered to the subject and (b) determining the frequency of central memory T cells and/or detecting the proliferation of central memory T cells in the sample, wherein the CD80 ECD fusion molecule comprises a human CD80 ECD polypeptide and a human IgG1 Fc domain.
- [0010]** In certain instances, the methods further comprise administering a CD80 ECD fusion molecule to the subject after determining the frequency of central memory T cells and/or detecting the proliferation of central memory T cells in the sample. In certain instances, the subject has cancer.
- [0011]** Also provided herein are methods of treating cancer in a subject comprising (a) administering to the subject a CD80 ECD fusion molecule comprising a human CD80 ECD polypeptide and a human IgG1 Fc domain; and (b) determining the frequency of

central memory T cells and/or detecting the proliferation of central memory T cells in a sample obtained from the subject after the administration.

[0012] In certain instances, the methods further comprise determining the frequency of central memory T cells or detecting the proliferation of central memory T cells in a sample obtained from the subject prior to administration of the CD80 ECD fusion molecule.

[0013] In certain instances, the methods comprise determining the frequency of central memory T cells, but not detecting the proliferation of central memory T cells. In certain instances, the methods comprise detecting the proliferation of central memory T cells, but not determining the frequency of central memory T cells. In certain instances, the methods comprise determining the frequency of central memory T cells and detecting the proliferation of central memory T cells.

[0014] In certain instances, the frequency is determined using flow cytometry.

[0015] In certain instances, the proliferation is detected using flow cytometry. In certain instances, the proliferation is detected by measuring Ki67 expression. In certain instances, the proliferation is detected in a sample obtained at least 7 days after administration of the CD80 ECD fusion molecule.

[0016] Also provided herein are methods of treating cancer in a subject comprising administering to the subject a CD80 ECD fusion molecule comprising a human CD80 ECD polypeptide and a human IgG1 Fc domain, wherein (a) the frequency of memory T cells has been determined in a sample obtained from the subject prior to the administration and/or (b) the proliferation of memory T cells has been detected in a sample obtained from the subject prior to the administration.

[0017] In certain instances, the frequency of memory T cells has been determined, but the proliferation of memory T cells has not been detected. In certain instances, the proliferation of memory T cells has been detected, but the frequency of memory T cells has not been determined. In certain instances, the frequency of memory T cells has been determined and the proliferation of memory T cells has been detected.

[0018] In certain instances, the frequency has been determined using flow cytometry.

[0019] In certain instances, the proliferation has been detected using flow cytometry. In certain instances, the proliferation has been determined by measuring Ki67 expression. In certain instances, the proliferation has been detected in a sample obtained at least 7 days after administration of the CD80 ECD fusion molecule.

- [0020] In certain instances, the sample is a blood sample. In certain instances, the sample is a plasma sample.
- [0021] In certain instances, the central memory T cells are CD95+ and CD28+ cells. In certain instances, the central memory T cells are CD4+ central memory T cells. In certain instances, the central memory T cells are CD8+ central memory T cells. In certain instances, the central memory T cells are CD4+ and CD8+ central memory T cells.
- [0022] In certain instances, the sample obtained after administration of the CD80 ECD fusion molecule is obtained at least one week after the administration. In certain instances, the sample obtained after administration of the CD80 ECD fusion molecule is obtained at least two weeks after the administration. In certain instances, the sample obtained after administration of the CD80 ECD fusion molecule is obtained at least one month after the administration.
- [0023] In certain instances, the human CD80 ECD polypeptide comprises the amino acid sequence of SEQ ID NO:5. In certain instances, the human CD80 ECD polypeptide comprises the amino acid sequence of SEQ ID NO:3. In certain instances, the human CD80 ECD polypeptide comprises the amino acid sequence of SEQ ID NO:4.
- [0024] In certain instances, the human IgG1 Fc domain comprises the amino acid sequence of SEQ ID NO:14.
- [0025] In certain instances, the CD80 ECD fusion molecule comprises the amino acid sequence of SEQ ID NO:20. In certain instances, the CD80 ECD fusion molecule comprises the amino acid sequence of SEQ ID NO:21.
- [0026] In certain instances, the CD80 ECD fusion molecule comprises 10-60 molecules of sialic acid (SA). In certain instances, the CD80 ECD fusion molecule comprises 15-40 molecules of SA. In certain instances, the CD80 ECD fusion molecule comprises 15-25 molecules of SA. In certain instances, the CD80 ECD fusion molecule comprises 15-30 molecules of SA.
- [0027] In certain instances, the cancer is a solid tumor. In certain instances, the cancer is selected from the group consisting of colorectal cancer, breast cancer, gastric cancer, non-small cell lung cancer, melanoma, squamous cell carcinoma of the head and neck, ovarian cancer, pancreatic cancer, renal cell carcinoma, hepatocellular carcinoma, bladder cancer, and endometrial cancer. In certain instances, the cancer is recurrent or progressive after a therapy selected from the group consisting of surgery, chemotherapy, radiation therapy, and a combination thereof.

[0028] In certain instances, the CD80 ECD fusion molecule is administered in combination with a programmed cell death 1 (PD-1) / programmed cell death ligand 1 (PD-L1) inhibitor. In certain instances, the PD-1/PD-L1 inhibitor is an antibody. In certain instances, the PD-1/PD-L1 inhibitor is an anti-PD-1 antibody. In certain instances, the anti-PD-1 antibody comprises the heavy chain and light chain CDRs of nivolumab, pidilizumab, or pembrolizumab. In certain instances, the anti-PD-1 antibody comprises the heavy chain and light chain variable regions of nivolumab, pidilizumab, or pembrolizumab. In certain instances, the anti-PD-1 antibody is nivolumab, pidilizumab, or pembrolizumab. In certain instances, the PD-1/PD-L1 inhibitor is an anti-PD-L1 antibody. In certain instances, the anti-PD-L1 antibody comprises the heavy chain and light chain CDRs of avelumab, durvalumab, atezolizumab, or BMS-936559. In certain instances, the anti-PD-L1 antibody comprises the heavy chain and light chain variable regions of avelumab, durvalumab, atezolizumab, or BMS-936559. In certain instances, the anti-PD-L1 antibody is avelumab, durvalumab, atezolizumab, or BMS-936559. In certain instances, the PD-1/PD-L1 inhibitor is a PD-1 fusion molecule. In certain instances, the fusion molecule is AMP-224. In certain instances, the PD-1/PD-L1 inhibitor is AUR-012.

[0029] In certain instances, the CD80 ECD fusion molecule is administered in combination with a cancer vaccine. In certain instances, the cancer vaccine is a personalized cancer vaccine. In certain instances, the CD80 ECD fusion molecule and the cancer vaccine are administered concurrently or sequentially.

[0030] The present disclosure encompasses methods of treating cancer in a subject in need thereof, comprising administering a CD80 extracellular domain (ECD) or CD80 ECD fusion molecule, wherein the CD80 ECD or CD80 ECD fusion molecule is administered in an amount effective to increase the number of central memory T cells in the subject. In some embodiments, the number of central memory T cells is increased for at least one week, at least two weeks, or at least one month. In some embodiments, the number of central memory T cells is determined in a blood or plasma sample of the subject. In some embodiments, the central memory T cells are CD95⁺ and CD28⁺ cells. The present disclosure also encompasses methods of treating cancer in a subject in need thereof, comprising: (a) administering to the subject a CD80 extracellular domain (ECD) or CD80 ECD fusion molecule; and (b) determining the concentration of central memory T cells in a sample obtained from the subject after administration of the CD80 ECD or

CD80 ECD fusion molecule. In some embodiments, the methods further comprise determining the concentration of central memory T cells in a sample obtained from the subject prior to administration of the CD80 ECD or CD80 ECD fusion molecule. In some embodiments, the methods further comprise increasing the dose or frequency of the CD80 ECD or CD80 ECD fusion molecule administered to the subject if the concentration of central memory T cells in the sample obtained after administration is not larger than the concentration of central memory T cells in the sample obtained prior to administration. In some embodiments, the sample for determining the concentration of central memory T cells is a blood or plasma sample. In some embodiments, the central memory T cells are CD95+ and CD28+ cells.

[0031] The present disclosure also encompasses methods of increasing the number of central memory T cells in a subject in need thereof, comprising administering a CD80 extracellular domain (ECD) or CD80 ECD fusion molecule, wherein the CD80 ECD or CD80 ECD fusion molecule is administered in an amount effective to increase the number of central memory T cells in the subject. In some embodiments, the number of central memory T cells is increased for at least one week, at least two weeks, or at least one month. In some embodiments, the number of central memory T cells is determined in a blood or plasma sample of the subject. In some embodiments, the central memory T cells are CD95+ and CD28+ cells.

[0032] The present disclosure also encompasses a method of increasing the number of central memory T cells in a subject in need thereof, comprising: (a) administering to the subject a CD80 extracellular domain (ECD) or CD80 ECD fusion molecule; and (b) determining the concentration of central memory T cells in a sample obtained from the subject after administration of the CD80 ECD or CD80 ECD fusion molecule. In some embodiments, the method further comprises determining the concentration of central memory T cells in a sample obtained from the subject prior to the CD80 ECD or CD80 ECD fusion molecule administration. In some embodiments, the method further comprises increasing the dose or frequency of the CD80 ECD or CD80 ECD fusion molecule administered to the subject if the concentration of central memory T cells in the sample obtained after administration is not larger than the concentration of central memory T cells in the sample obtained prior to administration. In some embodiments, the sample for determining the concentration of central memory T cells is a blood or plasma sample. In some embodiments, the central memory T cells are CD95+ and CD28+ cells.

- [0033] The present disclosure also encompasses methods of detecting central memory T cells in a subject, for example a cancer subject, the methods comprising, for example, determining the concentration of central memory T cells in a sample obtained from the subject after administration of a CD80 ECD or CD80 ECD fusion molecule to the subject.
- [0034] In some of the above methods, a CD80 ECD is administered. In other methods, a CD80 ECD fusion molecule is administered. In either case, in some embodiments CD80 ECD or the CD80 ECD portion of the fusion molecule comprises an amino acid sequence selected from (a) amino acids 35 to end of SEQ ID NO:1, (b) SEQ ID NO:3, (c) SEQ ID NO:4, and (d) SEQ ID NO:5.
- [0035] In some embodiments where a CD80 ECD fusion molecule is administered, the CD80 ECD fusion molecule comprises a fusion partner comprising an Fc domain. In some embodiments the Fc domain is a human IgG1 Fc domain. In some embodiments, the Fc domain comprises the amino acid sequence of SEQ ID NO:14. In some embodiments, the CD80 ECD fusion molecule comprises the sequence of SEQ ID NO:20 or SEQ ID NO: 21.
- [0036] In some embodiments of the above methods, the CD80 ECD comprises 10-60 mol sialic acid (SA) to mol of protein, 15-40 mol SA to mol of protein, 15-25 mol SA to mol of protein, or 15-30 mol SA to mol of protein. In some embodiments, the CD80 ECD fusion molecule comprises 10-60 mol sialic acid (SA) to mol of protein, 15-40 mol SA to mol of protein, 15-25 mol SA to mol of protein, or 15-30 mol SA to mol of protein.
- [0037] In the methods herein, in some embodiments, the CD80 ECD or CD80 ECD fusion molecule is administered in combination with a programmed cell death 1 (PD-1) / programmed cell death ligand 1 (PD-L1) inhibitor. In some embodiments, the PD-1/PD-L1 inhibitor is an antibody. In some embodiments, the PD-1/PD-L1 inhibitor is an anti-PD-1 antibody. In some embodiments, the anti-PD-1 antibody comprises the heavy chain and light chain CDRs of nivolumab, pidilizumab, or pembrolizumab. In some embodiments, the anti-PD-1 antibody comprises the heavy chain and light chain variable regions of nivolumab, pidilizumab, or pembrolizumab. In some embodiments, the anti-PD-1 antibody is nivolumab, pidilizumab, or pembrolizumab. In some embodiments, the PD-1/PD-L1 inhibitor is an anti-PD-L1 antibody. In some embodiments, the anti-PD-L1 antibody comprises the heavy chain and light chain CDRs of avelumab, durvalumab, atezolizumab, or BMS-936559. In some embodiments, the anti-PD-L1 antibody comprises the heavy chain and light chain variable regions of avelumab, durvalumab,

atezolizumab, or BMS-936559. In some embodiments, the anti-PD-L1 antibody is avelumab, durvalumab, atezolizumab, or BMS-936559. In some embodiments, the PD-1/PD-L1 inhibitor is a PD-1 fusion molecule. In some embodiments, the fusion molecule is AMP-224. In some embodiments, the PD-1/PD-L1 inhibitor is AUR-012.

[0038] In some embodiments of the methods herein, the subject has cancer and the subject's cancer is a solid tumor. In some embodiments, the cancer is selected from colorectal cancer, breast cancer, gastric cancer, non-small cell lung cancer, melanoma, squamous cell carcinoma of the head and neck, ovarian cancer, pancreatic cancer, renal cell carcinoma, hepatocellular carcinoma, bladder cancer, and endometrial cancer. In some embodiments, the cancer is recurrent or progressive after a therapy selected from surgery, chemotherapy, radiation therapy, or a combination thereof.

[0039] In some embodiments of the above methods, the CD80 ECD or CD80 ECD fusion molecule is administered in combination with a cancer vaccine. In some embodiments, the cancer vaccine is a personalized cancer vaccine. In some embodiments, the CD80 ECD or CD80 ECD fusion molecule and the cancer vaccine are administered concurrently or sequentially.

[0040] The present disclosure also encompasses cancer vaccine compositions comprising at least one tumor-specific antigen or tumor-associated antigen and a CD80 extracellular domain (ECD) or CD80-ECD fusion molecule. In some embodiments, the vaccine compositions further comprise autologous immune cells from a subject to be treated with the vaccine. In some such embodiments, the autologous immune cells comprise antigen-presenting cells.

[0041] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the claims. The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All references cited herein, including patent applications and publications, are incorporated herein by reference in their entireties for any purpose.

BRIEF DESCRIPTION OF THE DRAWINGS

[0042] **Fig. 1** shows that there is an increase in the frequency of central memory CD4⁺ T cells (CD4⁺ T_{cm}) in cynomolgus monkeys after treatment with human CD80 ECD-Fc

(hCD80 ECD-Fc) at 10 and 50 mg/kg doses but not after treatment with a 1 mg/kg dose or with a vehicle control. Data is presented as the percentage (%) of pre-dose frequency of central memory CD4⁺ T cells. Dotted vertical lines represent the timing when hCD80 ECD-Fc was administered.

[0043] **Fig. 2** shows changes in the frequency of central memory CD8⁺ T cells (CD8⁺ Tcm) in cynomolgus monkeys after treatment with 1, 10, or 50 mg/kg hCD80 ECD-Fc or vehicle control. Data is presented as the percentage (%) of pre-dose frequency of central memory CD8⁺ T cells. Dotted vertical lines represent the timing when hCD80 ECD-Fc was administered.

[0044] **Fig. 3** shows changes in proliferating (Ki67⁺) central memory CD4⁺ T cells after treatment with 1, 10, or 50 mg/kg hCD80 ECD-Fc or vehicle control. Data is presented as the percentage (%) of average pre-dose frequency of central memory CD4⁺ T cells. Dotted vertical lines represent the timing when hCD80 ECD-Fc was administered. Maximum frequency of Ki67⁺ CD4⁺ Tcm occurred 7 days after first dose.

[0045] **Fig 4** shows changes in proliferating (Ki67⁺) central memory CD8⁺ T cells after treatment with 1, 10, or 50 mg/kg hCD80 ECD-Fc or vehicle control. Data is presented as the percentage (%) of average pre-dose frequency of central memory CD8⁺ T cells and expressed as mean value +/- standard deviation. Dotted vertical lines represent the timing when hCD80 ECD-Fc was administered. Maximum frequency of Ki67⁺ CD8⁺ Tcm occurred 7 days after second dose.

DESCRIPTION OF PARTICULAR EMBODIMENTS

Definitions

[0046] Unless otherwise defined, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0047] In this application, the use of “or” means “and/or” unless stated otherwise. In the context of a multiple dependent claim, the use of “or” refers back to more than one preceding independent or dependent claim in the alternative only. Also, terms such as “element” or “component” encompass both elements and components comprising one

unit and elements and components that comprise more than one subunit unless specifically stated otherwise.

[0048] As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[0049] The terms “**polypeptide**” and “**protein**” are used interchangeably to refer to a polymer of amino acid residues, and are not limited to a minimum length. Such polymers of amino acid residues may contain natural or non-natural amino acid residues, and include, but are not limited to, peptides, oligopeptides, dimers, trimers, and multimers of amino acid residues. Both full-length proteins and fragments thereof are encompassed by the definition. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. Furthermore, for purposes of the present invention, a “polypeptide” refers to a protein that includes modifications, such as deletions, additions, and substitutions (generally conservative in nature), to the native sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts that produce the proteins or errors due to PCR amplification.

[0050] A “**fusion molecule**” as used herein refers to a molecule composed of two or more different molecules that do not occur together in nature being covalently or noncovalently joined to form a new molecule. For example, fusion molecules may be comprised of a polypeptide and a polymer such as PEG, or of two different polypeptides. A “**fusion protein**” refers to a fusion molecule composed of two or more polypeptides that do not occur in a single molecule in nature.

[0051] A “**CD80 extracellular domain**” or “**CD80 ECD**” refers to an extracellular domain polypeptide of human CD80, including natural and engineered variants thereof. A “**CD80 ECD fusion molecule**” refers to a molecule comprising a CD80 ECD and a fusion partner such as an Fc domain, albumin, or PEG. The fusion partner may be covalently attached, for example, to the N- or C- terminal of the CD80 ECD or at an internal location.

[0052] As used herein, “**central memory T cells**” or “**Tcm**” refers to T cells, including CD4⁺ or CD8⁺ T cells, that are identified as CD95⁺ and CD28⁺, CD95⁺ and CD27⁺ or as CD95⁺, CD28⁺, and CD27⁺.

- [0053] The terms “**programmed cell death protein 1**” and abbreviations “**PD-1**” and “**PD1**” refer to the full-length, mature human PD-1 protein, which is an immunoinhibitory receptor belonging to the CD28 family.
- [0054] The terms “**programmed cell death 1 ligand 1**” and “**PD-L1**” (PD-L1; B7 homolog-1; B7-H1; or CD274) and “**Programmed Death Ligand-2**” (PD-L2; B7-DC; or CD273) are two cell surface glycoprotein ligands for PD-1 that downregulate T-cell activation and cytokine secretion upon binding to PD-1. The term “PD-L1” as used herein refers to full-length, mature, human PD-L1 unless specifically noted otherwise.
- [0055] The term “**immune stimulating agent**” as used herein refers to a molecule that stimulates the immune system by either acting as an agonist of an immune-stimulatory molecule, including a co-stimulatory molecule, or acting as an antagonist of an immune inhibitory molecule, including a co-inhibitory molecule. An immune stimulating agent may be a biologic, such as an antibody or antibody fragment, other protein, or vaccine, or may be a small molecule drug. An “**immune stimulatory molecule**” includes a receptor or ligand that acts to enhance, stimulate, induce, or otherwise “turn-on” an immune response. Immune stimulatory molecules as defined herein include co-stimulatory molecules. An “**immune inhibitory molecule**” includes a receptor or ligand that acts to reduce, inhibit, suppress, or otherwise “turn-off” an immune response. Immune inhibitory molecules as defined herein include co-inhibitory molecules. Such immune stimulatory and immune inhibitory molecules may be, for example, receptors or ligands found on immune cells such as T cells, or found on cells involved in innate immunity such as NK cells.
- [0056] The term “**PD-1/PD-L1 inhibitor**” refers to a moiety that disrupts the PD-1/PD-L1 signaling pathway. In some embodiments, the inhibitor inhibits the PD-1/PD-L1 signaling pathway by binding to PD-1 and/or PD-L1. In some embodiments, the inhibitor also binds to PD-L2. In some embodiments, a PD-1/PD-L1 inhibitor blocks binding of PD-1 to PD-L1 and/or PD-L2. Nonlimiting exemplary PD-1/PD-L1 inhibitors include antibodies that bind to PD-1; antibodies that bind to PD-L1; fusion proteins, such as AMP-224; and polypeptides, such as AUR-012.
- [0057] The term “**antibody that inhibits PD-1**” refers to an antibody that binds to PD-1 or binds to PD-L1 and thereby inhibits PD-1 and/or PD-L1 signaling. In some embodiments, an antibody that inhibits PD-1 binds to PD-1 and blocks binding of PD-L1 and/or PD-L2 to PD-1. In some embodiments, an antibody that inhibits PD-1 binds to

PD-L1 and blocks binding of PD-1 to PD-L1. An antibody that inhibits PD-1 that binds to PD-L1 may be referred to as an anti-PD-L1 antibody. An antibody that inhibits PD-1 that binds to PD-1 may be referred to as an anti-PD-1 antibody.

- [0058] With reference to CD80 ECDs and CD80 ECD fusion molecules, the term “**blocks binding of**” a ligand, and grammatical variants thereof, refers to the ability to inhibit an interaction between CD80 and a CD80 ligand, such as CD28, CTLA4, or PD-L1. Such inhibition may occur through any mechanism, including by the CD80 ECDs or CD80 ECD fusion molecules competing for binding with CD80 ligands.
- [0059] With reference to anti-PD-1 antibodies and PD-1 fusion molecules or peptides the term “**blocks binding of**” a ligand, such as PD-L1, and grammatical variants thereof, are used to refer to the ability to inhibit the interaction between PD-1 and a PD-1 ligand, such as PD-L1. Such inhibition may occur through any mechanism, including direct interference with ligand binding, e.g., because of overlapping binding sites on PD-1, and/or conformational changes in PD-1 induced by the antibody that alter ligand affinity, etc., or, in the case of a PD-1 fusion molecule or peptide, by competing for binding with a PD-1 ligand.
- [0060] “**Affinity**” or “**binding affinity**” refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (*e.g.*, a polypeptide) and its binding partner (*e.g.*, a ligand). In some embodiments, “binding affinity” refers to intrinsic binding affinity, which reflects a 1:1 interaction between members of a binding pair (*e.g.*, polypeptide and ligand). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_d).
- [0061] The term “**antibody**” as used herein refers to a molecule comprising at least complementarity-determining region (CDR) 1, CDR2, and CDR3 of a heavy chain and at least CDR1, CDR2, and CDR3 of a light chain, wherein the molecule is capable of binding to antigen. The term antibody includes, but is not limited to, fragments that are capable of binding antigen, such as Fv, single-chain Fv (scFv), Fab, Fab', and (Fab')₂. The term antibody also includes, but is not limited to, chimeric antibodies, humanized antibodies, and antibodies of various species such as mouse, human, cynomolgus monkey, etc.
- [0062] In some embodiments, an antibody comprises a heavy chain variable region and a light chain variable region. In some embodiments, an antibody comprises at least one heavy chain comprising a heavy chain variable region and at least a portion of a heavy

chain constant region, and at least one light chain comprising a light chain variable region and at least a portion of a light chain constant region. In some embodiments, an antibody comprises two heavy chains, wherein each heavy chain comprises a heavy chain variable region and at least a portion of a heavy chain constant region, and two light chains, wherein each light chain comprises a light chain variable region and at least a portion of a light chain constant region. As used herein, a single-chain Fv (scFv), or any other antibody that comprises, for example, a single polypeptide chain comprising all six CDRs (three heavy chain CDRs and three light chain CDRs) is considered to have a heavy chain and a light chain. In some such embodiments, the heavy chain is the region of the antibody that comprises the three heavy chain CDRs and the light chain in the region of the antibody that comprises the three light chain CDRs.

[0063] The term “**heavy chain variable region**” refers to a region comprising heavy chain HVR1, framework (FR) 2, HVR2, FR3, and HVR3. In some embodiments, a heavy chain variable region also comprises at least a portion of an FR1 and/or at least a portion of an FR4.

[0064] The term “**heavy chain constant region**” refers to a region comprising at least three heavy chain constant domains, C_H1 , C_H2 , and C_H3 . Nonlimiting exemplary heavy chain constant regions include γ , δ , and α . Nonlimiting exemplary heavy chain constant regions also include ϵ and μ . Each heavy constant region corresponds to an antibody isotype. For example, an antibody comprising a γ constant region is an IgG antibody, an antibody comprising a δ constant region is an IgD antibody, and an antibody comprising an α constant region is an IgA antibody. Further, an antibody comprising a μ constant region is an IgM antibody, and an antibody comprising an ϵ constant region is an IgE antibody. Certain isotypes can be further subdivided into subclasses. For example, IgG antibodies include, but are not limited to, IgG1 (comprising a γ_1 constant region), IgG2 (comprising a γ_2 constant region), IgG3 (comprising a γ_3 constant region), and IgG4 (comprising a γ_4 constant region) antibodies; IgA antibodies include, but are not limited to, IgA1 (comprising an α_1 constant region) and IgA2 (comprising an α_2 constant region) antibodies; and IgM antibodies include, but are not limited to, IgM1 and IgM2.

[0065] The term “**heavy chain**” refers to a polypeptide comprising at least a heavy chain variable region, with or without a leader sequence. In some embodiments, a heavy chain comprises at least a portion of a heavy chain constant region. The term “full-length heavy

chain” refers to a polypeptide comprising a heavy chain variable region and a heavy chain constant region, with or without a leader sequence.

- [0066] The term “**light chain variable region**” refers to a region comprising light chain HVR1, framework (FR) 2, HVR2, FR3, and HVR3. In some embodiments, a light chain variable region also comprises an FR1 and/or an FR4.
- [0067] The term “**light chain constant region**” refers to a region comprising a light chain constant domain, C_L. Nonlimiting exemplary light chain constant regions include λ and κ .
- [0068] The term “**light chain**” refers to a polypeptide comprising at least a light chain variable region, with or without a leader sequence. In some embodiments, a light chain comprises at least a portion of a light chain constant region. The term “full-length light chain” refers to a polypeptide comprising a light chain variable region and a light chain constant region, with or without a leader sequence.
- [0069] The term “**hypervariable region**” or “**HVR**” refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops (“hypervariable loops”). Generally, native four-chain antibodies comprise six HVRs; three in the V_H (H1, H2, H3), and three in the V_L (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the “**complementarity determining regions**” (“**CDRs**”), the latter being of highest sequence variability and/or involved in antigen recognition. Exemplary hypervariable loops occur at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3). (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987).) Exemplary CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) occur at amino acid residues 24-34 of L1, 50-56 of L2, 89-97 of L3, 31-35B of H1, 50-65 of H2, and 95-102 of H3. (Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991)). The terms hypervariable regions (HVRs) and complementarity determining regions (CDRs) both refer to portions of the variable region that form the antigen binding regions.
- [0070] A “**chimeric antibody**” as used herein refers to an antibody comprising at least one variable region from a first species (such as mouse, rat, cynomolgus monkey, etc.) and at least one constant region from a second species (such as human, cynomolgus monkey, etc.). In some embodiments, a chimeric antibody comprises at least one mouse variable region and at least one human constant region. In some embodiments, a chimeric

antibody comprises at least one cynomolgus variable region and at least one human constant region. In some embodiments, a chimeric antibody comprises at least one rat variable region and at least one mouse constant region. In some embodiments, all of the variable regions of a chimeric antibody are from a first species and all of the constant regions of the chimeric antibody are from a second species.

[0071] A “**humanized antibody**” as used herein refers to an antibody in which at least one amino acid in a framework region of a non-human variable region has been replaced with the corresponding amino acid from a human variable region. In some embodiments, a humanized antibody comprises at least one human constant region or fragment thereof. In some embodiments, a humanized antibody is a Fab, an scFv, a (Fab')₂, etc.

[0072] A “**human antibody**” as used herein refers to antibodies produced in humans, antibodies produced in non-human animals that comprise human immunoglobulin genes, such as XenoMouse®, and antibodies selected using in vitro methods, such as phage display, wherein the antibody repertoire is based on a human immunoglobulin sequences.

[0073] The term “**leader sequence**” refers to a sequence of amino acid residues located at the N terminus of a polypeptide that facilitates secretion of a polypeptide from a mammalian cell. A leader sequence may be cleaved upon export of the polypeptide from the mammalian cell, forming a mature protein. Leader sequences may be natural or synthetic, and they may be heterologous or homologous to the protein to which they are attached. Nonlimiting exemplary leader sequences also include leader sequences from heterologous proteins. In some embodiments, an antibody lacks a leader sequence. In some embodiments, an antibody comprises at least one leader sequence, which may be selected from native antibody leader sequences and heterologous leader sequences.

[0074] The term “**isolated**” as used herein refers to a molecule that has been separated from at least some of the components with which it is typically found in nature. For example, a polypeptide is referred to as “isolated” when it is separated from at least some of the components of the cell in which it was produced. Where a polypeptide is secreted by a cell after expression, physically separating the supernatant containing the polypeptide from the cell that produced it is considered to be “isolating” the polypeptide. Similarly, a polynucleotide is referred to as “isolated” when it is not part of the larger polynucleotide (such as, for example, genomic DNA or mitochondrial DNA, in the case of a DNA polynucleotide) in which it is typically found in nature, or is separated from at least some of the components of the cell in which it was produced, e.g., in the case of an

RNA polynucleotide. Thus, a DNA polynucleotide that is contained in a vector inside a host cell may be referred to as “isolated” so long as that polynucleotide is not found in that vector in nature.

- [0075] The term “**reduce**” or “**reduces**” when applied to a parameter such as tumor volume means to lower the level of that parameter in an observable, measurable way. In some embodiments, the reduction may be statistically significant compared to an alternative treatment or control.
- [0076] The term “**increase**” or “**expand**” when applied to a parameter such as a type of cell, such as a type of T cell, means to increase in concentration (i.e., to expand or proliferate in number within a certain area such as within a tumor sample or within a volume of blood or plasma). In some embodiments, the expansion may be statistically significant compared to an alternative treatment or control.
- [0077] The terms “**subject**” and “**patient**” are used interchangeably herein to refer to a human. In some embodiments, methods of treating other mammals, including, but not limited to, rodents, simians, felines, canines, equines, bovines, porcines, ovines, caprines, mammalian laboratory animals, mammalian farm animals, mammalian sport animals, and mammalian pets, are also provided.
- [0078] The terms “**resistant**” or “**nonresponsive**” when used in the context of treatment with a therapeutic agent, means that the subject shows decreased response or lack of response to a standard dose of the therapeutic agent, relative to the subject’s response to the standard dose of the therapeutic agent in the past, or relative to the expected response of a similar subject with a similar disorder to the standard dose of the therapeutic agent. Thus, in some embodiments, a subject may be resistant to therapeutic agent although the subject has not previously been given the therapeutic agent, or the subject may develop resistance to the therapeutic agent after having responded to the agent on one or more previous occasions.
- [0079] The term “**sample**,” as used herein, refers to a composition that is obtained or derived from a subject that contains a cellular and/or other molecular entity that is to be characterized, quantitated, and/or identified, for example based on physical, biochemical, chemical and/or physiological characteristics. An exemplary sample is a tissue sample.
- [0080] The term “**tissue sample**” refers to a collection of similar cells obtained from a tissue of a subject. The source of the tissue sample may be solid tissue as from a fresh, frozen and/or preserved organ or tissue sample or biopsy or aspirate; blood or any blood

constituents; bodily fluids such as cerebral spinal fluid, amniotic fluid, peritoneal fluid, synovial fluid, or interstitial fluid; cells from any time in gestation or development of the subject. In some embodiments, a tissue sample is a synovial biopsy tissue sample and/or a synovial fluid sample. In some embodiments, a tissue sample is a synovial fluid sample. The tissue sample may also be primary or cultured cells or cell lines. Optionally, the tissue sample is obtained from a disease tissue/organ. The tissue sample may contain compounds that are not naturally intermixed with the tissue in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, antibiotics, or the like. A “control sample” or “control tissue”, as used herein, refers to a sample, cell, or tissue obtained from a source known, or believed, not to be afflicted with the disease for which the subject is being treated.

[0081] For the purposes herein a “**section**” of a tissue sample means a part or piece of a tissue sample, such as a thin slice of tissue or cells cut from a solid tissue sample.

[0082] The term “**cancer**” is used herein to refer to a group of cells that exhibit abnormally high levels of proliferation and growth. A cancer may be benign (also referred to as a benign tumor), pre-malignant, or malignant. Cancer cells may be solid cancer cells (i.e. “**solid tumors**”) or may be hematologic (e.g. lymphomic or leukemic) cancer cells. The term “**cancer growth**” is used herein to refer to proliferation or growth by a cell or cells that comprise a cancer that leads to a corresponding increase in the size or extent of the cancer.

[0083] Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular nonlimiting examples of such cancers include squamous cell cancer, small-cell lung cancer, pituitary cancer, esophageal cancer, astrocytoma, soft tissue sarcoma, non-small cell lung cancer (including squamous cell non-small cell lung cancer), adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, renal cell carcinoma, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, brain cancer, endometrial cancer, testis cancer, cholangiocarcinoma, gallbladder carcinoma, gastric cancer, melanoma, and various types of head and neck cancer (including squamous cell carcinoma of the head and neck).

[0084] “**Treatment**,” as used herein, refers to therapeutic treatment, for example, wherein the object is to reduce in severity or slow progression of the targeted pathologic condition or disorder as well as, for example, wherein the object is to inhibit recurrence of the condition or disorder. In certain embodiments, the term “**treatment**” covers any administration or application of a therapeutic for disease in a patient, and includes inhibiting or slowing the disease or progression of the disease; partially or fully relieving the disease, for example, by causing regression, or restoring or repairing a lost, missing, or defective function; stimulating an inefficient process; or causing the disease plateau to have reduced severity. The term “**treatment**” also includes reducing the severity of any phenotypic characteristic and/or reducing the incidence, degree, or likelihood of that characteristic. Those in need of treatment include those already with the disorder as well as those at risk of recurrence of the disorder or those in whom a recurrence of the disorder is to be prevented or slowed down.

[0085] The term “**efficacy**” as used herein may be determined from one or more parameters such as survival or disease-free survival over a period of time such as 1 year, 5 years, or 10 years, as well as parameters such as the reduction in growth of one or more tumors in a subject. Pharmacokinetic parameters such as bioavailability and underlying parameters such as clearance rate may also impact efficacy. Thus, an “enhanced efficacy” (i.e. an improvement in efficacy) may be due to improved pharmacokinetic parameters as well as improved potency, and may be measured by comparing clearance rates and tumor growth in test animals or in human subjects, as well as parameters such as survival, rate of recurrence, or disease-free survival.

[0086] The term “**effective amount**” or “**therapeutically effective amount**” refers to an amount of a drug effective to treat a disease or disorder in a subject. In certain embodiments, an effective amount refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. A therapeutically effective amount of a CD80 ECD or CD80 ECD fusion molecule may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the drug to elicit a desired response in the individual. A therapeutically effective amount encompasses an amount in which any toxic or detrimental effects of the drug are outweighed by the therapeutically beneficial effects. In some embodiments, the expression “effective amount” refers to an amount of the drug that is effective for treating the cancer.

- [0087] Administration “**in combination with**” one or more further therapeutic agents, such as an immune stimulating agent or cancer vaccine, includes simultaneous (concurrent) and consecutive (sequential) administration in any order.
- [0088] A “**pharmaceutically acceptable carrier**” refers to a non-toxic solid, semisolid, or liquid filler, diluent, encapsulating material, formulation auxiliary, or carrier conventional in the art for use with a therapeutic agent that together comprise a “**pharmaceutical composition**” for administration to a subject. A pharmaceutically acceptable carrier is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. The pharmaceutically acceptable carrier is appropriate for the formulation employed. For example, if the therapeutic agent is to be administered orally, the carrier may be a gel capsule. If the therapeutic agent is to be administered subcutaneously, the carrier ideally is not irritable to the skin and does not cause injection site reaction.
- [0089] The term “**antigen**” is used to denote a chemical compound such as a polypeptide that is specifically recognized by an antibody. As used herein, a “**tumor-specific antigen**” refers to an antigen only expressed in significant amounts by tumor cells, such as a mutant polypeptide antigen corresponding to a protein that is mutated in tumor cells. As used herein, a “**tumor-associated antigen**” refers to an antigen corresponding to a polypeptide that is overexpressed by tumor cells and that may also be expressed by normal cells of the same or different tissue.
- [0090] The term “**cancer vaccine**” as used herein refers to a treatment composition that is administered to promote a specific immune response against tumor antigens. In some embodiments, the cancer vaccine may comprise tumor-specific and/or tumor-associated antigens or cells presenting tumor-specific and/or tumor associated antigens in order to promote an immune response against those antigens. Such a vaccine composition may also comprise other agents that promote an immune response such as immune stimulating agents.
- [0091] The term “**personalized cancer vaccine**” or “**personalized vaccine**” refers to a cancer vaccine that comprises cells or antigens taken from the patient to be treated and optionally expanded or amplified *ex vivo* prior to reintroduction to the patient. For example, a personalized cancer vaccine may include tumor-specific antigens from the patient or may include immune cells or other hematopoietic cells that have been taken from the patient and allowed to proliferate *ex vivo*.

Exemplary CD80 Extracellular Domain and Extracellular Domain Fusion Molecules

- [0092]** Methods of cancer treatment with CD80 ECD or CD80 ECD fusion molecules are provided herein, as are cancer vaccines comprising CD80 ECD or CD80 ECD fusion molecules. CD80 ECDs, for example, may comprise the ECDs of human CD80 isoform 1, isoform 2, and isoform 3 (see SEQ ID NOs: 1-3). In some embodiments, CD80 ECDs and may comprise the amino acid sequence of SEQ ID NO:5.
- [0093]** CD80 ECD fusion molecules may comprise fusion partners such as polymers, polypeptides, lipophilic moieties, and succinyl groups. Exemplary polypeptide fusion partners include, but are not limited to, serum albumin and an IgG Fc domain. Further exemplary polymer fusion partners include, but are not limited to, polyethylene glycol, including polyethylene glycols having branched and/or linear chains. The amino acid sequences of certain exemplary Fc domains are shown in SEQ ID NOs: 9-16 herein.
- [0094]** In certain embodiments, the CD80 ECD or CD80 ECD fusion molecule lacks a signal peptide. In certain embodiments, the CD80 ECD or CD80 ECD fusion molecule includes at least one signal peptide, which may be selected from a native CD80 signal peptide (SEQ ID NO: 7 or amino acids 1-34 of SEQ ID NO:1) and/or a heterologous signal peptide.
- [0095]** In the case of a CD80 ECD fusion molecule, the fusion partner may be linked to either the amino-terminus or the carboxy-terminus of the polypeptide. In certain embodiments, the polypeptide and the fusion partner are covalently linked. If the fusion partner is also a polypeptide (“the fusion partner polypeptide”), the polypeptide and the fusion partner polypeptide may be part of a continuous amino acid sequence. In such cases, the polypeptide and the fusion partner polypeptide may be translated as a single polypeptide from a coding sequence that encodes both the polypeptide and the fusion partner polypeptide. In some such cases, the two polypeptides are directly linked in sequence such that the N-terminal of one polypeptide immediately follows the C-terminal of the other with no intervening amino acids. In other cases, a linker peptide sequence is inserted in between the two polypeptides, such as a GS linker sequence. In certain embodiments, a CD80 ECD and the fusion partner are covalently linked through other means, such as, for example, a chemical linkage other than a peptide bond. In certain embodiments, the polypeptide and the fusion partner are noncovalently linked. In certain such embodiments, they may be linked, for example, using binding pairs. Exemplary

binding pairs include, but are not limited to, biotin and avidin or streptavidin, an antibody and its antigen, etc.

[0096] In some embodiments, the CD80 ECD fusion molecule comprises the sequence of SEQ ID NO: 20 or 21.

[0097] CD80 ECD fusion molecules may, depending on how they are produced, have different levels of particular glycosylation modifications. For example, a CD80 ECD fusion molecule may have different concentrations of sialic acid residues in relation to the concentration of the CD80 ECD protein. In some embodiments, a higher sialic acid content may have a longer clearance time in the body and thus an increased overall bioavailability.

[0098] In order to produce CD80 ECD fusion molecules with various levels of sialylation, CD80 ECD fusion molecules prepared from cell culture may be fractionated using anion exchange chromatography, for example. In some cases, CD80 ECD fusion molecules may be subjected to one or more initial purification processes prior to fractionation. Pooled fractions may be further analyzed using a 4,5-Methylenedioxy-1,2-phenylenediamine dihydrochloride (DMB)-high performance liquid chromatography (HPLC) based sialic acid assay in order to determine sialic acid content.

[0099] In some embodiments, the sialic acid content of the CD80 ECD fusion molecule is from 10 to 60 mol sialic acid (SA) to mol protein. In some embodiments, the sialic acid content of the CD80 ECD fusion molecule is from 15 to 60 mol SA to mol protein. For example, in some embodiments, the SA content is 10-40 mol SA/mol protein, such as 15-30 mol SA/mol protein, such as 15-25 mol SA/mol protein, such as 20-40 mol SA/mol protein, such as 20-30 mol SA/mol protein, such as 30-40 mol SA/mol protein, such as 10, 15, 20, 25, 30, 35, or 40 mol SA/mol protein. In some embodiments, the SA content is at least 15 mol SA/mol protein, such as at least 20 mol SA/mol protein, at least 25 mol SA/mol protein, at least 30 mol SA/mol protein, at least 35 mol SA/mol protein, or at least 40 mol SA/mol protein. In some such embodiments, the fusion partner is an Fc domain, such as a human IgG1, IgG2, or IgG4 Fc domain.

[0100] In some embodiments, the SA content of the CD80 ECD fusion molecule is increased or is maintained at a relatively high level in comparison to current CD80 ECD fusion molecules. In some embodiments, an increase in SA content, such as by 5, 10, 15, 20, 30, 40 or 50 mol SA to mol of CD80 ECD protein, may lead to an enhanced efficacy in at least one mouse syngeneic or xenograft tumor model. For example, in some

embodiments, tumor growth in a mouse tumor model may be further reduced by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 98% when there is an increase in SA content, such as by 5, 10, 15, 20, 30, 40 or 50 mol SA to mol of CD80 ECD protein.

[0101] For example, in some embodiments, a CD80 ECD Fc fusion molecule, such as a fusion molecule comprising a human IgG1 Fc domain comprising between 10 and 60 mol SA/mol protein is capable of at least 80%, such as at least 90%, such as at least 95%, such as at least 98% tumor cell growth inhibition in at least one mouse syngeneic or xenograft cancer model over a period of at least ten days or at least two weeks or at least three weeks, such as ten days to two weeks or two to three weeks following inoculation with tumor cells. In some such embodiments, the molecule comprises at least 15 mol SA/mol protein, such as at least 20 mol SA/mol protein, or a range from 15-30, 15-25, or 20-30 mol SA/mol protein. In some embodiments, the mouse model is a CT26, MC38, or B16 mouse tumor model. In some embodiments, the mice are given one to three doses of the molecule at 0.3 to 3.0 mg/kg, such as at 0.3 to 0.6 mg/kg, for example over a period of one week, once tumors have reached a minimum volume. In some embodiments, the Fc domain comprises the amino acid sequence of SEQ ID NO:14. In some embodiments, the CD80 ECD fusion molecule comprises the sequence of SEQ ID NO: 20 or 21.

[0102] In some embodiments, the CD80 ECD Fc fusion molecule reduces growth of CT26 tumor cells in mice over a period of at least ten days or at least two weeks or at least three weeks, such as ten days to two weeks or two to three weeks, after inoculation by a greater degree than a CD80 ECD Fc fusion protein with the identical amino acid sequence but a lower level of SA per mol of protein. In some embodiments, the CD80 ECD Fc fusion molecule reduces growth of CT26 tumors in mice over a period of at least ten days or at least two weeks, such as over ten days to two weeks or two to three weeks, after inoculation by a greater degree than an anti-CTLA4 antibody, such as anti-CTLA4 antibody clone 9D9. In some such embodiments, the CD80 ECD Fc molecule is dosed one to three times at 0.3 mg/kg, 0.6 mg/kg, or 3.0 mg/kg while the anti-CTLA4 antibody is dosed the same number of times at 1.5 or 10 mg/kg. In some such embodiments, the model is a CT26, MC38, or B16 murine tumor model.

Exemplary Fc Domain Fusion Partners

- [0103]** In some embodiments, the CD80 ECD fusion molecule has an Fc domain as fusion partner. In some embodiments, the Fc domain is derived from human IgG1, IgG2, IgG3, or IgG4. In some embodiments, the Fc domain has a wild-type sequence, such as a wild-type human IgG1 or IgG2 (e.g. IgG2a) sequence. In other embodiments, the Fc domain is either a natural or engineered variant. In some embodiments, an Fc domain is chosen that has altered interactions of the Fc with one or more Fc gamma receptors. In some embodiments, an Fc domain is chosen that has altered interactions of the Fc with one or more complement factors. In some embodiments, an Fc domain is chosen that has altered interactions of the Fc with one or more Fc gamma receptors and that has altered interactions with one or more complement factors.
- [0104]** In some embodiments, the Fc domain comprises at least one point mutation as described in WO 2014/144960. In some embodiments, the Fc domain is a human Fc domain with a substitution at one or more of positions E233, L234, L235, P238, D265, N297, A327, P329, or P331 (wherein the numbering of these positions is according to the EU index as in Kabat). In some embodiments, the Fc domain is a human Fc domain with a mutation at L234, L235, and/or P331. In some embodiments, the Fc domain is a human Fc domain with the substitutions L234F, L235E, and P331S. (See, e.g., SEQ ID NO:12.) In some embodiments, the Fc domain has an amino acid substitution at position N297. (See, e.g., SEQ ID NO: 13.) In some embodiments, the Fc domain comprises a C237S mutation. (See, e.g., SEQ ID NO: 9.)
- [0105]** In some embodiments, a mutated Fc fusion partner causes the CD80 ECD Fc fusion molecule to have altered interactions with one or more Fc gamma receptors compared to those of a CD80 ECD fusion molecule with the same amino acid sequence except for the Fc domain mutations. In some embodiment, the Fc has reduced affinity for Fc gamma receptors such as one or more of FcRN, RI, RIIA, RIIB, and RIII compared to a wild-type Fc domain. In some embodiments, the Fc has reduced affinity for all of FcRN, RI, RIIA, RIIB, and RIII compared to a wild-type Fc domain.
- [0106]** In some embodiments, a mutated Fc fusion partner causes the CD80 ECD Fc fusion molecule to have altered interactions with at one or more complement factors such as C1, C2, C3, C4, and their cleavage products, such as C4a, C4b, C2a, C2b, C3a, and C3b. In some embodiments, a mutated Fc fusion partner causes the CD80 ECD Fc fusion

molecule to have altered interactions with one or more complement factors compared to those of a CD80 ECD fusion molecule with the same amino acid sequence except for the Fc domain mutations.

[0107] In some embodiments the CD80 ECD and the fusion partner, such as an Fc fusion partner, are directly linked such that the N- or C-terminal amino acid of the Fc immediately precedes or follows the N- or C-terminal amino acid of the CD80 ECD sequence. (See, e.g., SEQ ID NOs: 20 and 21.) In other embodiments, the CD80 ECD and fusion partner are joined by a linker molecule, such as by a linker peptide sequence, such as by a GS linker sequence.

[0108] CD80 ECD and CD80 ECD fusion molecules may also include the CD80 and CD80 ECD fusion molecules described, for example, in United States Patent Appl. No. 15/340,238, filed November 1, 2016, which is incorporated herein by reference in its entirety.

Central Memory T Cells and T Cell Subsets

[0109] Several different types or subsets of T cells may be found in the body. Naïve T cells (T_n) having a specific epitope specificity are produced in the thymus. After naïve T cells encounter the appropriate antigen, they proliferate and differentiate into effector cells, which can travel to sites of inflammation. Following an infection or a vaccination, for example, the vast majority of the effector T cells die by apoptosis, while a small fraction develop into various types of memory T cells, which can remain in bodily tissues, and which can help guard against re-infection with the antigen. Multiple types or subsets of memory T cells may persist in the body. These have been identified in part due to their different combinations of surface protein markers. As noted earlier, “central memory T cells” or “T_{cm}” are T cells, including CD4⁺ or CD8⁺ T cells, which are identified as CD95⁺ and CD28⁺, CD95⁺ and CD27⁺ or as CD95⁺, CD28⁺, and CD27⁺. Such cells encompass at least three further memory T cell subtypes. First, T_{cm} as defined herein encompass a type of memory T cell that may appear early in differentiation from naïve T cells (T_n cells) called a stem central memory T cell (T_{scm}). T_{scm} overexpress CD95 and are also CD45RO[−], CCR7⁺, and CD28⁺. Second, T_{cm} includes cells that are CD45RO⁺, CCR7⁺, CD95⁺, and CD28⁺, which may be referred to elsewhere as T_{cm} or a component of T_{cm}. Third, T_{cm} may include a subset of memory T cells called transitional memory T cells (T_{tm}), which are CD45⁺, CCR7[−], CD95⁺, and CD28⁺. All

of these Tscm, Tcm, and Ttm cells are CD95+/CD28+ and thus, are within the scope of Tcm as used herein. Furthermore, in some embodiments, Tcm may also express CD62L.

[0110] Tscm cells may be precursors of some or all of the other types of memory T cells, including other Tcm, Ttm, as well as effector memory T cells (Tem), and terminal effector memory cells (Tte). Tem are CD45RO+, CCR7-, CD28-, and CD95+ and Tte are CD45RO-, CCR7-, CD28-, and CD95+. Moreover, Tem do not express CD62L.

[0111] Tcm may be found in tissues such as lymph nodes, spleen, and blood, while Tem, in contrast, may be found initially in peripheral nonlymphoid tissues such as lung, liver, and intestine, but can migrate to other tissues such as lymph nodes.

[0112] In some embodiments, the number or concentration of Tcm is determined, for example, either prior to or following administration of CD80 ECD or CD80 ECD fusion molecule to the patient.

Therapeutic Compositions and Methods

Methods of Increasing Central Memory T Cells and Methods of Treating Cancer

[0113] The present disclosure provides, for example, methods of increasing the number of central memory T cells (Tcm) in a patient in need thereof, comprising administering a human CD80 extracellular domain (ECD) or CD80 ECD fusion molecule, wherein the CD80 ECD or CD80 ECD fusion molecule is administered in an amount effective to increase the number of Tcm in the patient. In some embodiments, the number of Tcm in the patient is determined by analyzing the number or concentration of Tcm in a blood or plasma sample from the patient or in a sample of diseased tissue from the patient, such as a tumor sample. In such embodiments, an increase or decrease in the number of Tcm in the patient is based on whether the number or concentration of Tcm in the tested sample is increased or decreased relative to that of a sample taken at a different time-point, such as prior to administration. In some embodiments, the number of central memory T cells in the patient is increased for at least one week after administration, such as for at least two weeks or for at least one month after administration.

[0114] The present disclosure also provides methods of increasing the number of Tcm cells in a patient in need thereof, comprising: administering to the patient a human CD80 extracellular domain (ECD) or CD80 ECD fusion molecule, and determining the concentration of central memory T cells in a sample from the patient after administration.

In some embodiments, the concentration of central memory T cells is also determined in a sample obtained prior to administration. In some embodiments, the concentrations of Tcm in the pre- and post-administration samples are compared to determine if the Tcm concentration has increased after administration. In some embodiments, if the concentration of Tcm has not increased, more CD80 ECD or CD80 ECD fusion molecule is administered. In some embodiments the Tcm concentration is measured in a blood or plasma sample while in other embodiments, it is measured in a sample of diseased tissue, such as a tumor sample. In some embodiments, the concentration of central memory T cells in samples from the patient remains increased compared to the pre-administration level for at least one week after administration, such as for at least two weeks or for at least one month after administration.

[0115] The present disclosure provides, for example, methods of treating cancer in a patient in need thereof, comprising administering a human CD80 extracellular domain (ECD) or CD80 ECD fusion molecule, wherein the CD80 ECD or CD80 ECD fusion molecule is administered in an amount effective to increase the number of central memory T cells (Tcm) in the patient. In some embodiments, the number of Tcm in the patient is determined by analyzing the number or concentration of Tcm in a blood or plasma sample from the patient or in a tumor sample from the patient. In such embodiments, an increase or decrease in the number of Tcm in the patient is based on whether the number or concentration of Tcm in the tested sample is increased or decreased relative to that of a sample taken at a different time-point, such as prior to administration. In some embodiments, the number of central memory T cells in the patient is increased for at least one week after administration, such as for at least two weeks or for at least one month after administration.

[0116] The present disclosure also provides methods of treating cancer in a patient in need thereof, comprising: administering to the subject a human CD80 extracellular domain (ECD) or CD80 ECD fusion molecule, and determining the concentration of central memory T cells in the sample after administration. In some embodiments, the concentration of central memory T cells is also determined in a sample obtained prior to administration. The present disclosure also encompasses methods of detecting central memory T cells in a subject, for example in a cancer subject, the methods comprising, for example, determining the concentration of central memory T cells in a sample obtained from the subject before and/or after administration of a CD80 ECD or CD80 ECD fusion

molecule to the subject. In some embodiments, the concentrations of Tcm in the pre- and post-administration samples are compared to determine if the Tcm concentration has increased after administration. In some embodiments, if the concentration of Tcm has not increased, more CD80 ECD or CD80 ECD fusion molecule is administered. In some embodiments the Tcm concentration is measured in a blood or plasma sample while in other embodiments, it is measured in a tumor sample. In some embodiments, the concentration of central memory T cells in samples from the patient remains increased compared to the pre-administration level for at least one week after administration, such as for at least two weeks or for at least one month after administration.

[0117] In some embodiments, the cancer may be benign (also referred to as a benign tumor), pre-malignant, or malignant. In some embodiments, the cancer may be a non-hematologic or “solid tumor” form of cancer, or alternatively, the cancer may comprise hematologic (e.g. lymphoma or leukemia) cancer cells. In some embodiments, the CD80 ECD or CD80 ECD fusion molecule is effective to reduce cancer growth in a human or animal subject, or in a mouse syngeneic or xenograft model for the cancer being treated. In some embodiments, the CD80 ECD or CD80 ECD fusion molecule is effective to reduce tumor volume, such as in a mouse syngeneic or xenograft model for the cancer being treated. Changes in tumor volume may be measured, for example, by monitoring the size (e.g. diameter) and optionally the shape of a primary tumor in the animal. Tumor growth may be measured, for example, as changes in tumor volume over time.

[0118] Examples of particular cancers that may be treated include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular nonlimiting examples of such cancers include but are not limited to squamous cell cancer, small-cell lung cancer, pituitary cancer, esophageal cancer, astrocytoma, soft tissue sarcoma, non-small cell lung cancer (including squamous cell non-small cell lung cancer), adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, renal cell carcinoma, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, brain cancer, endometrial cancer, testis cancer, cholangiocarcinoma, gallbladder carcinoma, gastric cancer, melanoma, and various types of head and neck cancer (including squamous cell carcinoma of the head and neck).

[0119] In any of the above method embodiments, the CD80 ECD or CD80 ECD fusion molecule administered to the subject may inhibit tumor growth in a mouse syngeneic xenograft cancer model over a period of 1 week, 10 days, 2 weeks, or 3 weeks, for example, by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98%. In some embodiments, the CD80 ECD fusion molecule may inhibit tumor growth in a CT26 mouse xenograft tumor model by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% at two weeks or at three weeks post-inoculation. In some such cases, the fusion molecule may be dosed one to three times at 0.3 to 3 mg/kg, such as at 0.3 to 0.6 mg/kg. In any of the above method embodiments, administration of the CD80 ECD or CD80 ECD fusion molecule administered to the subject may reduce the volume of at least one tumor in a human or animal subject by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98%, for example, over a period of one month, two months, three months, six months, or one year. In some cases, the CD80 ECD Fc fusion molecule may be capable of resulting in complete tumor regression in a mouse tumor model such as a CT26 model, for example in a significant portion of tested mice, such as at least 40%, or at least 50% of mice.

[0120] In any of these methods, the CD80 ECD or CD80 ECD fusion molecule may be a CD80 ECD Fc comprising 10-60 mol SA to mol of CD80 ECD Fc protein, such as 15-60 mol SA/mol protein. In some embodiments, the content is 10-40 mol SA/mol protein, such as 15-40 mol SA/mol protein, such as 20-40 mol SA/mol protein, 20-30 mol SA/mol protein, 15-25 mol SA/mol protein, 15-30 mol SA to mol of protein, or 30-40 mol SA/mol protein. In some embodiments, the SA content is at least 15, such as at least 20, at least 25, at least 30, at least 35, or at least 40 mol SA/mol protein. In some embodiments, the SA content is 15, 20, 25, 30, 35, or 40 mol SA/mol protein. In some embodiments, the Fc domain is a human IgG1, IgG2, or IgG4 Fc domain. In some embodiments, the Fc domain comprises the amino acid sequence of SEQ ID NO:14. In some embodiments, the fusion molecule comprises the amino acid sequence of SEQ ID NO:20 or 21. Such CD80 ECD and CD80 ECD fusion molecules are described, for example, in United States Patent Appl. No. 15/340,238, filed November 1, 2016, which is incorporated herein by reference in its entirety.

Combination Treatments with Immune Stimulating Agents Including PD-1/PD-L1 Inhibitors

- [0121] In some embodiments, the CD80 ECD or CD80 ECD fusion molecule is administered in the methods herein in combination with an effective amount of at least one immune stimulating agent. Immune stimulating agents may include, for example, a small molecule drug or a biologic. Examples of biologic immune stimulating agents include, but are not limited to, antibodies, antibody fragments, fragments of receptor or ligand polypeptides, for example that block receptor-ligand binding, vaccines and cytokines.
- [0122] In some embodiments, the at least one immune stimulating agent comprises an agonist of an immune stimulatory molecule, including a co-stimulatory molecule, while in some embodiments, the at least one immune stimulating agent comprises an antagonist of an immune inhibitory molecule, including a co-inhibitory molecule. In some embodiments, the at least one immune stimulating agent comprises an agonist of an immune-stimulatory molecule, including a co-stimulatory molecule, found on immune cells, such as T cells. In some embodiments, the at least one immune stimulating agent comprises an antagonist of an immune inhibitory molecule, including a co-inhibitory molecule, found on immune cells, such as T cells. In some embodiments, the at least one immune stimulating agent comprises an agonist of an immune stimulatory molecule, including a co-stimulatory molecule, found on cells involved in innate immunity, such as NK cells. In some embodiments, the at least one immune stimulating agent comprises an antagonist of an immune inhibitory molecule, including a co-inhibitory molecule, found on cells involved in innate immunity, such as NK cells. In some embodiments, the combination enhances the antigen-specific T cell response in the treated subject and/or enhances the innate immunity response in the subject. In some embodiments, the combination results in an improved anti-tumor response in an animal cancer model, such as a syngeneic or xenograft model, compared to administration of either the CD80 ECD or CD80 ECD fusion molecule or immune stimulating agent alone. In some embodiments, the combination results in a synergistic response in an animal cancer model, such as a syngeneic or xenograft model, compared to administration of either the CD80 ECD or CD80 ECD fusion molecule or immune stimulating agent alone.
- [0123] In any of the above combination therapy method embodiments, the combination of the CD80 ECD or CD80 ECD fusion molecule with the immune stimulating agent,

such as a PD-1/PD-L1 inhibitor, that is administered to the subject may inhibit tumor growth in a mouse syngeneic or xenograft cancer model over a period of 1 week, 10 days, 2 weeks, or 3 weeks, for example, by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98%. In any of the above combination therapy method embodiments, the combination of the CD80 ECD or CD80 ECD fusion molecule with the immune stimulating agent, such as a PD-1/PD-L1 inhibitor, that is administered to the subject may reduce the volume of at least one tumor in the subject or in an animal model by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%, for example, over a period of one month, two months, three months, six months, or one year.

[0124] In any of the combination therapy methods, the CD80 ECD or CD80 ECD fusion molecule may be a CD80 ECD Fc comprising 10-60 mol SA to mol of CD80 ECD Fc protein, such as 15-60 mol SA/mol protein. In some embodiments, the content is 10-40 mol SA/mol protein, such as 15-40 mol SA/mol protein, such as 20-40 mol SA/mol protein, 20-30 mol SA/mol protein, 15-25 mol SA/mol protein, 15-30 mol SA to mol of protein, or 30-40 mol SA/mol protein. In some embodiments, the SA content is at least 15, such as at least 20, at least 25, at least 30, at least 35, or at least 40 mol SA/mol protein. In some embodiments, the SA content is 15, 20, 25, 30, 35, or 40 mol SA/mol protein. In some embodiments, the Fc domain is a human IgG1, IgG2, or IgG4 Fc domain. In some embodiments, the Fc domain comprises the amino acid sequence of SEQ ID NO:14. In some embodiments, the fusion molecule comprises the amino acid sequence of SEQ ID NO:20 or 21. In any of the combination treatment embodiments herein, the CD80 ECD or CD80 ECD fusion molecule may be a molecule described in US Application No. 15,340,238, filed November 1, 2016, for example.

[0125] In certain embodiments, an immune stimulating agent targets a stimulatory or inhibitory molecule that is a member of the immunoglobulin super family (IgSF). For example, an immune stimulating agent may be an agent that targets (or binds specifically to) another member of the B7 family of polypeptides. An immune stimulating agent may be an agent that targets a member of the TNF family of membrane bound ligands or a co-stimulatory or co-inhibitory receptor binding specifically to a member of the TNF family. Exemplary TNF and TNFR family members that may be targeted by immune stimulating agents include CD40 and CD40L, OX-40, OX-40L, GITR, GITRL, CD70, CD27L,

CD30, CD30L, 4-1BBL, CD137 (4-1BB), TRAIL/Apo2-L, TRAILR1/DR4, TRAILR2/DR5, TRAILR3, TRAILR4, OPG, RANK, RANKL, TWEAKR/Fn14, TWEAK, BAFFR, EDAR, XEDAR, TACI, APRIL, BCMA, LT β R, LIGHT, DcR3, HVEM, VEGI/TL1A, TRAMP/DR3, EDAR, EDA1, XEDAR, EDA2, TNFR1, Lymphotoxin α /TNF β , TNFR2, TNF α , LT β R, Lymphotoxin α 1 β 2, FAS, FASL, RELT, DR6, TROY and NGFR.

[0126] In some embodiments, an immune stimulating agent may comprise (i) an antagonist of a protein that inhibits T cell activation (e.g., immune checkpoint inhibitor) such as CTLA4 (e.g. an anti-CTLA4 antibody, e.g. YERVOY (ipilimumab) or tremelimumab), LAG-3 (e.g. an anti-LAG-3 antibody, for example, BMS-986016 (WO10/19570, WO14/08218), or IMP-731 or IMP-321 (WO08/132601, WO09/44273), TIM3, Galectin 9, CEACAM-1, BTLA, CD69, Galectin-1, TIGIT, CD113, GPR56, VISTA, B7-H3 (e.g. MGA271 (WO11/109400)), B7-H4, 2B4, CD48, GARP, PD1H, LAIR1, TIM-1, TIM-4, and ILT4 and/or may comprise (ii) an agonist of a protein that stimulates T cell activation such as B7-2, CD28, 4-1BB (CD137) (e.g. a CD137 agonist antibody such as urelumab or PF-05082566 (WO12/32433)), 4-1BBL, ICOS, ICOS-L, OX40 (e.g. an OX40 agonist antibody, for example, MEDI-6383, MEDI-6469 or MOXR0916 (RG7888; WO06/029879)), OX40L, GITRL, CD70, CD27 (e.g. an agonistic CD27 antibody such as varlilumab (CDX-1127)), CD40, CD40L, DR3, and CD28H. In some embodiments, the agonist of a protein that stimulates T cell activation is an antibody.

[0127] In some embodiments, an immune stimulating agent may comprise an agent that inhibits or is an antagonist of a cytokine that inhibits T cell activation (e.g., IL-6, IL-10, TGF- β , VEGF, and other immunosuppressive cytokines), and in some embodiments an immune stimulating agent may comprise an agent that is an agonist of a cytokine, such as IL-2, IL-7, IL-12, IL-15, IL-21 and IFN α (e.g., the cytokine itself) that stimulates T cell activation. TGF- β inhibitors include, e.g., GC1008, LY2157299, TEW7197 and IMC-TR1. In some embodiments, immune stimulating agents may comprise an antagonist of a chemokine, such as CXCR2 (e.g., MK-7123), CXCR4 (e.g. AMD3100), CCR2, or CCR4 (mogamulizumab).

[0128] In some embodiments, the at least one immune stimulating agent comprises a Toll-like receptor agonist, e.g., a TLR2/4 agonist (e.g., Bacillus Calmette-Guerin); a

TLR7 agonist (e.g., Hiltonol or Imiquimod); a TLR7/8 agonist (e.g., Resiquimod); or a TLR9 agonist (e.g., CpG7909).

- [0129] In some embodiments, immune stimulating agents may include antagonists of inhibitory receptors on NK cells or agonists of activating receptors on NK cells. In some embodiments, the at least one immune stimulating agent is an antagonist of KIR, e.g. the antibody lirilumab.
- [0130] In some embodiments, an immune stimulating agent may comprise an anti-GITR agonist antibody such as TRX-518 (WO06/105021, WO09/009116), MK-4166 (WO11/028683) or the GITR antibody disclosed in WO2015/031667.
- [0131] Immune stimulating agents may also include agents that enhance tumor antigen presentation, e.g., dendritic cell vaccines, GM-CSF secreting cellular vaccines, CpG oligonucleotides, and imiquimod, or therapies that enhance the immunogenicity of tumor cells (e.g., anthracyclines).
- [0132] Immune stimulating agents may also include certain vaccines such as mesothelin-targeting vaccines or attenuated listeria cancer vaccines, such as CRS-207.
- [0133] Immune stimulating agents may also comprise agents that deplete or block Treg cells, such as agents that specifically bind to CD25.
- [0134] Immune stimulating agents may also comprise agents that inhibit a metabolic enzyme such as indoleamine dioxigenase (IDO), dioxigenase, arginase, or nitric oxide synthetase. IDO antagonists include, for example, INCB-024360 (WO2006/122150, WO07/75598, WO08/36653, WO08/36642), indoximod, NLG-919 (WO09/73620, WO09/1156652, WO11/56652, WO12/142237) and F001287.
- [0135] Immune stimulating agents may also comprise agents that inhibit the formation of adenosine or inhibit the adenosine A2A receptor.
- [0136] Immune stimulating agents may also comprise agents that reverse/prevent T cell anergy or exhaustion and agents that trigger an innate immune activation and/or inflammation at a tumor site.
- [0137] The treatment combinations can also be further combined in a combinatorial approach that targets multiple elements of the immune pathway, such as one or more of the following: at least one agent that enhances tumor antigen presentation (e.g., dendritic cell vaccine, GM-CSF secreting cellular vaccines, CpG oligonucleotides, imiquimod); at least one agent that inhibits negative immune regulation e.g., by inhibiting CTLA4 pathway and/or depleting or blocking Treg or other immune suppressing cells; a therapy

that stimulates positive immune regulation, e.g., with agonists that stimulate the CD-137 and/or OX-40 pathway and/or stimulate T cell effector function; at least one agent that increases systemically the frequency of anti-tumor T cells; a therapy that depletes or inhibits Tregs, such as Tregs in the tumor, e.g., using an antagonist of CD25 (e.g., daclizumab) or by ex vivo anti-CD25 bead depletion; at least one agent that impacts the function of suppressor myeloid cells in the tumor; a therapy that enhances immunogenicity of tumor cells (e.g., anthracyclines); adoptive T cell or NK cell transfer including genetically modified cells, e.g., cells modified by chimeric antigen receptors (CAR-T therapy); at least one agent that inhibits a metabolic enzyme such as indoleamine dioxigenase (IDO), dioxigenase, arginase or nitric oxide synthetase; at least one agent that reverses/prevents T cell anergy or exhaustion; a therapy that triggers an innate immune activation and/or inflammation at a tumor site; administration of immune stimulatory cytokines or blocking of immuno repressive cytokines.

[0138] For example, the at least one immune stimulating agent may comprise one or more agonistic agents that ligate positive costimulatory receptors; one or more antagonists (blocking agents) that attenuate signaling through inhibitory receptors, such as antagonists that overcome distinct immune suppressive pathways within the tumor microenvironment; one or more agents that increase systemically the frequency of anti-tumor immune cells, such as T cells, deplete or inhibit Tregs (e.g., by inhibiting CD25); one or more agents that inhibit metabolic enzymes such as IDO; one or more agents that reverse/prevent T cell anergy or exhaustion; and one or more agents that trigger innate immune activation and/or inflammation at tumor sites.

[0139] In some embodiments, the CD80 ECD or CD80 ECD fusion molecule is administered in combination with an effective amount of a PD-1/PD-L1 inhibitor.

PD-1/PD-L1 Inhibitors

[0140] PD-1/PD-L1 inhibitors include antibodies, fusion proteins, and peptides. A nonlimiting exemplary fusion protein that is a PD-1/PD-L1 inhibitor is AMP-224 (Amplimmune, GlaxoSmithKline). A nonlimiting exemplary polypeptide that is a PD-1/PD-L1 inhibitor is AUR-012. Other exemplary PD-1/PD-L1 inhibitors include antibodies that inhibit PD-1, such as anti-PD-1 antibodies and anti-PD-L1 antibodies. Such antibodies may be humanized antibodies, chimeric antibodies, mouse antibodies, and human antibodies.

- [0141] In some embodiments, the PD-1/PD-L1 inhibitor is an anti-PD-L1 antibody, such as atezolizumab (Tecentriq®), durvalumab, avelumab, or BMS-936559.
- [0142] In some embodiments, the PD-1/PD-L1 inhibitor is an anti-PD-1 antibody. In one embodiment, the anti-PD-1 antibody is nivolumab. Nivolumab (also known as Opdivo®; formerly designated 5C4, BMS-936558, MDX-1106, or ONO-4538) is a fully human IgG4 (S228P) PD-1 immune checkpoint inhibitor antibody that selectively prevents interaction with PD-1 ligands (PD-L1 and PD-L2), thereby blocking the down-regulation of antitumor T-cell functions (U.S. Patent No. 8,008,449; Wang *et al.*, 2014 *Cancer Immunol Res.* 2(9):846-56). In some embodiments, nivolumab is administered at a dose of 3 mg/kg every 2 weeks or at a flat dose of 240 mg every 2 weeks. In another embodiment, the anti-PD-1 antibody is pembrolizumab. Pembrolizumab (also known as Keytruda®, formerly lambrolizumab, and MK-3475) is a humanized monoclonal IgG4 anti-PD-1 antibody. Pembrolizumab is described, for example, in U.S. Patent No. 8,900,587; *see also* [www \(dot\) cancer \(dot\) gov \(slash\) drugdictionary?cdrid=695789](http://www.cancer.gov/drugdictionary?cdrid=695789) (last accessed: March 27, 2017). Pembrolizumab has been approved by the FDA for the treatment of relapsed or refractory melanoma. For example, a flat dose of the anti-PD-1 antibody pembrolizumab can be 200 mg. In some embodiments, pembrolizumab may be administered at 200 mg every 3 weeks. In other embodiments, the anti-PD-1 Ab is MEDI0608 (formerly AMP-514). MEDI0608 is described, for example, in US Pat. No. 8,609,089, B2 or in [www \(dot\) cancer \(dot\) gov \(slash\) drugdictionary?cdrid=756047](http://www.cancer.gov/drugdictionary?cdrid=756047) (last accessed March 27, 2017). In some embodiments, the anti-PD-1 antibody is Pidilizumab (CT-011), which is a humanized monoclonal antibody. Pidilizumab is described in US Pat. No. 8,686,119 B2 or WO 2013/014668 A1.

Additional Combination Therapies

- [0143] CD80 ECDs or CD80 ECD fusion molecules may also be provided before, substantially contemporaneous with, or after other modes of treatment, for example, surgery, chemotherapy, radiation therapy, or the administration of another biologic.
- [0144] For treatment of cancer for example, CD80 ECDs or CD80 ECD fusion molecules may be administered in conjunction with one or more additional anti-cancer agents, such as the chemotherapeutic agent, growth inhibitory agent, anti-angiogenesis agent and/or anti-neoplastic composition. Nonlimiting examples of chemotherapeutic agent, growth inhibitory agent, anti-angiogenesis agent, anti-cancer agent and anti-neoplastic

composition that can be used in combination with the antibodies of the present invention are provided in the following definitions.

[0145] A “**chemotherapeutic agent**” is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include, but are not limited to, alkylating agents such as thiotepa and Cytosan[®] cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gammaII and calicheamicin omegaII (*see, e.g.*, Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994))); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, Adriamycin[®] doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine,

azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitio stanol, mepitio stanane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK[®] polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.*, Taxol[®] paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), Abraxane[®] Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumburg, Illinois), and Taxotere[®] docetaxel (Rhône-Poulenc Rorer, Antony, France); chloranbucil; Gemzar[®] gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin, oxaliplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; Navelbine[®] vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (Camptosar, CPT-11) (including the treatment regimen of irinotecan with 5-FU and leucovorin); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; combretastatin; leucovorin (LV); oxaliplatin, including the oxaliplatin treatment regimen (FOLFOX); inhibitors of PKC- α , Raf, H-Ras, EGFR (*e.g.*, erlotinib (Tarceva[®])) and VEGF-A that reduce cell proliferation and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0146] Further nonlimiting exemplary chemotherapeutic agents include anti-hormonal agents that act to regulate or inhibit hormone action on cancers such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including Nolvadex[®] tamoxifen), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and Fareston[®] toremifene; aromatase

inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, Megase[®] megestrol acetate, Aromasin[®] exemestane, formestanie, fadrozole, Rivisor[®] vorozole, Femara[®] letrozole, and Arimidex[®] anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC- α , Ralf and H-Ras; ribozymes such as a VEGF expression inhibitor (*e.g.*, Angiozyme[®] ribozyme) and a HER2 expression inhibitor; vaccines such as gene therapy vaccines, for example, Allovectin[®] vaccine, Leuvectin[®] vaccine, and Vaxid[®] vaccine; Proleukin[®] rIL-2; Lurtotecan[®] topoisomerase 1 inhibitor; Abarelix[®] rmRH; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0147] An “**anti-angiogenesis agent**” or “**angiogenesis inhibitor**” refers to a small molecular weight substance, a polynucleotide (including, *e.g.*, an inhibitory RNA (RNAi or siRNA)), a polypeptide, an isolated protein, a recombinant protein, an antibody, or conjugates or fusion proteins thereof, that inhibits angiogenesis, vasculogenesis, or undesirable vascular permeability, either directly or indirectly. It should be understood that the anti-angiogenesis agent includes those agents that bind and block the angiogenic activity of the angiogenic factor or its receptor. For example, an anti-angiogenesis agent is an antibody or other antagonist to an angiogenic agent, *e.g.*, antibodies to VEGF-A (*e.g.*, bevacizumab (Avastin[®])) or to the VEGF-A receptor (*e.g.*, KDR receptor or Flt-1 receptor), anti-PDGFR inhibitors such as Gleevec[®] (Imatinib Mesylate), small molecules that block VEGF receptor signaling (*e.g.*, PTK787/ZK2284, SU6668, Sutent[®]/SU11248 (sunitinib malate), AMG706, or those described in, *e.g.*, international patent application WO 2004/113304). Anti-angiogenesis agents also include native angiogenesis inhibitors, *e.g.*, angiostatin, endostatin, *etc.* See, *e.g.*, Klagsbrun and D’Amore (1991) *Annu. Rev. Physiol.* 53:217-39; Streit and Detmar (2003) *Oncogene* 22:3172-3179 (*e.g.*, Table 3 listing anti-angiogenic therapy in malignant melanoma); Ferrara & Alitalo (1999) *Nature Medicine* 5(12):1359-1364; Tonini *et al.* (2003) *Oncogene* 22:6549-6556 (*e.g.*, Table 2 listing known anti-angiogenic factors); and, Sato (2003) *Int. J. Clin. Oncol.* 8:200-206 (*e.g.*, Table 1 listing anti-angiogenic agents used in clinical trials).

[0148] A “**growth inhibitory agent**” as used herein refers to a compound or composition that inhibits growth of a cell (such as a cell expressing VEGF) either *in vitro* or *in vivo*. Thus, the growth inhibitory agent may be one that significantly reduces the percentage of cells (such as a cell expressing VEGF) in S phase. Examples of growth inhibitory agents include, but are not limited to, agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in Mendelsohn and Israel, eds., *The Molecular Basis of Cancer*, Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami *et al.* (W.B. Saunders, Philadelphia, 1995), *e.g.*, p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (Taxotere[®], Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (Taxol[®], Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

[0149] The term “**anti-neoplastic composition**” refers to a composition useful in treating cancer comprising at least one active therapeutic agent. Examples of therapeutic agents include, but are not limited to, *e.g.*, chemotherapeutic agents, growth inhibitory agents, cytotoxic agents, agents used in radiation therapy, anti-angiogenesis agents, other cancer immunotherapeutic agents aside from PD-1/PD-L1 inhibitors, apoptotic agents, anti-tubulin agents, and other-agents to treat cancer, such as anti-HER-2 antibodies, anti-CD20 antibodies, an epidermal growth factor receptor (EGFR) antagonist (*e.g.*, a tyrosine kinase inhibitor), HER1/EGFR inhibitor (*e.g.*, erlotinib (Tarceva[®]), platelet derived growth factor inhibitors (*e.g.*, Gleevec[®] (Imatinib Mesylate)), a COX-2 inhibitor (*e.g.*, celecoxib), interferons, CTLA-4 inhibitors (*e.g.*, anti-CTLA antibody ipilimumab (YERVOY[®])), PD-L2 inhibitors (*e.g.*, anti-PD-L2 antibodies), TIM3 inhibitors (*e.g.*, anti-TIM3 antibodies), cytokines, antagonists (*e.g.*, neutralizing antibodies) that bind to one or more of the following targets ErbB2, ErbB3, ErbB4, PDGFR-beta, BlyS, APRIL, BCMA, PD-

L2, CTLA-4, TIM3, or VEGF receptor(s), TRAIL/Apo2, and other bioactive and organic chemical agents, *etc.* Combinations thereof are also included in the invention.

Routes of Administration and Carriers

[0150] In various embodiments, polypeptides and fusion molecules may be administered *in vivo* by various routes, including, but not limited to, oral, intra-arterial, parenteral, intranasal, intravenous, intramuscular, intracardiac, intraventricular, intratracheal, buccal, rectal, intraperitoneal, intradermal, topical, transdermal, and intrathecal, or otherwise by implantation or inhalation. The subject compositions may be formulated into preparations in solid, semi-solid, liquid, or gaseous forms; including, but not limited to, tablets, capsules, powders, granules, ointments, solutions, suppositories, enemas, injections, inhalants, and aerosols. A nucleic acid molecule encoding a polypeptide may be coated onto gold microparticles and delivered intradermally by a particle bombardment device, or “gene gun,” as described in the literature (see, e.g., Tang et al., *Nature* 356:152-154 (1992)). The appropriate formulation and route of administration may be selected according to the intended application.

[0151] In various embodiments, polypeptide-comprising compositions are provided in formulations with a wide variety of pharmaceutically acceptable carriers (see, e.g., Gennaro, *Remington: The Science and Practice of Pharmacy with Facts and Comparisons: Drugfacts Plus*, 20th ed. (2003); Ansel et al., *Pharmaceutical Dosage Forms and Drug Delivery Systems*, 7th ed., Lippencott Williams and Wilkins (2004); Kibbe et al., *Handbook of Pharmaceutical Excipients*, 3rd ed., Pharmaceutical Press (2000)). Various pharmaceutically acceptable carriers, which include vehicles, adjuvants, and diluents, are available. Moreover, various pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are also available. Non-limiting exemplary carriers include saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof.

[0152] In various embodiments, compositions comprising polypeptides and fusion molecules may be formulated for injection, including subcutaneous administration, by dissolving, suspending, or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids, or propylene glycol; and if desired, with conventional additives such as

solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives. In various embodiments, the compositions may be formulated for inhalation, for example, using pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen, and the like. The compositions may also be formulated, in various embodiments, into sustained release microcapsules, such as with biodegradable or non-biodegradable polymers. A non-limiting exemplary biodegradable formulation includes poly lactic acid-glycolic acid polymer. A non-limiting exemplary non-biodegradable formulation includes a polyglycerin fatty acid ester. Certain methods of making such formulations are described, for example, in EP 1 125 584 A1.

[0153] Pharmaceutical packs and kits comprising one or more containers, each containing one or more doses of a polypeptide or combination of polypeptides are also provided. In some embodiments, a unit dosage is provided wherein the unit dosage contains a predetermined amount of a composition comprising a polypeptide or combination of polypeptides, with or without one or more additional agents. In some embodiments, such a unit dosage is supplied in a single-use prefilled syringe for injection. In various embodiments, the composition contained in the unit dosage may comprise saline, sucrose, or the like; a buffer, such as phosphate, or the like; and/or be formulated within a stable and effective pH range. Alternatively, in some embodiments, the composition may be provided as a lyophilized powder that may be reconstituted upon addition of an appropriate liquid, for example, sterile water. In some embodiments, the composition comprises one or more substances that inhibit protein aggregation, including, but not limited to, sucrose and arginine. In some embodiments, a composition of the invention comprises heparin and/or a proteoglycan.

[0154] Pharmaceutical compositions are administered in an amount effective for treatment or prophylaxis of the specific indication. The therapeutically effective amount is typically dependent on the weight of the subject being treated, his or her physical or health condition, the extensiveness of the condition to be treated, or the age of the subject being treated.

CD80 ECD and ECD Fusion Molecules in Combination with or as Components of Cancer Vaccines

[0155] The present disclosure also includes methods of administering a CD80 ECD or CD80 ECD fusion molecule in combination with a cancer vaccine composition

comprising at least one tumor-specific or tumor-associated antigen. The disclosure also provides cancer vaccine compositions comprising a CD80 ECD or CD80 ECD fusion molecule and at least one tumor-specific or tumor-associated antigen. In either situation (whether the CD80 ECD or CD80 ECD fusion molecule is administered separately or is a component of a cancer vaccine), the cancer vaccine may be a personalized cancer vaccine.

[0156] In some embodiments, the tumor-specific or tumor-associated antigen may be an antigen commonly found associated with the tumor. In some embodiments, the tumor-specific or tumor-associated antigen may be an antigen found on the particular patient's tumor and that has been, for example, produced or amplified *ex vivo* and then reintroduced as a vaccine. In some embodiments, the antigen may be provided on the surface of immune cells such as antigen-presenting cells, which are then administered to the patient.

[0157] The present disclosure also provides for a CD80 ECD or CD80 ECD fusion protein as a component of a cancer vaccine composition. For example, a cancer vaccine composition may comprise a tumor-specific or tumor-associated antigen in combination with a CD80 ECD or CD80 ECD fusion protein.

[0158] In such vaccine compositions and methods, the CD80 ECD or CD80 ECD fusion protein may be capable of altering the memory T cell subsets in the patient administered the vaccine.

EXAMPLES

[0159] The examples discussed below are intended to be purely exemplary of the invention and should not be considered to limit the invention in any way. The examples are not intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (for example, amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1: Effect of CD80 ECD-Fc Administration on CD4⁺ and CD8⁺ Central Memory T Cells in Cynomolgus Monkeys

- [0160] A study was conducted in monkey (*Macaca fascicularis*) naive cynomolgus using human CD80 ECD-Fc, consisting of the extracellular domain (ECD) of human CD80 linked to the Fc domain of wild-type human IgG1. Human CD80 ECD-Fc was tested at 1mg/kg, 10mg/kg, and 50mg/kg administered weekly for 4 weeks.
- [0161] Sixteen monkeys were administered vehicle or human CD80-ECD-Fc on day 1, 8, 15 and 22. Each experimental group was composed of 4 animals, 2 female and 2 male. One animal of each sex from each dose group was euthanized at day 26. The remaining animals were retained and observed for a post-dosing recovery period of six weeks.
- [0162] Whole venous blood samples were collected at the following timepoints: two predose samples at least one week apart, 24 hours post-first dose on day 2, day 4, predose on day 8, predose on day 15 and on the days of scheduled necropsy, day 26 and day 64.
- [0163] Flow cytometry analysis was performed on whole blood samples to evaluate frequency, activation, and proliferation of T cells (CD8⁺ T cells and CD4⁺ T cells), T regulatory cells (Treg), NK cells, and T cell memory subset repertoire (naïve, central memory, and effector memory).
- [0164] Treatment with human CD80 ECD-Fc did not induce overt changes to the frequencies of major lymphocyte (NK cells, B cells, total CD4⁺ or CD8⁺ T cells, or Treg) in peripheral blood of cynomolgus monkeys. Human CD80 ECD-Fc induced dose-dependent expansion and proliferation of central memory T cell subsets (Tcm). Expansion of central memory CD4⁺ and CD8⁺ T cells expressing CD95 and CD28 was observed in the groups dosed with 10 and 50 mg/kg but not in the group dosed with 1 mg/kg. The central memory CD4⁺ and CD8⁺ T cell populations continued to expand in frequency after each dose of human CD80 ECD-Fc and then were reduced by the end of study (**Figure 1 and 2**). The proliferation of central memory CD4⁺ and CD8⁺ T cells as measured by Ki67 expression was also increased in the groups treated with the 10 mg/kg and 50 mg/kg. Ki67 expression was maximal in CD4⁺ Tcm at 7 days after the first dose and in CD8⁺ Tcm at 7 days after the second dose; Ki67 expression returned to baseline levels by the end of the study (**Figure 3 and 4**).

TABLE OF SEQUENCES

The table below provides a listing of certain sequences referenced herein.

SEQ. ID. NO.	Description	Sequence
1	Human CD80 precursor (with signal sequence) amino acid sequence	MGHTRRQGTSPSKCPYLNFFQLLVLAGLSHFCSG VIHVTKEVKEVATLSCGHNVSVLELAQTRIYWQ KEKKMVLTMMSGDMNIWPEYKNRTIFDITNNLS IVILALRPSDEGTYECVVLKYEKDAFKREHLAEV TLSVKADFPTPSISDFEIPSTNIRRIICSTSGGFPEPH LSWLENGEELNAINTTVSQDPETELYAVSSKLDF NMTTNHSFMCLIKYGHRLRVNQTFNWNTTKQEH FPDNLLPSWAITLISVNGIFVICCLTYCFAPRCRER RRNERLRRESVRPV
2	Mouse CD80 precursor (with signal sequence) amino acid sequence	MACNCQLMQDTPLLKFPCPRILLFVLLIRLSQ VSSDVDEQLSKSVKDKVLLPCRYNSPHEDESE DRIYWQKHDKVVLVSVIAGKLKVWPEYKNRT LYDNNTTYSLLGLVLSDRGTYSVVQKKERG TYEVKHLALVKLSIKADFSTPNITESGNPSAD TKRITCFASGGFPKPRFSWLENGRELPGINTTI SQDPESELYTISSQLDFNTTRNHTIKCLIKYGD AHVSEDFTWEKPPEDPPDSKNTLVLFAGGFG AVITVVVIVVVIKCFCKHRSCFRNEASRETNN SLTFGPPEALAEQTVFL
3	Human CD80 Isoform 2 (without signal sequence)	VIHVTKEVKEVATLSCGHNVSVLELAQTRIY WQKEKKMVLTMMSGDMNIWPEYKNRTIFDI TNNLSIVILALRPSDEGTYECVVLKYEKDAFK REHLAEVTLSVKADFPTPSISDFEIPSTNIRRIIC STSGGFPEPHLSWLENGEELNAINTTVSQDPE TELYAVSSKLDFNMTTNHSFMCLIKYGHRLRV NQTFNWNTSFAPRCRERRRNERLRRESVRPV
4	Human CD80 Isoform 3 (without signal sequence)	VIHVTKEVKEVATLSCGHNVSVLELAQTRIY WQKEKKMVLTMMSGDMNIWPEYKNRTIFDI TNNLSIVILALRPSDEGTYECVVLKYEKDAFK REHLAEVTLVKGFAAPRCRERRRNERLRRESV RPV
5	Human CD80 ECD sequence	VIHVTKEVKEVATLSCGHNVSVLELAQTRIYWQ KEKKMVLTMMSGDMNIWPEYKNRTIFDITNNLS

	(without signal sequence)	IVILALRPSDEGTYECVVLKYEKDAFKREHLAEV TLSVKADFPTPSISDFEIPTSNIRRIICSTSGGFPEPH LSWLENGEELNAINTTVSQDPETELYAVSSKLDF NMTTNHSFMCLIKYGHRLRVNQTFNWNTTKQEH FPDN
6	Mouse CD80 ECD sequence (without signal sequence)	VDEQLSKSVKDKVLLPCRYNSPHEDESEDRIY WQKHDKVVLVSVIAGKLKVWPEYKNRTLYDN TTYSLIILGLVLSDRGTYSCVVQKKERGTYEV KHLALVKLSIKADFSTPNITESGNPSADTKRIT CFASGGFPKPRFSWLENGRELPGINTTISQDP ESELYTISSQLDFNTTRNHTIKCLIKYGDAHV SEDFTWEKPPEDPPDSKN
7	Human CD80 signal sequence	MGHTRRQGTSPSKCPYLNFFQLLVLAGLSHFCSG
8	Mouse CD80 signal sequence	MACNCQLMQDTPLLKFCPRLILLFVLLIRLSQVS SD
9	Fc C237S	EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSGDSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
10	Fc	ERKCCVECPCP APPVAGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV HQDWLNGKEY KCKVSNKGLP APIEKTISK KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPE NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
11	Fc	ESKYGPPCPS CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPV LDSGDSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLGK
12	Human IgG1 Fc L234F, L235E, P331S	EPKSSDKTHTCPPCPAPEFEGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN

	mutant	GKEYKCKVSNKALPASIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
13	Human IgG1 Fc N297 mutant	EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYGSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
14	Fc human IgG1	EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
15	Fc human IgG3	ELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCPEPK SCDTPPPCPRCPEPKSCDTPPPCPRCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV QFKWYVDGVEVHNAKTKPREEQYNSTFRVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFL YSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQK SLSLSPGK
16	Fc human IgG4	ESKYGPCCPPCPAPEFLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEG NVFSCSVMHEALHNHYTQKSLSLSPGK
17	Mouse CD80 ECD mouse Fc IgG2a (Fc portion underlined)	VDEQLSKSVKDKVLLPCRYNSPHEDESEDRIYW QKHDKVVL SVIAGKLKVWPEYKNRTLYDNTTY SLIILGLVLSDRGTYSVVQKKERGTYEVKHLAL VKLSIKADFSTPNITESGNPSADTKRITCFASGGFP KPRFSWLENGRELPGINTTISQDPESELYTISSQLD FNTTRNHTIKCLIKYGDAHVSEDFTWKPPEDPP DSKNEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPK <u>IKDVL</u> MISLSPIVTCVVVDVSEDDPDVQISWFN <u>NVEVHTAQ</u> TQTHREDYNSTLRVVSALPIQHQDW <u>MSGKEFKCKVNNKDL</u> PAPIERTISKPKGSVRAPO <u>VYVLPPPEEEMTKKQVTL</u> TCMVTDFMPEDIYVE <u>WTNNGKTELNYKNTEP</u> VLDSDGSYFMYSKLRV <u>EKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTP</u> <u>GK</u>

18	Mouse CD80 ECD Human Fc IgG1 WT (Fc portion underlined)	VDEQLSKSVKDKVLLPCRYNSPHEDESEDRIYW QKHDKVVL SVIAGKLK VWPEYKNRTL YDNTTY SLIILGLVL SDRGTYS CVVQKKERGTYEVKHLAL VKLSIKADFSTPNITESGNPSADTKRITCFASGGFP KPRFSWLENGRELPGINTTISQDPESELYTISSQLD FNTTRNHTIKCLIKYGDAHVSEDFTWEKPPEDPP DSKNEPKSSDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK
19	Mouse CD80 ECD Fc IgG1 MT (234, 235, 331) (Fc portion underlined; mutants shown in bold)	VDEQLSKSVKDKVLLPCRYNSPHEDESEDRIYW QKHDKVVL SVIAGKLK VWPEYKNRTL YDNTTY SLIILGLVL SDRGTYS CVVQKKERGTYEVKHLAL VKLSIKADFSTPNITESGNPSADTKRITCFASGGFP KPRFSWLENGRELPGINTTISQDPESELYTISSQLD FNTTRNHTIKCLIKYGDAHVSEDFTWEKPPEDPP DSKNEPKSSDKTHTCPPCPAPE F EGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALP A SIEKTISKAKGQPR EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK
20	Human CD80 ECD Human Fc IgG1 WT (Fc portion underlined)	VIHVTKEVKEVATLSCGHNVSV EELAQTRIYWQ KEKKMVL TMMSGDMNIWPEYKNRTIFDITNNLS IVILALRPSDEGTYECVVLKYEKDAFKREHLAEV TL SVKADFPTPSISDFEIPTSNIRRIICSTSGGFPEPH LSWLENGEELNAINTTVSQDPETEL YAVSSKLDF NMTTNHSFMCLIKYGHLRVNQTFNWNTTKQEH FPDNEPKSSDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK
21	Human CD80 ECD Human Fc IgG1 L234F, L235E, P331S MT (Fc portion)	VIHVTKEVKEVATLSCGHNVSV EELAQTRIYWQ KEKKMVL TMMSGDMNIWPEYKNRTIFDITNNLS IVILALRPSDEGTYECVVLKYEKDAFKREHLAEV TL SVKADFPTPSISDFEIPTSNIRRIICSTSGGFPEPH LSWLENGEELNAINTTVSQDPETEL YAVSSKLDF NMTTNHSFMCLIKYGHLRVNQTFNWNTTKQEH

	underlined; mutants in bold)	<u>FPDNEPKSSDKTHTCPPCPAPEFEGGPSVFLFPPK</u> <u>PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV</u> <u>DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ</u> <u>DWLNGKEYKCKVSNKALPASIEKTISKAKGQPR</u> <u>EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA</u> <u>VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLT</u> <u>TVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSP</u> <u>GK</u>
22	human PD-1 precursor (with signal sequence) UniProtKB/Sw iss-Prot: Q15116.3, 01- OCT-2014	MQIPQAPWPV VWAVLQLGWR PGWFLDSPDR PWNPPPTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAKIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV VGVVGGLLGS LVLLVWVLAV ICSRAARGTI GARRTGQPLK EDPSAVPVFS VDYGELDFQW REKTPEPPVP CVPEQTEYAT IVFPSGMGTS SPARRGSADG PRSAQPLRPE DGHCSWPL
23	human PD-1 (mature, without signal sequence)	PGWFLDSPDR PWNPPPTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAKIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV VGVVGGLLGS LVLLVWVLAV ICSRAARGTI GARRTGQPLK EDPSAVPVFS VDYGELDFQW REKTPEPPVP CVPEQTEYAT IVFPSGMGTS SPARRGSADG PRSAQPLRPE DGHCSWPL
24	human PD-L1 precursor (with signal sequence) UniProtKB/Sw iss-Prot: Q9NZQ7.1, 01-OCT-2014	MRIFAVFIFM TYWHLLNAFT VTPPKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME DKNIIQFVHG EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV NAPYNKINQR ILVVDPTSE HELTCQAEGY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL FNVSTSLRIN TTTNEIFYCT FRRLDPEENH TAEVIPELP LAHPPNERTH LVILGAILLC LGVALTFIFR LRKGRMMDVK KCGIQDTNSK KQSDTHLEET
25	human PD-L1 (mature, without signal sequence)	FT VTPPKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME DKNIIQFVHG EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV NAPYNKINQR ILVVDPTSE HELTCQAEGY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL

		FNVTSTLRIN TTTNEIFYCT FRRLDPEENH TAE LVIPELP LAHPPNER TH LVILGAILLC LGVALTFIFR LRKGRMMDVK KCGIQDTNSK KQSDTHLEET
--	--	--

WHAT IS CLAIMED IS:

1. A method of determining the activity of a CD80 extracellular domain (ECD) fusion molecule in a subject comprising determining the frequency of central memory T cells and/or detecting the proliferation of central memory T cells in a sample obtained from the subject after administration of the CD80 ECD fusion molecule to the subject, wherein the CD80 ECD fusion molecule comprises a human CD80 ECD polypeptide and a human IgG1 Fc domain.
2. A method of detecting central memory T cell frequency and/or proliferation in a subject comprising (a) obtaining a sample from the subject after a CD80 ECD fusion molecule has been administered to the subject and (b) determining the frequency of central memory T cells and/or detecting the proliferation of central memory T cells in the sample, wherein the CD80 ECD fusion molecule comprises a human CD80 ECD polypeptide and a human IgG1 Fc domain.
3. The method of claim 1 or 2, further comprising administering a CD80 ECD fusion molecule to the subject after determining the frequency of central memory T cells and/or detecting the proliferation of central memory T cells in the sample.
4. The method of any one of claims 1-3, wherein the subject has cancer.
5. A method of treating cancer in a subject comprising (a) administering to the subject a CD80 ECD fusion molecule comprising a human CD80 ECD polypeptide and a human IgG1 Fc domain; and (b) determining the frequency of central memory T cells and/or detecting the proliferation of central memory T cells in a sample obtained from the subject after the administration.
6. The method of claim 5, further comprising determining the frequency of central memory T cells or detecting the proliferation of central memory T cells in a sample obtained from the subject prior to administration of the CD80 ECD fusion molecule.
7. The method of any one of claims 1-6, comprising determining the frequency of central memory T cells, but not detecting the proliferation of central memory T cells.

8. The method of any one of claims 1-6, comprising detecting the proliferation of central memory T cells, but not determining the frequency of central memory T cells.
9. The method of any one of claims 1-6, comprising determining the frequency of central memory T cells and detecting the proliferation of central memory T cells.
10. The method of any one of claims 1-7 and 9, wherein the frequency is determined using flow cytometry.
11. The method of any one of claims 1-6, 8, and 9, wherein the proliferation is detected using flow cytometry.
12. The method of any one of claims 1-6, 8, 9, and 11, wherein the proliferation is detected by measuring Ki67 expression.
13. The method of any one of claims 1-6, 8, 9, 11, and 12, wherein the proliferation is detected in a sample obtained at least 7 days after administration of the CD80 ECD fusion molecule.
14. A method of treating cancer in a subject comprising administering to the subject a CD80 ECD fusion molecule comprising a human CD80 ECD polypeptide and a human IgG1 Fc domain, wherein (a) the frequency of memory T cells has been determined in a sample obtained from the subject prior to the administration and/or (b) the proliferation of memory T cells has been detected in a sample obtained from the subject prior to the administration.
15. The method of claim 14, wherein the frequency of memory T cells has been determined, but the proliferation of memory T cells has not been detected.
16. The method of claim 14, wherein the proliferation of memory T cells has been detected, but the frequency of memory T cells has not been determined.
17. The method of claim 14, wherein the frequency of memory T cells has been determined and the proliferation of memory T cells has been detected.

18. The method of any one of claims 14, 15, and 17, wherein the frequency has been determined using flow cytometry.
19. The method of any one of claims 14, 16, and 17, wherein the proliferation has been detected using flow cytometry.
20. The method of any one of claims 14, 16, 17, and 19, wherein the proliferation has been determined by measuring Ki67 expression.
21. The method of any one of claims 14, 16, 17, 19, and 20, wherein the proliferation has been detected in a sample obtained at least 7 days after administration of the CD80 ECD fusion molecule.
22. The method of any one of claims 1-21, wherein the sample is a blood sample.
23. The method of any one of claims 1-22, wherein the sample is a plasma sample.
24. The method of any one of claims 1-23, wherein the central memory T cells are CD95+ and CD28+ cells.
25. The method of any one of claims 1-24, wherein the central memory T cells are CD4+ central memory T cells.
26. The method of any one of claims 1-24, wherein the central memory T cells are CD8+ central memory T cells.
27. The method of any one of claims 1-24, wherein the central memory T cells are CD4+ and CD8+ central memory T cells.
28. The method of any one of claims 1-27, wherein the sample obtained after administration of the CD80 ECD fusion molecule is obtained at least one week after the administration.
29. The method of any one of claims 1-27, wherein the sample obtained after administration of the CD80 ECD fusion molecule is obtained at least two weeks after the administration.

30. The method of any one of claims 1-27, wherein the sample obtained after administration of the CD80 ECD fusion molecule is obtained at least one month after the administration.
31. The method of any one of claims 1-30, wherein the human CD80 ECD polypeptide comprises the amino acid sequence of SEQ ID NO:5.
32. The method of any one of claims 1-30, wherein the human CD80 ECD polypeptide comprises the amino acid sequence of SEQ ID NO:3.
33. The method of any one of claims 1-30, wherein the human CD80 ECD polypeptide comprises the amino acid sequence of SEQ ID NO:4.
34. The method of any one of claims 1-33, wherein the human IgG1 Fc domain comprises the amino acid sequence of SEQ ID NO:14.
35. The method of any one of claims 1-30, wherein the CD80 ECD fusion molecule comprises the amino acid sequence of SEQ ID NO:20.
36. The method of any one of claims 1-30, wherein the CD80 ECD fusion molecule comprises the amino acid sequence of SEQ ID NO:21.
37. The method of any one of claims 1-36, wherein the CD80 ECD fusion molecule comprises 10-60 molecules of sialic acid (SA).
38. The method of any one of claims 1-36, wherein the CD80 ECD fusion molecule comprises 15-40 molecules of SA.
39. The method of any one of claims 1-36, wherein the CD80 ECD fusion molecule comprises 15-25 molecules of SA.
40. The method of any one of claims 1-36, wherein the CD80 ECD fusion molecule comprises 15-30 molecules of SA.
41. The method of any one of claims 4-40, wherein the cancer is a solid tumor.

42. The method of any one of claims 4-41, wherein the cancer is selected from the group consisting of colorectal cancer, breast cancer, gastric cancer, non-small cell lung cancer, melanoma, squamous cell carcinoma of the head and neck, ovarian cancer, pancreatic cancer, renal cell carcinoma, hepatocellular carcinoma, bladder cancer, and endometrial cancer.
43. The method of any one of claims 4-42, wherein the cancer is recurrent or progressive after a therapy selected from the group consisting of surgery, chemotherapy, radiation therapy, and a combination thereof.
44. The method of any one of claims 1-43, wherein the CD80 ECD fusion molecule is administered in combination with a programmed cell death 1 (PD-1) / programmed cell death ligand 1 (PD-L1) inhibitor.
45. The method of claim 44, wherein the PD-1/PD-L1 inhibitor is an antibody.
46. The method of claim 45, wherein the PD-1/PD-L1 inhibitor is an anti-PD-1 antibody.
47. The method of claim 46, wherein the anti-PD-1 antibody comprises the heavy chain and light chain CDRs of nivolumab, pidilizumab, or pembrolizumab.
48. The method of claim 47, wherein the anti-PD-1 antibody comprises the heavy chain and light chain variable regions of nivolumab, pidilizumab, or pembrolizumab.
49. The method of claim 48, wherein the anti-PD-1 antibody is nivolumab, pidilizumab, or pembrolizumab.
50. The method of claim 45, wherein the PD-1/PD-L1 inhibitor is an anti-PD-L1 antibody.
51. The method of claim 50, wherein the anti-PD-L1 antibody comprises the heavy chain and light chain CDRs of avelumab, durvalumab, atezolizumab, or BMS-936559.
52. The method of claim 51, wherein the anti-PD-L1 antibody comprises the heavy chain and light chain variable regions of avelumab, durvalumab, atezolizumab, or BMS-936559.

53. The method of claim 52, wherein the anti-PD-L1 antibody is avelumab, durvalumab, atezolizumab, or BMS-936559.
54. The method of claim 44, wherein the PD-1/PD-L1 inhibitor is a PD-1 fusion molecule.
55. The method of claim 54, wherein the fusion molecule is AMP-224.
56. The method of claim 44, wherein the PD-1/PD-L1 inhibitor is AUR-012.
57. The method of any one of claims 1-56, wherein the CD80 ECD fusion molecule is administered in combination with a cancer vaccine.
58. The method of claim 57, wherein the cancer vaccine is a personalized cancer vaccine.
59. The method of claim 57 or 58, wherein the CD80 ECD fusion molecule and the cancer vaccine are administered concurrently or sequentially.

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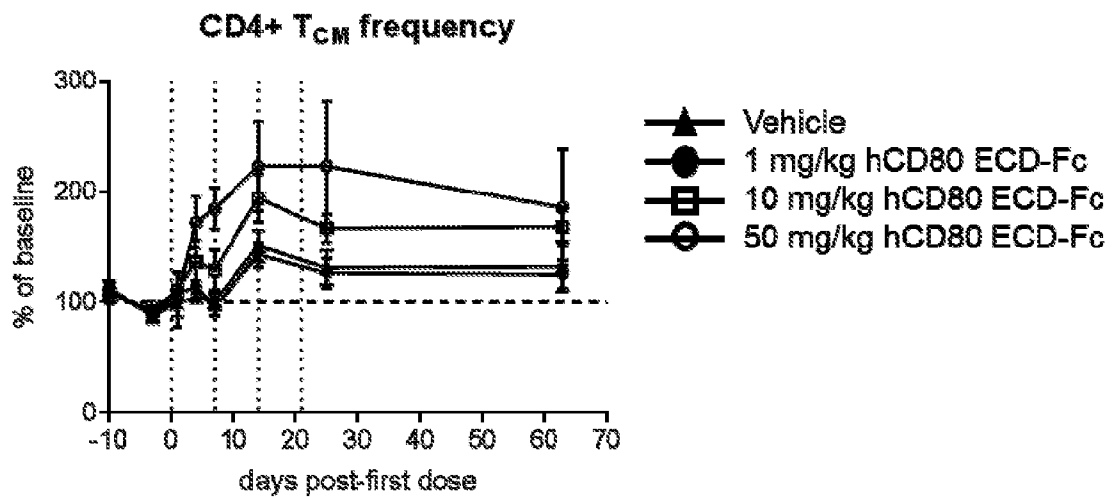


Fig. 1

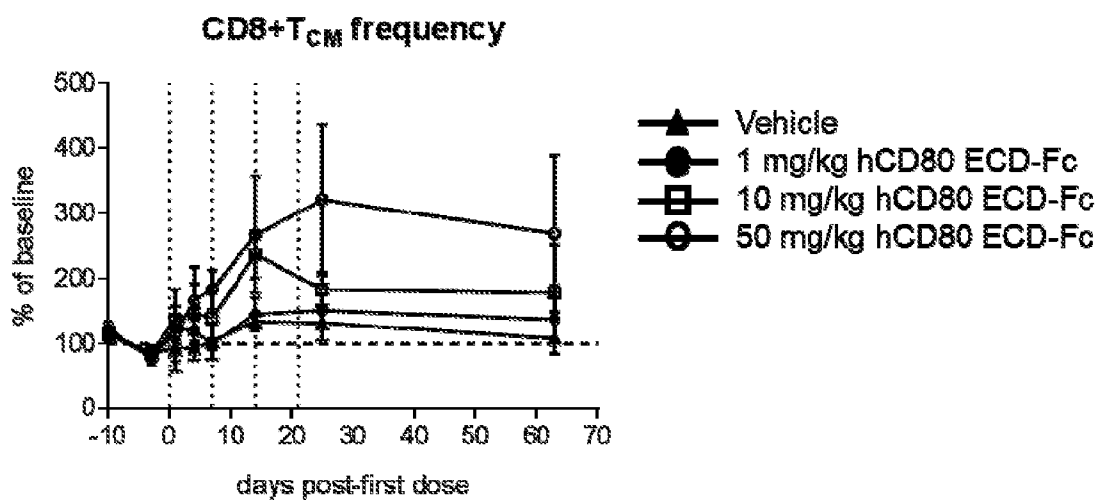


Fig. 2

2/2

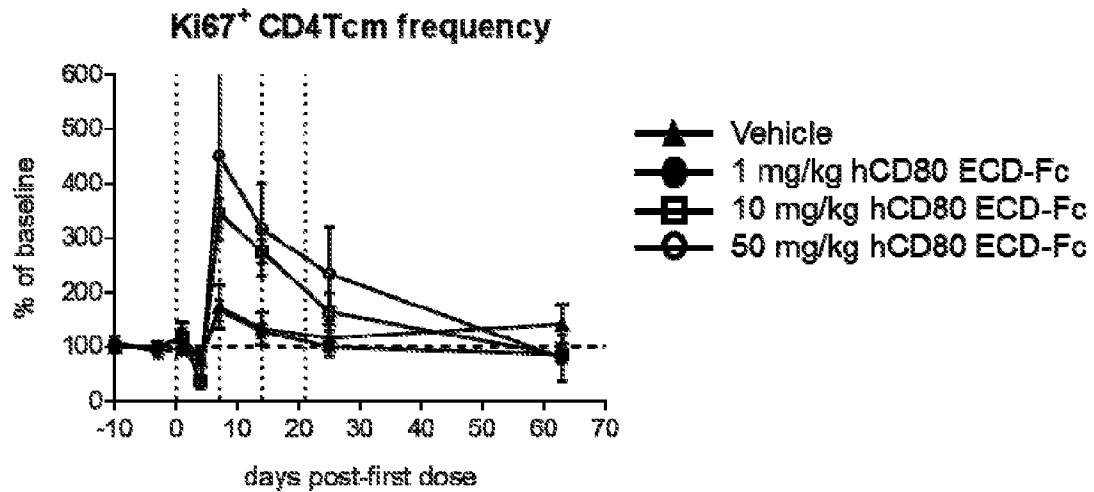


Fig. 3

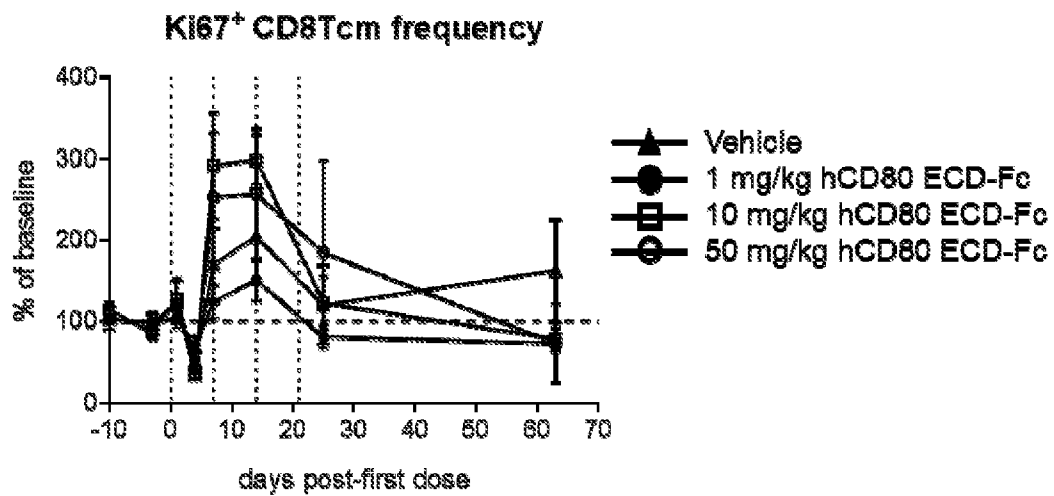


Fig. 4

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SEQUENCE LISTING

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BRENNAN, THOMAS
SENNINO, BARBARA
BARBEE, SUSANNAH D.
JEFFRY, URSULA

<120> METHODS OF TREATMENT WITH CD80 EXTRACELLULAR DOMAIN POLYPEPTIDES

<130> 3986.007PC01

<150> 62/491,479
<151> 2017-04-28

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<210> 1
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Leu Asn Phe Phe Gln Leu Leu Val Leu Ala Gly Leu Ser His Phe Cys
20 25 30

Ser Gly Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr Leu
35 40 45

Ser Cys Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile
50 55 60

Tyr Trp Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp
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Met Asn Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr
85 90 95

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130 135 140

Pro Ser Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile
145 150 155 160

Ile Cys Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu
165 170 175

Glu Asn Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp
180 185 190

Pro Glu Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met
195 200 205

Thr Thr Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg
210 215 220

Val Asn Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro
225 230 235 240

Asp Asn Leu Leu Pro Ser Trp Ala Ile Thr Leu Ile Ser Val Asn Gly
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<210> 2
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 20 25 30

Gln Val Ser Ser Asp Val Asp Glu Gln Leu Ser Lys Ser Val Lys Asp
 35 40 45

Lys Val Leu Leu Pro Cys Arg Tyr Asn Ser Pro His Glu Asp Glu Ser
 50 55 60

Glu Asp Arg Ile Tyr Trp Gln Lys His Asp Lys Val Val Leu Ser Val
 65 70 75 80

Ile Ala Gly Lys Leu Lys Val Trp Pro Glu Tyr Lys Asn Arg Thr Leu
 85 90 95

Tyr Asp Asn Thr Thr Tyr Ser Leu Ile Ile Leu Gly Leu Val Leu Ser
 100 105 110

Asp Arg Gly Thr Tyr Ser Cys Val Val Gln Lys Lys Glu Arg Gly Thr
 115 120 125

Tyr Glu Val Lys His Leu Ala Leu Val Lys Leu Ser Ile Lys Ala Asp
 130 135 140

Phe Ser Thr Pro Asn Ile Thr Glu Ser Gly Asn Pro Ser Ala Asp Thr
 145 150 155 160

Lys Arg Ile Thr Cys Phe Ala Ser Gly Gly Phe Pro Lys Pro Arg Phe
 165 170 175

Ser Trp Leu Glu Asn Gly Arg Glu Leu Pro Gly Ile Asn Thr Thr Ile

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180

185

190

Ser Gln Asp Pro Glu Ser Glu Leu Tyr Thr Ile Ser Ser Gln Leu Asp
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Phe Asn Thr Thr Arg Asn His Thr Ile Lys Cys Leu Ile Lys Tyr Gly
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Asp Ala His Val Ser Glu Asp Phe Thr Trp Glu Lys Pro Pro Glu Asp
225 230 235 240

Pro Pro Asp Ser Lys Asn Thr Leu Val Leu Phe Gly Ala Gly Phe Gly
245 250 255

Ala Val Ile Thr Val Val Val Ile Val Val Ile Ile Lys Cys Phe Cys
260 265 270

Lys His Arg Ser Cys Phe Arg Arg Asn Glu Ala Ser Arg Glu Thr Asn
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Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile Tyr Trp
20 25 30

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Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp Met Asn
35 40 45

Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr Asn Asn
50 55 60

Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly Thr Tyr
65 70 75 80

Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg Glu His
85 90 95

Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr Pro Ser
100 105 110

Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile Ile Cys
115 120 125

Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu Glu Asn
130 135 140

Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp Pro Glu
145 150 155 160

Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met Thr Thr
165 170 175

Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg Val Asn
180 185 190

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Arg Arg Asn Glu Arg Leu Arg Arg Glu Ser Val Arg Pro Val
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Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile Tyr Trp
20 25 30

Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp Met Asn
35 40 45

Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr Asn Asn
50 55 60

Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly Thr Tyr
65 70 75 80

Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg Glu His
85 90 95

Leu Ala Glu Val Thr Leu Ser Val Lys Gly Phe Ala Pro Arg Cys Arg
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<210> 5

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<223> Human CD80 ECD sequence (without signal sequence)

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Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp Met Asn
35 40 45

Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr Asn Asn
50 55 60

Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly Thr Tyr
65 70 75 80

Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg Glu His
85 90 95

Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr Pro Ser
100 105 110

Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile Ile Cys
115 120 125

Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu Glu Asn
130 135 140

Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp Pro Glu
145 150 155 160

Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met Thr Thr
165 170 175

Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg Val Asn
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Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro Asp Asn
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<211> 209
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<213> Artificial Sequence

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20 25 30

Trp Gln Lys His Asp Lys Val Val Leu Ser Val Ile Ala Gly Lys Leu
35 40 45

Lys Val Trp Pro Glu Tyr Lys Asn Arg Thr Leu Tyr Asp Asn Thr Thr
50 55 60

Tyr Ser Leu Ile Ile Leu Gly Leu Val Leu Ser Asp Arg Gly Thr Tyr
65 70 75 80

Ser Cys Val Val Gln Lys Lys Glu Arg Gly Thr Tyr Glu Val Lys His
85 90 95

Leu Ala Leu Val Lys Leu Ser Ile Lys Ala Asp Phe Ser Thr Pro Asn
100 105 110

Ile Thr Glu Ser Gly Asn Pro Ser Ala Asp Thr Lys Arg Ile Thr Cys
115 120 125

Phe Ala Ser Gly Gly Phe Pro Lys Pro Arg Phe Ser Trp Leu Glu Asn
130 135 140

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

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Glu Asp Phe Thr Trp Glu Lys Pro Pro Glu Asp Pro Pro Asp Ser Lys
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<211> 34

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<213> Artificial Sequence

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<223> Human CD80 signal sequence

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Ser Gly

<210> 8

<211> 37

<212> PRT

<213> Artificial Sequence

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<223> Mouse CD80 signal sequence

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 1 5 10 15

Pro Cys Pro Arg Leu Ile Leu Leu Phe Val Leu Leu Ile Arg Leu Ser
 20 25 30

Gln Val Ser Ser Asp

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 <223> Fc C237S

<400> 9

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
 1 5 10 15

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
 20 25 30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
 35 40 45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
 50 55 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
 65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
 85 90 95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
 100 105 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
 115 120 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
 130 135 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
 145 150 155 160

3986-007PC01_SequenceListing_ST25.txt

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
165 170 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
180 185 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
195 200 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
210 215 220

Ser Leu Ser Leu Ser Pro Gly Lys
225 230

<210> 10
<211> 228
<212> PRT
<213> Artificial Sequence

<220>
<223> Fc

<400> 10

Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val
1 5 10 15

Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
20 25 30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
35 40 45

His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
50 55 60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
65 70 75 80

Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn

85

90

95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro
 100 105 110

Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln
 115 120 125

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
 130 135 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 145 150 155 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 165 170 175

Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 180 185 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
 195 200 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
 210 215 220

Ser Pro Gly Lys
 225

<210> 11
 <211> 229
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Fc

<400> 11

Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe
 1 5 10 15

3986-007PC01_SequenceListing_ST25.txt

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
20 25 30

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
35 40 45

Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
50 55 60

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
65 70 75 80

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
85 90 95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser
100 105 110

Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
115 120 125

Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln
130 135 140

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
145 150 155 160

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
165 170 175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
180 185 190

Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser
195 200 205

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
210 215 220

Leu Ser Leu Gly Lys
225

<210> 12
<211> 232
<212> PRT
<213> Artificial Sequence

<220>
<223> Human IgG1 Fc L234F, L235E, P331S mutant

<400> 12

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1 5 10 15

Pro Glu Phe Glu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
20 25 30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
35 40 45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
50 55 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
85 90 95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
100 105 110

Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
115 120 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
130 135 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser

3986-007PC01_SequenceListing_ST25.txt

145 150 155 160

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
165 170 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
180 185 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
195 200 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
210 215 220

Ser Leu Ser Leu Ser Pro Gly Lys
225 230

<210> 13
<211> 232
<212> PRT
<213> Artificial Sequence

<220>
<223> Human IgG1 Fc N297 mutant

<400> 13

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1 5 10 15

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
20 25 30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
35 40 45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
50 55 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65 70 75 80

3986-007PC01_SequenceListing_ST25.txt

Tyr Gly Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
85 90 95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
100 105 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
115 120 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
130 135 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145 150 155 160

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
165 170 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
180 185 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
195 200 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
210 215 220

Ser Leu Ser Leu Ser Pro Gly Lys
225 230

<210> 14
<211> 232
<212> PRT
<213> Artificial Sequence

<220>
<223> Fc human IgG1

<400> 14

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala

3986-007PC01_SequenceListing_ST25.txt

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1           5           10           15

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
      20                25                30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
      35                40                45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
      50                55                60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
      65                70                75                80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
      85                90                95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
      100               105               110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
      115               120               125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
      130               135               140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
      145               150               155               160

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
      165               170               175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
      180               185               190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
      195               200               205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys

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210

215

220

Ser Leu Ser Leu Ser Pro Gly Lys
 225 230

<210> 15

<211> 279

<212> PRT

<213> Artificial Sequence

<220>

<223> Fc human IgG3

<400> 15

Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Cys Pro Arg Cys
 1 5 10 15

Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro
 20 25 30

Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro Glu
 35 40 45

Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro Ala Pro
 50 55 60

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
 65 70 75 80

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
 85 90 95

Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp
 100 105 110

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
 115 120 125

Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
 130 135 140

3986-007PC01_SequenceListing_ST25.txt

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
145 150 155 160

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg
165 170 175

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
180 185 190

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
195 200 205

Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Asn
210 215 220

Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
225 230 235 240

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser
245 250 255

Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser
260 265 270

Leu Ser Leu Ser Pro Gly Lys
275

<210> 16

<211> 229

<212> PRT

<213> Artificial Sequence

<220>

<223> Fc human IgG4

<400> 16

Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe
1 5 10 15

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr

3986-007PC01_SequenceListing_ST25.txt

20

25

30

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
 35 40 45

Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
 50 55 60

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
 65 70 75 80

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
 85 90 95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser
 100 105 110

Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
 115 120 125

Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln
 130 135 140

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
 145 150 155 160

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
 165 170 175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
 180 185 190

Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser
 195 200 205

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
 210 215 220

Leu Ser Leu Gly Lys

225

<210> 17

<211> 442

<212> PRT

<213> Artificial Sequence

<220>

<223> Mouse CD80 ECD mouse Fc IgG2a

<400> 17

Val Asp Glu Gln Leu Ser Lys Ser Val Lys Asp Lys Val Leu Leu Pro
 1 5 10 15

Cys Arg Tyr Asn Ser Pro His Glu Asp Glu Ser Glu Asp Arg Ile Tyr
 20 25 30

Trp Gln Lys His Asp Lys Val Val Leu Ser Val Ile Ala Gly Lys Leu
 35 40 45

Lys Val Trp Pro Glu Tyr Lys Asn Arg Thr Leu Tyr Asp Asn Thr Thr
 50 55 60

Tyr Ser Leu Ile Ile Leu Gly Leu Val Leu Ser Asp Arg Gly Thr Tyr
 65 70 75 80

Ser Cys Val Val Gln Lys Lys Glu Arg Gly Thr Tyr Glu Val Lys His
 85 90 95

Leu Ala Leu Val Lys Leu Ser Ile Lys Ala Asp Phe Ser Thr Pro Asn
 100 105 110

Ile Thr Glu Ser Gly Asn Pro Ser Ala Asp Thr Lys Arg Ile Thr Cys
 115 120 125

Phe Ala Ser Gly Gly Phe Pro Lys Pro Arg Phe Ser Trp Leu Glu Asn
 130 135 140

Gly Arg Glu Leu Pro Gly Ile Asn Thr Thr Ile Ser Gln Asp Pro Glu
 145 150 155 160

3986-007PC01_SequenceListing_ST25.txt

Ser Glu Leu Tyr Thr Ile Ser Ser Gln Leu Asp Phe Asn Thr Thr Arg
165 170 175

Asn His Thr Ile Lys Cys Leu Ile Lys Tyr Gly Asp Ala His Val Ser
180 185 190

Glu Asp Phe Thr Trp Glu Lys Pro Pro Glu Asp Pro Pro Asp Ser Lys
195 200 205

Asn Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys
210 215 220

Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro
225 230 235 240

Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val Thr Cys
245 250 255

Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp
260 265 270

Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg
275 280 285

Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln
290 295 300

His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn
305 310 315 320

Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly
325 330 335

Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu Glu Glu
340 345 350

Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met
355 360 365

3986-007PC01_SequenceListing_ST25.txt

Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu
370 375 380

Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe
385 390 395 400

Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn
405 410 415

Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His His Thr
420 425 430

Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
435 440

<210> 18

<211> 441

<212> PRT

<213> Artificial Sequence

<220>

<223> Mouse CD80 ECD Human Fc IgG1 WT

<400> 18

Val Asp Glu Gln Leu Ser Lys Ser Val Lys Asp Lys Val Leu Leu Pro
1 5 10 15

Cys Arg Tyr Asn Ser Pro His Glu Asp Glu Ser Glu Asp Arg Ile Tyr
20 25 30

Trp Gln Lys His Asp Lys Val Val Leu Ser Val Ile Ala Gly Lys Leu
35 40 45

Lys Val Trp Pro Glu Tyr Lys Asn Arg Thr Leu Tyr Asp Asn Thr Thr
50 55 60

Tyr Ser Leu Ile Ile Leu Gly Leu Val Leu Ser Asp Arg Gly Thr Tyr
65 70 75 80

Ser Cys Val Val Gln Lys Lys Glu Arg Gly Thr Tyr Glu Val Lys His

3986-007PC01_SequenceListing_ST25.txt

85

90

95

Leu Ala Leu Val Lys Leu Ser Ile Lys Ala Asp Phe Ser Thr Pro Asn
 100 105 110

Ile Thr Glu Ser Gly Asn Pro Ser Ala Asp Thr Lys Arg Ile Thr Cys
 115 120 125

Phe Ala Ser Gly Gly Phe Pro Lys Pro Arg Phe Ser Trp Leu Glu Asn
 130 135 140

Gly Arg Glu Leu Pro Gly Ile Asn Thr Thr Ile Ser Gln Asp Pro Glu
 145 150 155 160

Ser Glu Leu Tyr Thr Ile Ser Ser Gln Leu Asp Phe Asn Thr Thr Arg
 165 170 175

Asn His Thr Ile Lys Cys Leu Ile Lys Tyr Gly Asp Ala His Val Ser
 180 185 190

Glu Asp Phe Thr Trp Glu Lys Pro Pro Glu Asp Pro Pro Asp Ser Lys
 195 200 205

Asn Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro
 210 215 220

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
 225 230 235 240

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
 245 250 255

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
 260 265 270

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
 275 280 285

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His

290

295

300

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
 305 310 315 320

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
 325 330 335

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
 340 345 350

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
 355 360 365

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
 370 375 380

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
 385 390 395 400

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
 405 410 415

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
 420 425 430

Lys Ser Leu Ser Leu Ser Pro Gly Lys
 435 440

<210> 19

<211> 441

<212> PRT

<213> Artificial Sequence

<220>

<223> Mouse CD80 ECD Fc IgG1 MT (234, 235, 331)

<400> 19

Val Asp Glu Gln Leu Ser Lys Ser Val Lys Asp Lys Val Leu Leu Pro
 1 5 10 15

3986-007PC01_SequenceListing_ST25.txt

Cys Arg Tyr Asn Ser Pro His Glu Asp Glu Ser Glu Asp Arg Ile Tyr
20 25 30

Trp Gln Lys His Asp Lys Val Val Leu Ser Val Ile Ala Gly Lys Leu
35 40 45

Lys Val Trp Pro Glu Tyr Lys Asn Arg Thr Leu Tyr Asp Asn Thr Thr
50 55 60

Tyr Ser Leu Ile Ile Leu Gly Leu Val Leu Ser Asp Arg Gly Thr Tyr
65 70 75 80

Ser Cys Val Val Gln Lys Lys Glu Arg Gly Thr Tyr Glu Val Lys His
85 90 95

Leu Ala Leu Val Lys Leu Ser Ile Lys Ala Asp Phe Ser Thr Pro Asn
100 105 110

Ile Thr Glu Ser Gly Asn Pro Ser Ala Asp Thr Lys Arg Ile Thr Cys
115 120 125

Phe Ala Ser Gly Gly Phe Pro Lys Pro Arg Phe Ser Trp Leu Glu Asn
130 135 140

Gly Arg Glu Leu Pro Gly Ile Asn Thr Thr Ile Ser Gln Asp Pro Glu
145 150 155 160

Ser Glu Leu Tyr Thr Ile Ser Ser Gln Leu Asp Phe Asn Thr Thr Arg
165 170 175

Asn His Thr Ile Lys Cys Leu Ile Lys Tyr Gly Asp Ala His Val Ser
180 185 190

Glu Asp Phe Thr Trp Glu Lys Pro Pro Glu Asp Pro Pro Asp Ser Lys
195 200 205

Asn Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro
210 215 220

3986-007PC01_SequenceListing_ST25.txt

Ala Pro Glu Phe Glu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
225 230 235 240

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
245 250 255

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
260 265 270

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
275 280 285

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
290 295 300

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
305 310 315 320

Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
325 330 335

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
340 345 350

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
355 360 365

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
370 375 380

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
385 390 395 400

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
405 410 415

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
420 425 430

3986-007PC01_SequenceListing_ST25.txt

Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440

<210> 20
<211> 440
<212> PRT
<213> Artificial Sequence

<220>
<223> Human CD80 ECD Human Fc IgG1 WT

<400> 20

Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr Leu Ser Cys
1 5 10 15

Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile Tyr Trp
20 25 30

Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp Met Asn
35 40 45

Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr Asn Asn
50 55 60

Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly Thr Tyr
65 70 75 80

Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg Glu His
85 90 95

Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr Pro Ser
100 105 110

Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile Ile Cys
115 120 125

Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu Glu Asn
130 135 140

Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp Pro Glu
145 150 155 160

3986-007PC01_SequenceListing_ST25.txt

Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met Thr Thr
165 170 175

Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg Val Asn
180 185 190

Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro Asp Asn
195 200 205

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
210 215 220

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
225 230 235 240

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
245 250 255

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
260 265 270

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
275 280 285

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
290 295 300

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
305 310 315 320

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
325 330 335

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
340 345 350

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
355 360 365

3986-007PC01_SequenceListing_ST25.txt

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
370 375 380

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
385 390 395 400

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
405 410 415

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
420 425 430

Ser Leu Ser Leu Ser Pro Gly Lys
435 440

<210> 21

<211> 440

<212> PRT

<213> Artificial Sequence

<220>

<223> Human CD80 ECD Human Fc IgG1 L234F, L235E, P331S MT

<400> 21

Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr Leu Ser Cys
1 5 10 15

Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile Tyr Trp
20 25 30

Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp Met Asn
35 40 45

Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr Asn Asn
50 55 60

Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly Thr Tyr
65 70 75 80

3986-007PC01_SequenceListing_ST25.txt

Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg Glu His
85 90 95

Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr Pro Ser
100 105 110

Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile Ile Cys
115 120 125

Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu Glu Asn
130 135 140

Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp Pro Glu
145 150 155 160

Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met Thr Thr
165 170 175

Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg Val Asn
180 185 190

Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro Asp Asn
195 200 205

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
210 215 220

Pro Glu Phe Glu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
225 230 235 240

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
245 250 255

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
260 265 270

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
275 280 285

3986-007PC01_SequenceListing_ST25.txt

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
290 295 300

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
305 310 315 320

Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
325 330 335

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
340 345 350

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
355 360 365

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
370 375 380

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
385 390 395 400

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
405 410 415

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
420 425 430

Ser Leu Ser Leu Ser Pro Gly Lys
435 440

<210> 22

<211> 288

<212> PRT

<213> Artificial Sequence

<220>

<223> human PD-1 precursor (with signal sequence) UniProtKB/Swiss-Prot:
Q15116.3, 01-OCT-2014

<400> 22

Met Gln Ile Pro Gln Ala Pro Trp Pro Val Val Trp Ala Val Leu Gln

3986-007PC01_SequenceListing_ST25.txt

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1           5           10           15

Leu Gly Trp Arg Pro Gly Trp Phe Leu Asp Ser Pro Asp Arg Pro Trp
      20           25           30

Asn Pro Pro Thr Phe Ser Pro Ala Leu Leu Val Val Thr Glu Gly Asp
      35           40           45

Asn Ala Thr Phe Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser Phe Val
      50           55           60

Leu Asn Trp Tyr Arg Met Ser Pro Ser Asn Gln Thr Asp Lys Leu Ala
      65           70           75           80

Ala Phe Pro Glu Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg Phe Arg
      85           90           95

Val Thr Gln Leu Pro Asn Gly Arg Asp Phe His Met Ser Val Val Arg
      100          105          110

Ala Arg Arg Asn Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile Ser Leu
      115          120          125

Ala Pro Lys Ala Gln Ile Lys Glu Ser Leu Arg Ala Glu Leu Arg Val
      130          135          140

Thr Glu Arg Arg Ala Glu Val Pro Thr Ala His Pro Ser Pro Ser Pro
      145          150          155          160

Arg Pro Ala Gly Gln Phe Gln Thr Leu Val Val Gly Val Val Gly Gly
      165          170          175

Leu Leu Gly Ser Leu Val Leu Leu Val Trp Val Leu Ala Val Ile Cys
      180          185          190

Ser Arg Ala Ala Arg Gly Thr Ile Gly Ala Arg Arg Thr Gly Gln Pro
      195          200          205

Leu Lys Glu Asp Pro Ser Ala Val Pro Val Phe Ser Val Asp Tyr Gly

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210

215

220

Glu Leu Asp Phe Gln Trp Arg Glu Lys Thr Pro Glu Pro Pro Val Pro
225 230 235 240

Cys Val Pro Glu Gln Thr Glu Tyr Ala Thr Ile Val Phe Pro Ser Gly
245 250 255

Met Gly Thr Ser Ser Pro Ala Arg Arg Gly Ser Ala Asp Gly Pro Arg
260 265 270

Ser Ala Gln Pro Leu Arg Pro Glu Asp Gly His Cys Ser Trp Pro Leu
275 280 285

<210> 23

<211> 268

<212> PRT

<213> Artificial Sequence

<220>

<223> human PD-1 (mature, without signal sequence)

<400> 23

Pro Gly Trp Phe Leu Asp Ser Pro Asp Arg Pro Trp Asn Pro Pro Thr
1 5 10 15

Phe Ser Pro Ala Leu Leu Val Val Thr Glu Gly Asp Asn Ala Thr Phe
20 25 30

Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser Phe Val Leu Asn Trp Tyr
35 40 45

Arg Met Ser Pro Ser Asn Gln Thr Asp Lys Leu Ala Ala Phe Pro Glu
50 55 60

Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg Phe Arg Val Thr Gln Leu
65 70 75 80

Pro Asn Gly Arg Asp Phe His Met Ser Val Val Arg Ala Arg Arg Asn
85 90 95

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Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile Ser Leu Ala Pro Lys Ala
100 105 110

Gln Ile Lys Glu Ser Leu Arg Ala Glu Leu Arg Val Thr Glu Arg Arg
115 120 125

Ala Glu Val Pro Thr Ala His Pro Ser Pro Ser Pro Arg Pro Ala Gly
130 135 140

Gln Phe Gln Thr Leu Val Val Gly Val Val Gly Gly Leu Leu Gly Ser
145 150 155 160

Leu Val Leu Leu Val Trp Val Leu Ala Val Ile Cys Ser Arg Ala Ala
165 170 175

Arg Gly Thr Ile Gly Ala Arg Arg Thr Gly Gln Pro Leu Lys Glu Asp
180 185 190

Pro Ser Ala Val Pro Val Phe Ser Val Asp Tyr Gly Glu Leu Asp Phe
195 200 205

Gln Trp Arg Glu Lys Thr Pro Glu Pro Pro Val Pro Cys Val Pro Glu
210 215 220

Gln Thr Glu Tyr Ala Thr Ile Val Phe Pro Ser Gly Met Gly Thr Ser
225 230 235 240

Ser Pro Ala Arg Arg Gly Ser Ala Asp Gly Pro Arg Ser Ala Gln Pro
245 250 255

Leu Arg Pro Glu Asp Gly His Cys Ser Trp Pro Leu
260 265

<210> 24

<211> 290

<212> PRT

<213> Artificial Sequence

<220>

<223> human PD-L1 precursor (with signal sequence)

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<400> 24

Met Arg Ile Phe Ala Val Phe Ile Phe Met Thr Tyr Trp His Leu Leu
1 5 10 15

Asn Ala Phe Thr Val Thr Val Pro Lys Asp Leu Tyr Val Val Glu Tyr
20 25 30

Gly Ser Asn Met Thr Ile Glu Cys Lys Phe Pro Val Glu Lys Gln Leu
35 40 45

Asp Leu Ala Ala Leu Ile Val Tyr Trp Glu Met Glu Asp Lys Asn Ile
50 55 60

Ile Gln Phe Val His Gly Glu Glu Asp Leu Lys Val Gln His Ser Ser
65 70 75 80

Tyr Arg Gln Arg Ala Arg Leu Leu Lys Asp Gln Leu Ser Leu Gly Asn
85 90 95

Ala Ala Leu Gln Ile Thr Asp Val Lys Leu Gln Asp Ala Gly Val Tyr
100 105 110

Arg Cys Met Ile Ser Tyr Gly Gly Ala Asp Tyr Lys Arg Ile Thr Val
115 120 125

Lys Val Asn Ala Pro Tyr Asn Lys Ile Asn Gln Arg Ile Leu Val Val
130 135 140

Asp Pro Val Thr Ser Glu His Glu Leu Thr Cys Gln Ala Glu Gly Tyr
145 150 155 160

Pro Lys Ala Glu Val Ile Trp Thr Ser Ser Asp His Gln Val Leu Ser
165 170 175

Gly Lys Thr Thr Thr Thr Asn Ser Lys Arg Glu Glu Lys Leu Phe Asn
180 185 190

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Val Thr Ser Thr Leu Arg Ile Asn Thr Thr Thr Asn Glu Ile Phe Tyr
195 200 205

Cys Thr Phe Arg Arg Leu Asp Pro Glu Glu Asn His Thr Ala Glu Leu
210 215 220

Val Ile Pro Glu Leu Pro Leu Ala His Pro Pro Asn Glu Arg Thr His
225 230 235 240

Leu Val Ile Leu Gly Ala Ile Leu Leu Cys Leu Gly Val Ala Leu Thr
245 250 255

Phe Ile Phe Arg Leu Arg Lys Gly Arg Met Met Asp Val Lys Lys Cys
260 265 270

Gly Ile Gln Asp Thr Asn Ser Lys Lys Gln Ser Asp Thr His Leu Glu
275 280 285

Glu Thr
290

<210> 25
<211> 272
<212> PRT
<213> Artificial Sequence

<220>
<223> human PD-L1 (mature, without signal sequence)

<400> 25

Phe Thr Val Thr Val Pro Lys Asp Leu Tyr Val Val Glu Tyr Gly Ser
1 5 10 15

Asn Met Thr Ile Glu Cys Lys Phe Pro Val Glu Lys Gln Leu Asp Leu
20 25 30

Ala Ala Leu Ile Val Tyr Trp Glu Met Glu Asp Lys Asn Ile Ile Gln
35 40 45

Phe Val His Gly Glu Glu Asp Leu Lys Val Gln His Ser Ser Tyr Arg
50 55 60

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Gln Arg Ala Arg Leu Leu Lys Asp Gln Leu Ser Leu Gly Asn Ala Ala
65 70 75 80

Leu Gln Ile Thr Asp Val Lys Leu Gln Asp Ala Gly Val Tyr Arg Cys
85 90 95

Met Ile Ser Tyr Gly Gly Ala Asp Tyr Lys Arg Ile Thr Val Lys Val
100 105 110

Asn Ala Pro Tyr Asn Lys Ile Asn Gln Arg Ile Leu Val Val Asp Pro
115 120 125

Val Thr Ser Glu His Glu Leu Thr Cys Gln Ala Glu Gly Tyr Pro Lys
130 135 140

Ala Glu Val Ile Trp Thr Ser Ser Asp His Gln Val Leu Ser Gly Lys
145 150 155 160

Thr Thr Thr Thr Asn Ser Lys Arg Glu Glu Lys Leu Phe Asn Val Thr
165 170 175

Ser Thr Leu Arg Ile Asn Thr Thr Thr Asn Glu Ile Phe Tyr Cys Thr
180 185 190

Phe Arg Arg Leu Asp Pro Glu Glu Asn His Thr Ala Glu Leu Val Ile
195 200 205

Pro Glu Leu Pro Leu Ala His Pro Pro Asn Glu Arg Thr His Leu Val
210 215 220

Ile Leu Gly Ala Ile Leu Leu Cys Leu Gly Val Ala Leu Thr Phe Ile
225 230 235 240

Phe Arg Leu Arg Lys Gly Arg Met Met Asp Val Lys Lys Cys Gly Ile
245 250 255

Gln Asp Thr Asn Ser Lys Lys Gln Ser Asp Thr His Leu Glu Glu Thr
260 265 270

3986-007PC01_SequenceListing_ST25.txt