

NOVEL RATIONALLY DESIGNED PROTEIN COMPOSITIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of co-pending U.S. provisional patent applications Serial Nos. 62/752,293 filed October 29, 2018, and 62/811,116 filed February 27, 2019, which applications are incorporated herein by reference in entirety.

BACKGROUND OF INVENTION

IL2

[0002] Interleukin-2 (IL2) is a cytokine that plays a central role in both the resting and activated states of the immune system. During the resting stage, IL2 primarily ensures the development and survival of CD4⁺ Foxp3⁺ regulatory T-cells (T_{reg}s). However during an immune response, it promotes the proliferation and expansion of effector and memory T-cells and natural killer (NK) cells. It also enhances the effector function of these cells. IL2 is secreted by a variety of cells upon activation (CD8⁺ T-cells, NK and NKT-cells, DCs, and mast cells) but most of it comes from CD4⁺ T-cells; and it can work in an autocrine or paracrine manner.

[0003] Three polypeptide chains are involved in making up the IL2 receptor which bind to IL2 with different affinities when present in various combinations (Table 1). The biologically important versions of the receptor can be differentiated by their affinity for IL2: the low affinity (CD25 alone), intermediate affinity ($\beta\gamma$) and high affinity ($\alpha\beta\gamma$). CD122 and the common γ chain are necessary for signaling upon IL2 binding, while CD25 increases receptor affinity but does not appear to signal.

Table 1 IL2 receptors based on the combination of subunits

Subunit	IL2 binding (Kd)	IL2 signals
CD25 (α) (Low affinity)	10^{-8} M	-
CD122 (β)	10^{-7} M	-
γ (Common γ chain)	Not detectable	-
$\alpha\beta$	10^{-10} M	-
$\alpha\gamma$	10^{-8} M	-
$\beta\gamma$ (Intermediate affinity)	10^{-9} M	+
$\alpha\beta\gamma$ (High affinity)	10^{-11} M	+

[0004] The diverse functions of IL2 are largely a result of the differential expression of various IL2 receptor subunits on different cell types. On resting immune cells, CD25 is mainly confined to T_{reg}s, which also express the other IL2 receptor subunits, thus making them the primary target of low level IL2 in the resting state. On effector T cells, CD25 is upregulated on recently activated T-cells, along with a small increase in CD122 levels while the common γ chain expression is relatively constant, resulting in increased sensitivity and high dependence on IL2 during the expansion phase. During later stages of an immune response, memory CD8+ T-cells and NK cells express very high levels of CD122 along with the γ chain allowing them to compete with T_{reg}s for IL2.

[0005] Given IL2's role as a general T-cell growth factor, it has been used in clinics for several decades as a cancer immunotherapy. However, given the pattern of expression of the receptors, a high dose of IL2 is needed to activate intermediate-affinity receptor-bearing effector T and NK cells sufficiently to counteract its actions on the high-affinity receptor-bearing T_{reg} cells. Another problem with IL2 administration is that it has a half-life of less than 30 minutes in blood and therefore needs to be continuously infused or repeatedly injected to sustain a high enough titer to have a therapeutic effect. The high dose regimen results in many side effects including pulmonary edema (due to CD25 expression in pulmonary epithelial cells), hypotension, vascular leak syndrome, etc. Furthermore, the expansion of T_{reg} cells in the patients poses a continued threat against the anti-tumor response.

[0006] To circumvent these limitations of IL2 therapy, chemical modification, e.g. PEGylation has been used to reduce or alter the receptor selectivity of IL2 and to increase its half-life. Another approach was to use a specific anti-IL2 antibody to form a complex with IL2 to achieve preferential targeting of CD122-expressing cells. However, both these approaches have so far met with limited clinical success in addition to manufacture challenges. Alternatively, there are mutants of IL2 that preferentially bind CD122, or no longer bind CD25, allowing the effector T cells to better compete with T_{reg} cells for IL2. However these molecules continue to have a short half-life like wild type IL2, therefore requiring repeated infusions.

Checkpoint antibodies

[0007] Under normal physiological conditions, immune checkpoints are molecular pathways that have evolved for the maintenance of self-tolerance and to protect tissues from damage when the immune system is responding to infections. Tumors co-opt these pathways by mis-expressing immune-checkpoint proteins, generating an immune-suppressive environment and evading the immune system. Over the past decade, antibodies that block the CTLA-4 and PD1/PDL1 pathways have been shown to reverse tumor-associated immunosuppression and have proved to be highly

successful in the clinic. However, despite their promise, these antibodies only work on a small fraction of patients for reasons that are not completely clear yet. As a result, there is a need for a better understanding of predictive biomarkers and therapeutics that can be used in combination with checkpoint antibodies.

BRIEF SUMMARY OF THE INVENTION

[0008] The present invention meets the above challenges by using rationally designed fusion protein compositions that can simultaneously enhance anti-tumor immunity or derepress tumor-associated immunosuppression along with direct activation of effector cells by IL2 without activating T_{reg}. The fusion proteins of the present invention comprise a portion of or an entire immune checkpoint antibody, and a mutant Interleukin-2 (IL2) polypeptide. The fusion proteins of the present invention can be used to treat tumors as monotherapies or in combination with (a) antibodies targeting at least another immunosuppressive pathway; (b) chemotherapy, targeted therapy or radiation therapy; (c) another mechanism of blocking an immunosuppressive pathway, e.g., aptamers or RNAi; or (d) another immunotherapy agent, e.g. a cytokine, a targeted therapeutic, etc.

[0009] In one aspect, the present invention provides a fusion protein that combines an immune checkpoint antibody (also called anti-checkpoint antibody or anti-CP antibody hereinafter) with an IL2 mutant that is less selective towards the high affinity receptor (or interchangeably, an intermediate-affinity receptor selective mutant, MutIL2). The fusion protein is comprised of a complete anti-CP antibody connected to MutIL2 on the C-terminal end of the heavy chain (FIG. 1A). This approach would add a linker sequence between the C-terminal end of the heavy chain and MutIL2. In addition, the presence of the Fc portion of the antibody allows for the use of the Fc neonate receptor pathway to enhance the half-life of the fusion protein by orders of magnitude. Moreover, the presence of a tumor-targeting antibody will help deliver IL2 preferentially into the tumor and reduce systemic side effects.

[00010] In one feature, the anti-CP antibody is an anti-PDL1 antibody. Preferably, the anti-PDL1 antibody has one or more of the following features that can increase the anti-tumor capability of the fusion protein: (a) it binds PDL1 with K_d <100 nM to purified protein as well as protein-expressed on the cell surface; (b) it blocks PDL1-PD1 interaction in vitro; and (c) it de-represses PDL1-mediated repression of effector T cells in activated PBMC cultures.

[00011] In one embodiment, the anti-PDL1 antibody is an antibody of any available isotype or any known mutant forms of the Fc region that inactivates, reduces or enhances ADCC and/or

CDC functions. In a preferred embodiment, the anti-PDL1 antibody is an antibody of IgG1 isotype. In some embodiments, the anti-PDL1 antibody includes a pair of heavy chain variable region and light chain variable region where their respective sequences consist essentially of the following: (a) SEQ ID NO: 3 and SEQ ID NO: 6; (b) SEQ ID NO: 9 and SEQ ID NO: 12; or (c) SEQ ID NO: 15 and SEQ ID NO: 18. In some embodiments, the anti-PDL1 antibody further includes a second pair of heavy and light chains variable regions substantially identical to those of the first pair. In other embodiments, the anti-PDL1 antibody includes a pair of heavy chain and light chain where their respective sequences consist essentially of the following: (a) SEQ ID NO: 2 and SEQ ID NO: 5; (b) SEQ ID NO: 8 and SEQ ID NO: 11; or (c) SEQ ID NO: 14 and SEQ ID NO: 17.

[00012] In one feature, the MutIL2 has one or both of the following features that can increase the anti-tumor capability of the fusion protein: (a) reduce ability to activate CTLL2 and human and mouse T_{reg} while retaining its ability to activate effector T cells or NK cells; and (b) a up to 10-fold to over 1000-fold increase in its relative ability to activate T_{eff}/T_{reg} compared to wild type IL2. In a further embodiment, the MutIL2 includes amino acid sequences substantially identical to the amino acid sequences selected from the group consisting of SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57 and combinations thereof.

[00013] In one feature, the linker sequence is a flexible serine-glycine linker or other known variants or linkers. In one embodiment, the linker sequence is SEQ ID NO: 19.

[00014] In one feature, the fusion protein comprising: an antigen binding part comprising a portion of or an entire anti-PDL1 antibody and a mutant Interleukin-2 (IL2) polypeptide, the antigen binding part includes a pair of heavy chain and light chain where their respective sequences consist essentially of the following: (a) SEQ ID NO: 2 and SEQ ID NO: 5; (b) SEQ ID NO: 8 and SEQ ID NO: 11; the mutant IL2 polypeptide includes amino acid sequence substantially identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 25, SEQ ID NO: 27; and the mutant IL2 polypeptide is connected to the C-terminals of the heavy chains. In one feature, the mutant IL2 polypeptide is connected to the C-terminals of the heavy chains through a linker sequence comprising SEQ ID NO: 19.

[00015] In another embodiment, an alternative molecule of the fusion protein comprises of one or more mutated amino acids to remove proteolytic sites for improving production. In another embodiment, an alternative molecule of the fusion protein comprises one or more mutated amino

acids to remove glycosylation sites without otherwise altering the function of the protein for generating a more homogeneous product. In another embodiment, an alternative molecule of the fusion protein comprises of an alternate secretory sequence instead of a natural secretory sequence for improving secretion.

[00016] In another aspect, the present invention provides a fusion protein that combines an immune checkpoint antibody (anti-CP antibody) with an IL2 mutant that is less selective towards the high affinity receptor (or interchangeably, an intermediate-affinity receptor selective mutant, MutIL2), the fusion protein is a bispecific design with a first arm corresponding to an anti-CP antibody and a second arm consisting of MutIL2 fused to the Fc portion (FIG. 1B). In addition, the presence of the Fc portion of the protein allows the use of the Fc neonate receptor pathway to enhance the half-life of the fusion protein by orders of magnitude.

[00017] In one feature, the anti-CP antibody is an anti-PDL1 antibody. Perfectly, the anti-PDL1 antibody has one or more of the following features that can increase the anti-tumor capability of the fusion protein: (a) it binds PDL1 with a $K_d < 100$ nM to purified protein as well as protein-expressed on cell surface; (b) it blocks PDL1-PD1 interaction in vitro; and (c) it de-represses PDL1-mediated repression of effector T cells in activated PBMC cultures.

[00018] In one embodiment, the bispecific antibody is an antibody of any available isotype or any known mutant forms of Fc region that inactivates, reduces or enhances ADCC and/or CDC functions. In a perfect embodiment, the bispecific antibody is an antibody of IgG1 isotype. In some embodiments, the first arm of the bispecific antibody includes a pair of heavy chain variable region and light chain variable region where their respective sequences consist essentially of the following: (a) SEQ ID NO: 3 and SEQ ID NO: 6; (b) SEQ ID NO: 9 and SEQ ID NO: 12; or (c) SEQ ID NO: 15 and SEQ ID NO: 18. In other embodiments, the anti-PDL1 antibody includes a pair of heavy chain and light chain where their respective sequences consist essentially of the following: (a) SEQ ID NO: 2 and SEQ ID NO: 5; (b) SEQ ID NO: 8 and SEQ ID NO: 11; or (c) SEQ ID NO: 14 and SEQ ID NO: 17.

[00019] In one feature, the MutIL2 has one or both of the following features that can increase the anti-tumor capability of the fusion protein: (a) reduce ability to activate CTLL2 and human and mouse T_{reg} while retaining its ability to activate effector T cells or NK cells; and (b) up to 10-fold to over 1000-fold increase in its relative ability to activate T_{eff}/T_{reg} compared to wild type IL2. In a further embodiment, the MutIL2 includes amino acid sequence substantially identical to the amino acid sequences selected from the group consisting of SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO:

27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57 and combinations thereof.

[00020] In another embodiment, an alternative molecule of the fusion protein comprises one or more mutated amino acids to remove proteolytic sites for improving production. In another embodiment, an alternative molecule of the fusion protein comprises one or more mutated amino acids to remove glycosylation sites without otherwise altering the function of the protein for generating a more homogeneous product. In another embodiment, an alternative molecule of the fusion protein comprises an alternate secretory sequence instead of a natural secretory sequence for improving secretion.

[00021] In another aspect, the present invention also provides a fusion protein that is a polypeptide consisting of an antigen-binding polypeptide and an IL2 mutant that is less selective towards the high affinity receptor (or interchangeably, an intermediate-affinity receptor selective mutant, MutIL2) (FIG. 2). This approach would add a linker sequence between the two portions for instance. The polypeptide is a smaller molecule that could be expressed in tumor-targeting bacteria. In some embodiments, the antigen-binding polypeptide is selected from the group consisting of an anti-CP scFv, a ligand or a portion of the ligand. In some embodiments, the anti-CP scFv is an anti-PDL1 scFv. In some embodiments, the ligand is PD1 or CTLA4. In some embodiments, the antigen-binding polypeptide is selected from the group consisting of immunotherapeutic antibodies, such as anti-GD2, anti-TGFbeta, anti-CD47, anti-OX40, anti-IBB or an immunotherapeutic antibody that can work in concert with MutIL2 of this invention.

[00022] In one feature, the MutIL2 has one or both of the following features that can increase the anti-tumor capability of the fusion protein: (a) reduce ability to activate CTLL2 and human and mouse T_{reg} while retaining its ability to activate effector T cells or NK cells; and (b) up to 10-fold to over 1000-fold increase in its relative ability to activate T_{eff}/T_{reg} compared to wild type IL2. In a further embodiment, the MutIL2 includes amino acid sequence substantially identical to the amino acid sequences selected from the group consisting of SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57 and combinations thereof.

[00023] In one feature, the linker sequence is a flexible serine-glycine linker or other known variants or linkers. In one embodiment, the linker sequence is SEQ ID NO: 19.

[00024] In another embodiment, an alternative molecule of the fusion protein comprises one or more mutated amino acids to remove proteolytic sites for improving production. In another embodiment, an alternative molecule of the fusion protein comprises one or more mutated amino acids to remove glycosylation sites without otherwise altering the function of the protein for generating a more homogeneous product. In another embodiment, an alternative molecule of the fusion protein comprises an alternate secretory sequence instead of a natural secretory sequence for improving secretion.

[00025] In another aspect, the present invention provides an engineered protein, comprising a therapeutic protein, e.g., a tumor-targeting agent, that is fused to or otherwise connected to a mutant of the IL2 polypeptide selective for an intermediate-affinity receptor. In some embodiments, the therapeutic protein is a therapeutic antibody, tumor-targeting antibody, a tumor antigen-binding polypeptide, or a tumor-targeting oligonucleotides, e.g. aptamers or small molecules. In some embodiments, the tumor-targeting antibody is an immune checkpoint antibody. In some embodiments, the present invention provides an engineered protein, comprising a therapeutic protein and a mutant Interleukin-2 (IL2) polypeptide. In some embodiments, therapeutic protein is a therapeutic antibody or a tumor antigen-binding polypeptide. In some embodiments, the tumor-targeting antibody or the therapeutic antibody is an anti-PD-L1 antibody, an anti-CD19 antibody, an anti-MUC1 antibody, an anti-CD22 antibody, an anti-HER2 antibody, an anti-CD20 antibody, an anti-CD80 antibody, an anti-BCMA antibody, an anti-EGFR antibody, or an anti-Mesothelin antibody. The tumor-targeting agents can help deliver IL2 into the tumor and reduce systemic side effects. And the mutant IL2 can directly activate effector cells to enhance the anti-tumor activity of the tumor-targeting agents.

[00026] In one feature, the MutIL2 has one or both of the following features that can increase the anti-tumor capability of the fusion protein: (a) reduce ability to activate CTLL2 and human and mouse T_{reg} while retaining its ability to activate effector T-cells or NK cells; and (b) up to 10-fold to over 1000-fold increase in its relative ability to activate T_{eff}/T_{reg} compared to wild type IL2. In a further embodiment, the MutIL2 includes amino acid sequences substantially identical to the amino acid sequences selected from the group consisting of SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57 and combinations thereof.

[00027] In another aspect, the present invention provides a pharmaceutical composition that includes a fusion protein of any aspect above. The pharmaceutical composition further includes a pharmaceutically acceptable excipient, carrier, or diluent.

[00028] In a related aspect, the present invention provides a method of treating a subject in need thereof for a pathological condition therapeutically, said method comprising administering to said subject a therapeutically effective amount of the fusion protein disclosed herein. The method may further include a step of administering a second and different therapeutic antibody against at least one cell-surface antigen indicative of said condition. The condition being treated may be a mammalian cancer, an infection, and so on.

[00029] Preferably, the spectrum of mammalian cancers to be treated is selected from the a group consisting of ovarian cancer, colon cancer, breast cancer, lung cancer, myelomas, neuroblastic-derived CNS tumors, monocytic leukemias, B-cell derived leukemias, T-cell derived leukemias, B-cell derived lymphomas, T-cell derived lymphomas, mast cell derived tumors, melanoma, bladder cancer, gastric cancer, liver cancer, urothelial carcinoma, cutaneum carcinoma, renal cancer, head and neck cancer, pancreatic cancer, and combinations thereof. In some embodiments, the spectrum of mammalian cancers to be treated is selected from the a group consisting of melanoma, lung cancer, renal cancer, head neck cancer, gastric cancer, lymphoma, ovarian cancer, colon cancer, breast cancer, lung cancer, myelomas, brain tumors, leukemias, lymphomas, bladder cancer, liver cancer, urothelial carcinoma, cutaneous carcinoma, pancreatic cancer, and combinations thereof.

[00030] In yet another aspect, the invention provides a method of treating a subject in need thereof for similar conditions prophylactically, said method comprising administering to said subject a prophylactically effective amount of the pharmaceutical composition of the invention. The method may further include a step of administering a vaccine against said condition. In one embodiment, the condition is a cancer.

[00031] In a further aspect, the invention provides a mammalian expression system that produces the fusion protein described above.

BRIEF DESCRIPTION OF FIGURES

[00032] Figure 1A and 1B schematically depicts fusion protein design of the present invention.

[00033] Figure 2 schematically depicts alternate fusion protein design of the present invention.

- [00034] Figure 3A and 3B show representative data comparing binding of cell surface PDL1 by parental antibodies versus wild type and mutant IL2 fusion proteins of the present invention.
- [00035] Figure 4 shows representative ADCC activity of various fusion proteins.
- [00036] Figure 5 shows representative data comparing binding of cell surface trimeric (high-affinity) IL2 receptor by wild type IL2 fusion protein versus two mutant forms of IL2 fusion protein of the present invention.
- [00037] Figure 6 shows representative data comparing the ability of wild type IL2 fusion proteins versus various mutant forms of IL2 fusion protein of the present invention to trigger and support proliferation of CTLL2 cells (surrogate for T_{reg} cells) as measured by AlamarBlue assay.
- [00038] Figure 7 shows representative data comparing the ability of wild type IL2 fusion proteins versus various mutant forms of IL2 fusion protein of the present invention to support mouse T_{reg} cells survival and activation.
- [00039] Figure 8 shows representative data comparing the ability of wild type IL2 fusion proteins versus various mutant forms of IL2 fusion protein of the present invention to support mouse CD8+ T cells (example of T_{eff} cell) survival and proliferation.
- [00040] Figure 9 and 10 shows representative data comparing the ability of recombinant IL2 and wild type IL2 fusion proteins versus various mutant forms of IL2 fusion protein of the present invention to induce phosphorylation of Stat5 in T_{reg} and non-T_{reg} CD4⁺ cells.
- [00041] Figure 11 shows representative data comparing the ability of recombinant IL2 and wild type IL2 fusion proteins versus various mutant forms of IL2 fusion protein of the present invention to induce phosphorylation of Stat5 in CD8⁺ effector T cells.
- [00042] Figure 12 shows the pharmacokinetic profile of various mutant forms of IL2 fusion protein of the present invention.
- [00043] Figure 13 shows ability of the various mutant forms of IL2 fusion protein of the present invention to differentially induce preferential proliferation of CD8⁺ T cells and NK cells over T_{reg} cells.

DETAILED DESCRIPTION OF INVENTION

- [00044] Unless otherwise noted, technical terms are used according to conventional usage.
- [00045] As used herein, “a” or “an” may mean one or more. As used herein when used in conjunction with the word “comprising,” the words “a” or “an” may mean one or more than one. As

used herein “another” may mean at least a second or more. Furthermore, unless otherwise required by context, singular terms include pluralities and plural terms include the singular.

[00046] As used herein, “about” refers to a numeric value, including, for example, whole numbers, fractions, and percentages, whether or not explicitly indicated. The term “about” generally refers to a range of numerical values (e.g., +/-5-10% of the recited value) that one of ordinary skill in the art would consider equivalent to the recited value (e.g., having the same function or result). In some instances, the term “about” may include numerical values that are rounded to the nearest significant figure. Unless indicated otherwise, “about” is +/-10% of the recited value(s).

[00047] An “antigen-binding polypeptide” is a polypeptide comprising a portion that binds to an antigen. Examples of antigen-binding polypeptides include antibodies, antibody fragments (e.g., an antigen binding portion of an antibody), antibody derivatives, and antibody analogs.

[00048] The term “consist essentially of” or “substantially identical” as used herein, refers to at least 60%, or 80%, or, more preferably, 85%, 90%, 95%, or even 100%, identity, for example, to a selected amino acid sequence.

[00049] An antigen binding polypeptide or protein can have, for example, the structure of a naturally occurring antibody (also known as “immunoglobulin”. Each naturally occurring antibody is composed of two identical pairs of polypeptide chains, each pair having one “light” (about 25 kDa) and one “heavy” chain (about 50-70 kDa). The variable regions of each light/heavy chain pair form the antibody-binding site such that an intact antibody has two binding sites.

[00050] The variable regions of naturally occurring antibody chains exhibit the same general structure of relatively conserved framework regions (FR) joined by three hyper-variable regions, also called complementarity determining regions or CDRs. From N-terminus to C-terminus, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat et al. in *Sequences of Proteins of Immunological Interest*, 5th Ed., US Dept. of Health and Human Services, PHS, NIH, NIH Publication no. 91-3242, 1991. Other numbering systems for the amino acids in immunoglobulin chains include IMGT (international ImMunoGeneTics information system; Lefranc et al., *Dev. Comp. Immunol.* 29:185-203; 2005) and AHO (Honegger and Pluckthun, *J. Mol. Biol.* 309(3):657-670; 2001). Within the variable regions, the CDR 1, CDR 2 and CDR 3 regions are important, with CDR3 region being the most important. These can be identified by standard bioinformatics and mutagenesis experiments.

[00051] Antibodies can be obtained from sources such as serum or plasma that contain immunoglobulins having varied antigenic specificity. If such antibodies are subjected to affinity purification, they can be enriched for a particular antigenic specificity. Such enriched preparations of antibodies usually are made of less than about 10% antibody having specific binding activity for the particular antigen. Subjecting these preparations to several rounds of affinity purification can increase the proportion of antibody having specific binding activity for the antigen. Antibodies prepared in this manner are often referred to as “monospecific.” Monospecific antibody preparations can be made up of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 99.9% antibody having specific binding activity for the particular antigen.

[00052] The term “antibody” or “Ab” (and their plural forms), as used herein, broadly refers to any immunoglobulin (Ig) molecule comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains, or any functional fragment, mutant, variant, derivative or analog thereof, which retains the essential and specific epitope-binding features of an Ig molecule. Such fragment, mutant, variant, derivative or analog antibody formats are known in the art, and include, inter alia, Fab, F(ab'), F(ab')₂, Fv, single-chain antibodies (scFv), single-domain antibodies (sdAbs), complementarity determining region (CDR) fragments, chimeric antibodies, diabodies, triabodies, tetrabodies, and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide. Antibody fragments, derivatives and analogs may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies.

[00053] A Fab fragment is a monovalent fragment having the V_L, V_H, C_L and C_{H1} domains; a F(ab')₂ fragment is a bivalent fragment having two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment has the V_H and C_{H1} domains; an Fv fragment has the V_L and V_H domains of a single arm of an antibody; and a dAb fragment has a V_H domain, a V_L domain, or an antigen-binding fragment of a V_H or V_L domain (U.S. Pat. Nos. 6,846,634; 6,696,245, US App. Pub. 20/0202512; 2004/0202995; 2004/0038291; 2004/0009507; 2003/0039958, and Ward et al., *Nature* 341:544-546, 1989).

[00054] A single-chain antibody (scFv) is an antibody in which a V_L and a V_H region are joined via a linker (e.g., a synthetic sequence of amino acid residues) to form a continuous protein chain wherein the linker is long enough to allow the protein chain to fold back on itself and form a monovalent antigen binding site (see, e.g., Bird et al., 1988, *Science* 242:423-26 and Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-83). Diabodies are bivalent antibodies comprising two

polypeptide chains, where each polypeptide chain comprises V_H and V_L domains joined by a linker that is too short to allow for pairing between two domains on the same chain, thus allowing each domain to pair with a complementary domain on another polypeptide chain (see, e.g., Holliger et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:6444-48, and Poljak et al., 1994, *Structure* 2:1121-23). If the two polypeptide chains of a diabody are identical, then a diabody resulting from their pairing will have two identical antigen binding sites. Polypeptide chains having different sequences can be used to make a diabody with two different antigen-binding sites. Similarly, tribodies and tetrabodies are antibodies comprising three and four polypeptide chains, respectively, and forming three and four antigen binding sites, respectively, which can be the same or different.

[00055] Complementarity determining regions (CDRs) and framework regions (FR) of a given antibody may be identified using the system described by Kabat et al. *supra*; Lefranc et al., *supra* and/or Honegger and Pluckthun, *supra*. One or more CDRs may be incorporated into a molecule either covalently or noncovalently to make it an antigen binding protein. An antigen binding polypeptide may incorporate the CDR(s) as part of a larger polypeptide chain, may covalently link the CDR(s) to another polypeptide chain, or may incorporate the CDR(s) noncovalently. The CDRs permit the antigen binding protein to specifically bind to a particular antigen of interest.

[00056] A fusion protein may have one or more binding sites. If there is more than one binding site, the binding sites may be identical to one another or may be different. For example, a naturally occurring human immunoglobulin typically has two identical binding sites, while a “bispecific” or “bifunctional” antibody has two different binding sites.

[00057] The term “human antibody” or “humanized antibody” includes all antibodies that have one or more variable and constant regions derived from human immunoglobulin sequences. In one embodiment, all of the variable and constant domains are derived from human immunoglobulin sequences (a fully human or humanized antibody). These antibodies may be prepared in a variety of ways, including through the immunization with an antigen of interest of a mouse that is genetically modified to express antibodies derived from human heavy and/or light chain-encoding genes. A humanized antibody has a sequence that differs from the sequence of an antibody derived from a non-human species by one or more amino acid substitutions, deletions, and/or additions, such that the humanized antibody is less likely to induce an immune response, and/or induces a less severe immune response, as compared to the non-human species antibody, when it is administered to a human subject. In one embodiment, certain amino acids in the framework and constant domains of

the heavy and/or light chains of the non-human species antibody are mutated to produce the humanized antibody. In another embodiment, the constant domain(s) from a human antibody are fused to the variable domain(s) of a non-human species. In another embodiment, one or more amino acid residues in one or more CDR sequences of a non-human antibody are changed to reduce the likely immunogenicity of the non-human antibody when it is administered to a human subject, wherein the changed amino acid residues either are not critical for immunospecific binding of the antibody to its antigen, or the changes to the amino acid sequence that are made are conservative changes, such that the binding of the humanized antibody to the antigen is not significantly worse than the binding of the non-human antibody to the antigen. Examples of how to make humanized antibodies may be found in U.S. Pat. Nos. 6,054,297, 5,886,152 and 5,877,293.

[00058] Activated T cells express PD1 on their cell surface. Binding of PD-L1 to PD1 activates PD1 and suppresses the PD1⁺ T cells. A “neutralizing antibody” or an “inhibitory antibody” is an antibody that blocks the activation of PD1 when an excess of the anti-PD-L1 antibody reduces the amount of said activation by at least about 20% using an assay such as those described herein in the Examples. In various embodiments, the antigen binding protein reduces the amount of activation of PD1 by at least 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, and 99.9%.

[00059] Fragments or analogs of antibodies can be readily prepared by those of ordinary skill in the art following the teachings of this specification and using techniques known in the art. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Computerized comparison methods can be used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. See, Bowie et al., 1991, *Science* 253:164.

[00060] A fusion protein “specifically binds” to an antigen (e.g., human PD-L1) if it binds to the antigen with a dissociation constant of 100 nanomolar or less.

[00061] An “antigen binding domain”, “antigen binding region,” or “antigen binding site” is a portion of an antigen binding protein that contains amino acid residues (or other moieties) that interact with an antigen and contribute to the antigen binding protein's specificity and affinity for the antigen. For an antibody to specifically bind to its antigen, it will include at least part of at least one of its CDR domains.

[00062] An “epitope” is the portion of a molecule that is bound by an antigen binding protein (e.g., by an antibody). An epitope can comprise non-contiguous portions of the molecule (e.g., in a polypeptide, amino acid residues that are not contiguous in the polypeptide's primary sequence but that, in the context of the polypeptide's tertiary and quaternary structure, are near enough to each other to be bound by an antigen binding protein).

[00063] As used herein, the terms “polynucleotide”, “oligonucleotide” and “nucleic acid” are used interchangeably throughout and include DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), analogs of the DNA or RNA generated using nucleotide analogs (e.g., peptide nucleic acids and non-naturally occurring nucleotide analogs), and hybrids thereof. The nucleic acid molecule can be single-stranded or double-stranded. In one embodiment, the nucleic acid molecules of the invention comprise a contiguous open reading frame encoding a fusion protein, an antibody, or a fragment, derivative, mutant, or variant thereof.

[00064] A “vector” is a nucleic acid that can be used to introduce another nucleic acid linked to it into a cell. One type of vector is a “plasmid”, which refers to a linear or circular double stranded DNA molecule into which additional nucleic acid segments can be ligated. Another type of vector is a viral vector (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), wherein additional DNA segments can be introduced into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors comprising a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. An “expression vector” is a type of vector that can direct the expression of a chosen polynucleotide.

[00065] A nucleotide sequence is “operably linked” to a regulatory sequence if the regulatory sequence affects the expression (e.g., the level, timing, or location of expression) of the nucleotide sequence. A “regulatory sequence” is a nucleic acid that affects the expression (e.g., the level, timing, or location of expression) of a nucleic acid to which it is operably linked. The regulatory sequence can, for example, exert its effects directly on the regulated nucleic acid, or through the action of one or more other molecules (e.g., polypeptides that bind to the regulatory sequence and/or the nucleic acid). Examples of regulatory sequences include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Further examples of regulatory sequences are described in, for example, Goeddel, 1990, *Gene Expression Technology: Methods in*

Enzymology 185, Academic Press, San Diego, Calif. and Baron et al., 1995, *Nucleic Acids Res.* 23:3605-06.

[00066] Terms such as “therapeutic” as used herein refer to the quality and ability to cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder. Thus a therapeutic protein or polypeptide is one that has the above quality and ability. A subject is successfully “treated” according to the methods of the present invention, for example, if the patient shows one or more of the following: a reduction in the number of or complete absence of cancer cells; a reduction in the tumor size; inhibition of or an absence of cancer cell infiltration into peripheral organs including the spread of cancer into soft tissue and bone; inhibition of or an absence of tumor metastasis; inhibition or an absence of tumor growth; relief of one or more symptoms associated with the specific cancer; reduced morbidity and mortality; and improvement in quality of life.

[00067] Preferably, the broad spectrum of mammalian cancers to be treated by compositions of the present invention is selected from the group consisting of ovarian cancer, colon cancer, breast cancer, lung cancer, myelomas, neuroblastic-derived CNS tumors, monocytic leukemias, B-cell derived leukemias, T-cell derived leukemias, B-cell derived lymphomas, T-cell derived lymphomas, mast cell derived tumors, melanoma, bladder cancer, gastric cancer, liver cancer, urothelial carcinoma, cutaneum carcinoma, renal cancer, head and neck cancer, pancreatic cancer, and combinations thereof. More broadly, any cancer where at least a fraction of the tumor cells express detectable amount of PD-L1 can potentially be treated by the composition of the invention.

[00068] Fusion proteins or polypeptides of the present disclosure can be produced using any standard methods known in the art. In one example, the polypeptides are produced by recombinant DNA methods by inserting a nucleic acid sequence (e.g., a cDNA) encoding the polypeptide into a recombinant expression vector and expressing the DNA sequence under conditions promoting expression.

[00069] Nucleic acids encoding any of the various fusion proteins or polypeptides disclosed herein may be synthesized chemically. Codon usage may be selected so as to improve expression in a cell. Such codon usage will depend on the cell type selected. Specialized codon usage patterns have been developed for *E. coli* and other bacteria, as well as mammalian cells, plant cells, yeast cells and insect cells. See for example: Mayfield et al., *Proc. Natl. Acad. Sci. USA*. 2003 100(2):438-42; Sinclair et al. *Protein Expr. Purif.* 2002 (1):96-105; Connell N D. *Curr. Opin.*

Biotechnol. 2001 12(5):446-9; Makrides et al. *Microbiol. Rev.* 1996 60(3):512-38; and Sharp et al. *Yeast.* 1991 7(7):657-78.

[00070] General techniques for nucleic acid manipulation are described for example in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Vols. 1-3, Cold Spring Harbor Laboratory Press, 2 ed., 1989, or F. Ausubel et al., *Current Protocols in Molecular Biology* (Green Publishing and Wiley-Interscience: New York, 1987) and periodic updates, herein incorporated by reference. The DNA encoding the polypeptide is operably linked to suitable transcriptional or translational regulatory elements derived from mammalian, viral, or insect genes. Such regulatory elements include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences that control the termination of transcription and translation. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants is additionally incorporated.

[00071] The recombinant DNA can also include any type of protein tag sequence that may be useful for purifying the protein. Examples of protein tags include but are not limited to a histidine tag, a FLAG tag, a myc tag, an HA tag, or a GST tag. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts can be found in *Cloning Vectors: A Laboratory Manual*, (Elsevier, N.Y., 1985).

[00072] The expression construct is introduced into the host cell using a method appropriate to the host cell. A variety of methods for introducing nucleic acids into host cells are known in the art, including, but not limited to, electroporation; transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (where the vector is an infectious agent). Suitable host cells include prokaryotes, yeast, mammalian cells, or bacterial cells.

[00073] Proteins disclosed herein can also be produced using cell-translation systems. For such purposes the nucleic acids encoding the polypeptide must be modified to allow in vitro transcription to produce mRNA and to allow cell-free translation of the mRNA in the particular cell-free system being utilized (eukaryotic such as a mammalian or yeast cell-free translation system or prokaryotic such as a bacterial cell-free translation system).

[00074] The bispecific molecule disclosed herein can also be produced using cell-translation systems. For the sake of manufacturing ease, there are several ways to ensure that the heterodimeric

bispecific molecule is preferentially produced in the cells by using complementary mutations on the two heavy molecules.

[00075] The fusion protein or polypeptide of the present disclosure can also be produced by chemical synthesis (e.g., by the methods described in *Solid Phase Peptide Synthesis*, 2nd ed., 1984, The Pierce Chemical Co., Rockford, Ill.). Modifications to the protein can also be produced by chemical synthesis.

[00076] Another version of the molecule of the fusion protein has mutations in one or more amino acids to remove proteolytic sites to improve production.

[00077] Another version of the molecule of the fusion protein has mutations in one or more amino acids to remove glycosylation sites without otherwise altering the function of the protein to generate a more homogeneous product.

[00078] Another version of the molecule of the fusion protein uses an alternate secretory sequence instead of the natural antibody secretory sequence to improve secretion.

[00079] The fusion proteins or polypeptides of the present disclosure can be purified by isolation/purification methods for proteins generally known in the field of protein chemistry. Non-limiting examples include extraction, recrystallization, salting out (e.g., with ammonium sulfate or sodium sulfate), centrifugation, dialysis, ultrafiltration, adsorption chromatography, ion exchange chromatography, hydrophobic chromatography, normal phase chromatography, reversed-phase chromatography, gel filtration, gel permeation chromatography, affinity chromatography, electrophoresis, countercurrent distribution or any combination of these. After purification, polypeptides may be exchanged into different buffers and/or concentrated by any of a variety of methods known to the art, including, but not limited to, filtration and dialysis.

[00080] The purified fusion protein or polypeptide is preferably at least 85% pure, more preferably at least 95% pure, and most preferably at least 98% pure. Regardless of the exact numerical value of the purity, the polypeptide is sufficiently pure for use as a pharmaceutical product.

Post-Translational Modifications of Polypeptides

[00081] In certain embodiments, the fusion protein or polypeptides of the invention may further comprise post-translational modifications. Exemplary post-translational protein modifications include phosphorylation, acetylation, methylation, ADP-ribosylation, ubiquitination, glycosylation, carbonylation, sumoylation, biotinylation or addition of a polypeptide side chain or of

a hydrophobic group. As a result, the modified soluble polypeptides may contain non-amino acid elements, such as lipids, poly- or mono-saccharide, and phosphates. A preferred form of glycosylation is sialylation, which conjugates one or more sialic acid moieties to the polypeptide. Sialic acid moieties improve solubility and serum half-life while also reducing the possible immunogenicity of the protein. See Raju et al. *Biochemistry*. 2001 31; 40(30):8868-76. Effects of such non-amino acid elements on the functionality of a polypeptide may be tested for its antagonizing role in PD-L1 or PD-1 function, e.g., its inhibitory effect on angiogenesis or on tumor growth.

[00082] In one embodiment, biological activity refers to its ability to bind to PD-L1, as assessed by KD, kon or koff rates. In one specific embodiment, the pegylated polypeptide protein shows an increase in binding to human PD-L1 relative to the unpegylated counterpart. In another embodiment, the biological activity refers to blockage of PD-L1/PD1 interaction.

Therapeutics, Vaccines & Administration

[00083] The present disclosure further features methods for treating conditions or preventing pre-conditions. Preferred examples are conditions that are characterized by cellular hyperproliferation and sustained infection. Techniques and dosages for administration vary depending on the type of specific polypeptide and the specific condition being treated. Because regulatory agencies require that a protein reagent to be used as a therapeutic be formulated with acceptably low levels of pyrogens, therapeutic formulations of the present invention can be distinguished from other formulations for being substantially pyrogen free, or at least contain no more than acceptable levels of pyrogen as determined by the appropriate regulatory agency (e.g., FDA).

[00084] Pharmaceutical formulations of the present invention may include at least one pharmaceutically acceptable diluent, carrier, or excipient. Excipients included in the formulations will have different purposes depending, for example, on the kind of gene construct or effector cells used, and the mode of administration. Examples of generally used excipients include, without limitation: saline, buffered saline, dextrose, water-for-injection, glycerol, ethanol, and combinations thereof, stabilizing agents, solubilizing agents and surfactants, buffers and preservatives, tonicity agents, bulking agents, and lubricating agents.

[00085] In another embodiment of the invention, a pharmaceutical formulation of the invention is administered into the patient. Exemplary administration modes include, but are not

limited to, intravenous injection. Other modes include, without limitation, intratumoral, intradermal, subcutaneous (s.c., s.q., sub-Q, Hypo), intramuscular (i.m.), intraperitoneal (i.p.), intra-arterial, intramedullary, intracardiac, intra-articular (joint), intrasynovial (joint fluid area), intracranial, intraspinal, and intrathecal (spinal fluids). Any known device useful for parenteral injection or infusion of the formulations can be used to effect such administration. As used herein, the terms “treat”, “treating”, and “treatment” have their ordinary and customary meanings, and include one or more of: blocking, ameliorating, or decreasing in severity and/or frequency a symptom of a disease (e.g., cancer) in a subject, and/or inhibiting the growth, division, spread, or proliferation of cancer cells, or progression of cancer (e.g., emergence of new tumors) in a subject. Treatment means blocking, ameliorating, decreasing, or inhibiting by about 5% to about 100% versus a subject in which the methods of the present invention have not been practiced. Preferably, the blocking, ameliorating, decreasing, or inhibiting is about 100%, 99%, 95%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, or 5% versus a subject in which the methods of the present invention have not been practiced.

[00086] The invention also provides a kit comprising one or more containers filled with quantities of gene constructs encoding the fusion protein or polypeptides of the invention, with pharmaceutically acceptable excipients. The kit may also include instructions for use. Associated with the kit may further be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

EXAMPLES

[00087] Example 1

[00088] Anti-PDL1 light chain (IgL) and anti-PD-L1 heavy chain (IgH) fused to MutIL2 were cloned into a single eukaryotic expression vector with two expression cassettes - pCHO 1.0 vector. Other similar vectors are commercially available. Moreover, the two genes could be cloned in separate vectors. The vector was transfected into Expi293 cells following the manufacturer's recommendations. The protein was secreted into the supernatant and purified to > 95% purity using a HiTrap Protein A column using standard procedures. The purified proteins were compared with wild type IL2 for their ability to preferentially activate T_{eff} cells than T_{reg} cells. In this application, fusions are named using both anti-PDL1 antibody and the specific MutIL2. For instance, E1M1 contains the E1 antibody and a MutIL2 IL2M1.

[00089] Example 2

[00090] To test the ability of the proteins to bind cell surface PDL1, we used DLD1 cells expressing transgenically expressed human PDL1. Similar results could be obtained either with transgenic expression in other cell lines or by using cells that naturally express PDL1 or are induced to express PDL1 by interferon γ treatment. Briefly, 10^5 cells were incubated with different concentrations of the protein for 30 minutes at 4°C. Cells were washed with PBS and incubated with a fluorescent, anti-human-IgG antibody for 30 minutes at 4°C. Cells were again washed with PBS and binding was detected by FACS. FIG. 3 shows that the fusions with different IL2 mutations do not affect their PDL1 binding (Table 2).

Table 2 EC50 for cell surface PDL1 binding by IL2 fusions

	Mean (pM)	SD
E1	1958	297
E1WT	2006	1236
E1M1	2444	782
E1M2	3204	1294
B2WT	1482	-
B2M1	688	-
B2M2	1427	-

[00091] Similarly, to test the ability of the antibody part to block PD1-PDL1 interactions, receptor blocking assays were performed in two distinct ELISA formats – with either biotinylated Fc-PDL1 binding to PD1-coated plates or biotinylated Fc-PD1 binding to PDL1-coated plates. In both cases, binding was detected by using streptavidin linked horse radish peroxidase. Table 3 shows that the exemplary PDL1 antibodies show high potency in blocking PD1-PDL1 interaction.

Table 3 IC50 for blocking PD1-PDL1 binding by anti-PDL1 antibodies

	PD1 coated	PDL1 coated
E1	1.26 nM	1.53 nM
B2	1.39 nM	0.80 nM
E3	92.74 nM	16.57 nM

[00092] Example 3

[00093] To test ADCC activity, we used a commercial ADCC assay kit (Promega) that relies on Jurkat cells with an Fc receptor-responsive luciferase gene. Co-incubation of these cells with PDL1⁺ MDA-MB-231 cells and an ADCC+ antibody triggers Fc receptor engagement which can be

measured as luciferase activity. FIG. 4 shows that various fusion proteins of the invention show ADCC activity.

[00094] Example 4

[00095] To test the ability of the mutant proteins to bind IL2 receptors on CTLL2 cells, 10^5 cells were incubated with different concentrations of the protein for 30 minutes at 4°C. Cells were washed with PBS and incubated with a fluorescent, anti-human-IgG antibody for 30 minutes at 4°C. Cells were again washed with PBS and binding was detected by FACS. FIG. 5 shows that fusions with different IL2 mutants show dramatic reduction in binding to the high affinity IL2 receptor on CTLL2 cells compared to the fusion with wildtype IL2.

[00096] Example 5

[00097] To test the ability of the mutant proteins to activate CTLL2 cells (surrogate for T_{reg} cells), 10^4 cells were cultured in the presence of different concentrations of mutant or wild type IL2 fusion protein for 44 hours. Relative viable cell counts were determined by incubating the cultures with AlamarBlue for 1-4 hours and measuring fluorescence intensity using a fluorescent plate reader. FIG. 6 shows that fusions with different IL2 mutants show a greater than 100-fold reduced ability to support proliferation of CTLL2 cells compared to the fusion with wild type IL2 (Table 4). This is true with fusions containing either PDL1 antibody. The wild type fusion is comparable to recombinant IL2.

Table 4 EC50 for CTLL2 proliferation by IL2 fusions

	Mean (pM)	SD
E1WT	29	6
E1M1	19520	707
E1M2	9753	3779
B2WT	88	6
B2M1	9901	5156
B2M2	3312	189

[00098] Example 6

[00099] To test the ability of the mutant proteins to activate various mouse T-cell subsets, we used cells from a variety of sources. Mouse spleens or lymph nodes were used to isolate CD4, CD8 or regulatory T cells using Stem Cell Technologies magnetic bead-based cell isolation kit following the manufacturer's recommendations. Mouse NK cells assays were done with total splenocytes. Other isolation procedures are commercially available. Human T cell subsets and NK cells were commercially purchased but can be isolated from PBMCs using kits similar to those for mouse cells.

Different cell types were incubated with varied amounts of mutant or wild type IL2 fusion protein. For instance, $2.5 \times 10^4 - 10^5$ mouse T cells were used for mouse T-cell experiments. For mouse NK cells, $1-5 \times 10^6$ splenocytes were used. For T_{reg} cultures, cells were activated with plate bound anti-CD3 antibodies and incubated as well with a mouse IL2 neutralizing antibody. After 4 days of growth, cells proliferation was measured by direct counting (with or without propidium iodide based exclusion of dead cells) or by CFSE dilution using FACS. In addition, expression of activation markers was also assessed e.g. CD44 for mouse CD8 cells. FIG. 7 shows that fusions with different IL2 mutants show a significant reduction in their ability to activate and support the survival of T_{reg} cells when compared to the fusion with wild type IL2. FIG. 8 and Table 5 show that IL2 mutant fusions are comparable to the wildtype IL2 fusion protein in their ability to support proliferation of mouse CD8+ T-cells. The wild type IL2 fusion is comparable to recombinant IL2 in both assays.

[000100] FIG. 6, FIG. 7 and FIG. 8 indicate that fusions with different IL2 mutants show a greater than 100-fold increase in their relative ability to activate T_{eff}/T_{reg} compared to wild type IL2 fusion.

Table 5 EC50 for mouse CD8 T cell survival and proliferation by IL2 fusions

	Mean (pM)	SD
E1WT	8095	1052
E1M1	7654	2348
E1M2	7947	1197
B2WT	5921	1461
B2M1	4875	1604
B2M2	7651	1969

[000101] Example 7

[000102] To test the ability of the mutant proteins to activate various human T-cell subsets, we used cells from peripheral blood from healthy human donors. PBMCs were prepared by standard procedures and incubated with recombinant IL2 or different IL2 fusions for 15 minutes. Phosphorylation of Stat5 triggered by engagement of the IL2 receptor on different T-cell subpopulations was assessed by staining for appropriate cell markers and analyzing by FACS. FIG. 9 and FIG.10 show that compared to the fusion with wild type IL2, the fusions with different IL2 mutants show a significant (about 100-fold) increase in their relative ability to activate non- T_{reg}/T_{reg} (data also summarized in Tables 6-8). FIG. 11 shows that IL2 mutant fusions are comparable to the wildtype IL2 fusion protein in their ability to induce phosphorylation of Stat5 in CD8+ T-cells (data also summarized in Table 9). Compared to the fusion with wild type IL2, the fusions with different

IL2 mutants show a significant (about 1000-fold) increase in their relative ability to activate CD8+ T-cells/T_{reg} CD4+ (see Table 10). The wild type IL2 fusion is comparable to recombinant IL2 in each assay. These data shows that the fusions with different IL2 mutants have reduced ability to activate human T_{reg} while retaining its ability to activate effector T-cells, and show a greater than 100-fold increase in its relative ability to activate T_{eff}/T_{reg} compared to the fusion with wild type IL2.

Table 6 EC50 for Stat5 phosphorylation by IL2 fusions in T_{reg} (CD4⁺CD25^{high}) cells

	Median (pM)	Range (pM)
IL2	0.46	0.16 -0.9
E1WT	0.36	0.2-0.39
E1M1	148.9	108 - 184
E1M2	198.5	128 - 202
B2WT	0.25	0.07 - 0.3
B2M1	91.2	53 -148
B2M2	146.0	89 - 198

Table 7 EC50 for Stat5 phosphorylation by IL2 fusions in non-T_{reg} (CD4⁺CD25⁻) cells

	Median (pM)	Range (pM)
IL2	47.0	33-217
E1WT	34.9	23 -61
E1M1	198.1	152 - 336
E1M2	207.3	188 - 340
B2WT	27.7	14 - 36
B2M1	83.8	80 - 94
B2M2	141.0	119 – 145

Table 8 EC50 ratios between non-T_{reg} and T_{reg} CD4⁺ for various IL2 fusions

	Ratio
IL2	102
E1WT	96
E1M1	1.3
E1M2	1.0
B2WT	111
B2M1	0.9
B2M2	1.0

Table 9 EC50 for Stat5 phosphorylation by IL2 fusions in CD8⁺ cells

	Mean (pM)	SD
IL2	1236	536

E1WT	258	92
E1M1	522	66
E1M2	520	199
B2WT	303	234
B2M1	342	221
B2M2	370	212

Table 10 EC50 ratios between CD8⁺ cells and Treg CD4⁺ for various IL2 fusions

	Ratio
IL2	2686
E1WT	714
E1M1	4
E1M2	3
B2WT	1216
B2M1	4
B2M2	3

[000103] Example 8

[000104] For PK studies, female homozygous Tg32 mice (6-8 week-old), were injected with test fusion protein intravenously via tail vein at a dose of 0.1-10 mg/kg into 5 animals per group. Blood was drawn at multiple time points and serum was prepared by centrifugation. Amount of fusion protein was estimated by sandwich ELISA. The titer was normalized to Day 1 after injection.

[000105] FIG. 12 shows the pharmacokinetics of four MutIL2 fusions. The half-lives of the fusions are shown in Table 11. This is significantly longer than the reported half-life of recombinant IL2 by a couple of hours.

Table 11: Serum half-life of various MutIL2 fusions

	E1M1	E1M2	B2M1	B2M2
Half Life(Days)	1.563	1.383	1.196	1.139

[000106] Example 9

[000107] For mouse pharmacodynamics and toxicology studies, wild type B6 mice were injected with test antibody intravenously via tail vein at a dose of 0.1-10 mg/kg and observed for several days for immune activation and adverse reaction. Blood was drawn on multiple days to assess T-cell and NK cell compartments for expansion. At the end of observation, mice were

sacrificed and spleens were analyzed similarly and lungs and livers were assessed for lymphocyte infiltration and other immune reactions.

[000108] FIG. 13 shows there is a large expansion of the NK and CD8⁺ T-cell compartments relative to the CD4⁺ compartment. The ability of the various mutant forms of the IL2 fusion proteins of the present invention to differentially induce preferential proliferation of CD8⁺ T-cells and NK cells over T_{reg} cells indicates the great potential of the fusion proteins to improve the anti-tumor activity of checkpoint antibodies and their clinical applications in cancer treatment.

[000109] Example 10

[000110] For detection of tumor targeting by the fusion proteins, either syngeneic tumors (e.g. MC38 in B6 mice) or syngeneic tumors expressing human PDL1 were used. Typically, 1x10⁶ tumor cells were implanted subcutaneously and allowed to grow until they reached 100 mm². The mice were injected with 0.1-10 mg/kg of the fusion protein. Tumor tissue and other organs were harvested at various time points and the accumulation of the fusion protein was determined by sandwich ELISA or immunohistochemistry. Alternatively, radioactively labeled proteins were used and detected using standard methods.

Sequences

[000111] Examples of sequences that form part of the fusion protein according to the present inventions are listed as follows.

[000112] Examples of the full-length sequences and variable region sequences for anti-PDL1 antibodies that block PD1-PDL1 interaction are as described below. The DNA sequences as examples are also described below. Other variations based on alternate codon usage of the DNA sequences are alternative under this invention.

[000113] **PDL1 antibody**

[000114] B2 Heavy chain

[000115] DNA sequence (SEQ ID NO:1)

ATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTCGCGATTCTTAAGGGTGTCCAGTGCGAAGTGCA
GCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGAGGGTCCCTGAGACTCTCCTGTGCAGCC
TCTGGATTCACTTTTAGTGACTATGACATGATCTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGG
AGTGGGTGGCAGTTATATCATATGATGGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCG
ATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCT
GAGGACACGGCTGTGTATTACTGTGCGAAAGAGTTCTTTGGTGCTTTTGATATCTGGGGCCAAG
GGACAATGGTCACCGTCTCTTCAGCTTCGACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCTCC

TCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAAC
CGGTGACGGTGTCTGTGAACTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCT
ACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACC
CAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG
CCCAAATCTTGTGACAAAACCTACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGAC
CGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTC
ACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGAC
GGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGT
GTGGTCAGCGTCTCACCCTGCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG
GTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCC
GAGAACCACAGGTGTACACCCTGCCCCATCCCGCAGGAGATGACCAAGAACCAGGTCAGCC
TGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCA
GCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTAC
AGCAAGCTCACCCTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG
CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

[000116] Protein sequence

[000117] Full length (SEQ ID NO:2)

MEFGLSWLFLVAILKGVQCEVQLVESGGGLVQPGGSLRLSCAASGFTFSDYDMIWVRQAPGKGLE
WVAVISYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKEFFGAFDIWGQGT
MVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSP
GK

[000118] Variable region (SEQ ID NO:3)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYDMIWVRQAPGKGLEWVAVISYDGSNKYYADSVK
GRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKEFFGAFDIWGQGTMTVSS

[000119] B2 Light chain

[000120] DNA sequence (SEQ ID NO:4)

ATGACTTGGACCCCACTCCTCTTCCTCACCCTCCTCCTCCACTGCACAGGAAGCTTATCCTCTTCT
GAGCTGACTCAGGACCCTGCTGTGTGCGTGGCCTTGGGACAGACAGTCACGATCACATGCCAAG
GAGACAGCCTCAATTACTATTATGCAAACCTGGTTCCAGCTGAAGCCAGGGCAGGCCCTGTACT
TGTCTCTTTGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATTCTCTGGCTCCTACTCGG

GAAGCACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGATGACGCTGACTATTACTGTAA
TTCGCGGGACAGCGGTGGTAATCCTTGGGTGTTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGT
CAACCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACA
AGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCCTGGAAGGC
AGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCCTCCAAACAAAGCAACAACAA
GTACGCGGCCAGCAGCTACCTGAGCCTGACGCCTGAGCAGTGGAAAGTCCCACAGAAGCTACAG
CTGCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGACAGTGGCCCCCTACAGAATGTTTCATG
A

[000121] Protein sequence

[000122] Full length (SEQ ID NO:5)

MTWTPLLFLTLLHCTGSLSSSELTQDPAVSVALGQTVTITCQGDSLNYYYANWFQLKPGQAPVLV
LFGKNNRPSGIPDRFSGSYSGSTASLTITGAQAEDDADYYCNSRDSGGNPWVFGGGTKLTVLGQPKA
APSVTLFPSSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTPSKQSNKYAASSYL
SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

[000123] Variable region (SEQ ID NO:6)

SSELTQDPAVSVALGQTVTITCQGDSLNYYYANWFQLKPGQAPVLVLFKGNNRPSGIPDRFSGSYSG
STASLTITGAQAEDDADYYCNSRDSGGNPWVFGGGTKLTVL

[000124] E1 Heavy chain

[000125] DNA sequence (SEQ ID NO:7)

ATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTCGCGATTCTTAAGGGTGTCCAGTGCCAAATCCA
GCTGGTACAATCTGGGGCTGAGGTGAAGATGCCTGGGGCCTCAGTGACGATTTCTGCGAGGCG
TCTGGATACAACCTTCATCAGCTACTATATACACTGGGTGCGACAGGCCCTGGACAAGGCCTTG
AGTGGATGGGATTCGTCGTCCTAGTGGTGGTGCCGACAGGCTACACACAGAAAGTTCCAGGGCAG
ACTCACCGTGACCAGGGACACGTCCACGAGCACAGTCTACATGGACCTGAACAGCCTGACATCT
GACGACACGGCCGTGTATTACTGTGTGCGAGAAATGAGTGGTGGCTGGTTTGATTTCTGGGGCC
AGGGAACCCTGGTCACCGTCTCCTCGGCTTCGACCAAGGGCCCATCGGTCTTCCCCCTGGCACC
CTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCC
GAACCGGTGACGGTGTCTGTGGAATCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTG
TCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGC
ACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTT
GAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGGG
GACCGTCAGTCTTCTCTTCCCCCAAAACCAAGGACACCCTCATGATCTCCCGGACCCCTGAG
GTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGG
ACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACC

GTGTGGTCAGCGTCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAA
GGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCC
CCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGCGAGGAGATGACCAAGAACCAGGTGAG
CCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGG
CAGCCGGAGAACAATAACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCT
ACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGA
TGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

[000126] Protein sequence

[000127] Full length (SEQ ID NO:8)

MEFGLSWLFLVAILKGVQCQIQLVQSGAEVKMPGASVTISCEASGYNFISYYIHWVRQAPGQGLEW
MGFVVPSSGGAAGYTQKFQGRLLTVTRDTSTSTVYMDLNSLTSDDAVYYCVREMSGGWDFWQGG
TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNKGKEYCKKVSNAKALPAIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSP
GK

[000128] Variable region (SEQ ID NO:9)

SCEASGYNFISYYIHWVRQAPGQGLEWMGFVVPSSGGAAGYTQKFQGRLLTVTRDTSTSTVYMDLNS
LTSDDAVYYCVREMSGGWDFWQGGTLVTVSS

[000129] E1 Light chain

[000130] DNA sequence (SEQ ID NO:10)

ATGGACATGCGCGTGCCCGCCCAGCTGCTGGGCCTGCTGCTGCTGTGGTTCCCCGGCTCGCGAT
GCGACATCGTGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCAT
CACTTGCCGGGCAAGTCAGGGCATTAGAAAATGATTTAGGCTGGTATCAGCAAAAACCAGGGAA
AGCCCCTAAGCTCCTGATCTATGCTGCATCCACTTTGCAAAGTGGGGTCCCATCAAGGTTACAGC
GGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTGAAGATGTGGCAG
TTTATTACTGTGAGCAATATTATAGTACTCCTCTCACTTTTCGGCCCTGGGACCAAAAGTGGATATC
AAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGG
AACTGCCTCTGTTGTGTGCCTGCTGAATAAATTCTATCCCAGAGAGGCCAAAAGTACAGTGGAAG
GTGGATAACGCCCTCCAATCGGGTAACCTCCAGGAGAGTGTACAGAGCAGGACAGCAAGGAC
AGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTC
TACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGA
GAGTGTGA

[000131] Protein sequence

[000132] Full length (SEQ ID NO:11)

MDMRVPAQLLGLLLWFPGRCDIVMTQSPSSLSASVGDRVITICRASQGIRNDLGWYQQKPGKAP
KLLIYAASLTQSGVPSRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPLTFGPGTKVDIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[000133] Variable region (SEQ ID NO:12)

DIVMTQSPSSLSASVGDRVITICRASQGIRNDLGWYQQKPGKAPKLLIYAASLTQSGVPSRFSGSGSG
TDFTLTISSLQAEDVAVYYCQQYYSTPLTFGPGTKVDIK

[000134] E3 Heavy chain

[000135] DNA sequence (SEQ ID NO:13)

ATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTCGCGATTCTTAAGGGTGTCCAGTGCCAAATCCA
GCTGGTACAATCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCT
TCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGGTGCGACAGGCCCTGGACAAGGGCTTG
AGTGGATGGGAGGGATCATCCCTATCTTTGGTACAGCAAACTACGCACAGAAGTTCCAGGGCAG
AGTCACGATTACCGCGGACAAATCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATC
TGAGGACACGGCCGTGTATTACTGTGCCGGAGGGGGAGCAGTGGCGGACAATAGTTACTGGGG
CCAGGGAACCTGGTCACCGTCTCCTCAGCTTCGACCAAGGGCCCATCGGTCTTCCCCCTGGCA
CCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCC
CCGAACCGGTGACGGTGTCTGTGGAAGTCAAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGG
TGTCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGG
GCACCCAGACCTACATCTGCAACGTGAATCACAAGCCAGCAACACCAAGGTGGACAAGAAAG
TTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGG
GGGACCGTCAGTCTTCTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCCGGACCCCTG
AGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGT
GGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGT
ACCGTGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG
CAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCA
GCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGCGAGGAGATGACCAAGAACCAGGT
CAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT
GGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGTCTGGACTCCGACGGCTCCTTCTTCC
TCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGT
GATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

[000136] Protein sequence

[000137] Full length (SEQ ID NO:14)

MEFGLSWLFLVAILKGVQCQIQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEW
MGGIIPFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDNAVYYCAGGGAVADNSYWGQGT
LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSP
GK

[000138] Variable region (SEQ ID NO:15)

QIQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIIPFGTANYAQKFQGR
VTITADKSTSTAYMELSSLRSEDNAVYYCAGGGAVADNSYWGQGT LVTVSS

[000139] E3 Light chain**[000140]** DNA sequence (SEQ ID NO:16)

ATGGACATGCGCGTGCCCGCCAGCTGCTGGGCCTGCTGCTGCTGTGGTTCCCGGGCTCGCGAT
GCGACATCCGGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCAT
CACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTATCAGCAGAAACCAGGGAA
AGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTACAAAGTGGGGTCCCATCAAGGTTTCAGC
GGCAGTGGATCTGGCACAGATTTCACTCTCACCATCAGCAGCCTGCAGCCTGAAGATTTTGCAA
CTTATTACTGTCTACAAGATTACAATTACCCTCGAACGTTTCGGCCAAGGGACCAAGGTGGAAAT
CAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTG
GAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAA
GGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGA
CAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGT
CTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGA
GAGTGTGTA

[000141] Protein sequence**[000142]** Full length (SEQ ID NO:17)

MDMRVPAQLLGLLLWFPGSRCDIRMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAP
KLLIYAASSLQSGVPSRFSGSGSGTDFLTISLQPEDFATYYCLQDYNYPRTFGQGTKVEIKRTVAAP
SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[000143] Variable region (SEQ ID NO:18)

DIRMTQSPSSLSASVGDRVITTCRASQGIRNDLGWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSG
 TDFTLTISLQPEDFATYYCLQDYNYPRTFGQGTKVEIK

[000144] Example sequence of the linker is listed below:

[000145] **Ser-Gly linker**

[000146] Protein sequence (SEQ ID NO:19)

GGGGSGGGSGGGSGGGGS

[000147] Example sequence of (a) IL2WT (Wild type IL2 sequence); (b) IL2Del (20 amino acid deletion); and (c) IL2M1-MNew (MutIL2: the mutants that have the desired features, combinations of these mutations could also be used) are listed below:

[000148] **IL2 mutants**

[000149] IL2WT

[000150] DNA sequence (SEQ ID NO:20)

GGCGCCCCACATCCTCCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCATCTGCTGCTGGATC
 TGCAGATGATTTTAAACGGCATCAACAACACTACAAGAACCCCAAGCTGACCCGGATGCTGACCTT
 CAAGTTCTACATGCCCAAGAAGGCCACCGAGCTGAAGCATTACAGTGTCTGGAGGAAGAGCT
 GAAGCCTTTAGAGGAAGTGCTCAATTTAGCCCAGTCCAAGAAGCTCCATTTAAGGCCACGTGAT
 TTAATCTCCAACATCAACGTGATCGTGCTGGAGCTGAAGGGCTCCGAGACCACCTTCATGTGCG
 AGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGATCACCTTCTGCCAGAG
 CATCATCTCCACTTTAACCTGA

[000151] Protein sequence (SEQ ID NO:21)

GAPTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
 EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

[000152] IL2Del

[000153] DNA sequence (SEQ ID NO:22)

GGCGCCCCACATCCTCCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCATCTGCTGCTGGATC
 TGCAGATGATTTTAAACGGCATCAACGCCACCGAGCTGAAGCATTACAGTGTCTGGAGGAAGA
 GCTGAAGCCTTTAGAGGAAGTGCTCAATTTAGCCCAGTCCAAGAAGCTCCATTTAAGGCCACGT
 GATTTAATCTCCAACATCAACGTGATCGTGCTGGAGCTGAAGGGCTCCGAGACCACCTTCATGT
 GCGAGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGATCACCTTCTGCCA
 GAGCATCATCTCCACTTTAACCTGA

[000154] Protein sequence (SEQ ID NO:23)

GAPTSSSTKKTQLQLEHLLLDLQMILNGINATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLIS
NINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

[000155] IL2M1: F42K, Y45R

[000156] DNA sequence (SEQ ID NO:24)

GGCGCCCCACATCCTCCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCATCTGCTGCTGGATC
TGCAGATGATTTTAAACGGCATCAACAACACTACAAGAACCCCAAGCTGACCCGGATGCTGACCAA
GAAGTTCCGCATGCCCAAGAAGGCCACCGAGCTGAAGCATTACAGTGTCTGGAGGAAGAGCT
GAAGCCTTTAGAGGAAGTGCTCAATTTAGCCAGTCCAAGAAGCTTCCATTTAAGGCCACGTGAT
TTAATCTCCAACATCAACGTGATCGTGCTGGAGCTGAAGGGCTCCGAGACCACCTTCATGTGCG
AGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGATCACCTTCTGCCAGAG
CATCATCTCCACTTTAACCTGA

[000157] Protein sequence (SEQ ID NO:25)

GAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTKKFRMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

[000158] IL2M2: F42A, Y45R

[000159] DNA sequence (SEQ ID NO:26)

GGCGCCCCACATCCTCCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCATCTGCTGCTGGATC
TGCAGATGATTTTAAACGGCATCAACAACACTACAAGAACCCCAAGCTGACCCGGATGCTGACCGC
CAAGTTCCGCATGCCCAAGAAGGCCACCGAGCTGAAGCATTACAGTGTCTGGAGGAAGAGCT
GAAGCCTTTAGAGGAAGTGCTCAATTTAGCCAGTCCAAGAAGCTTCCATTTAAGGCCACGTGAT
TTAATCTCCAACATCAACGTGATCGTGCTGGAGCTGAAGGGCTCCGAGACCACCTTCATGTGCG
AGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGATCACCTTCTGCCAGAG
CATCATCTCCACTTTAACCTGA

[000160] Protein sequence (SEQ ID NO:27)

GAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFRMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

[000161] IL2M3: E62R

[000162] DNA sequence (SEQ ID NO:28)

GGCGCCCCACATCCTCCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCATCTGCTGCTGGATC
TGCAGATGATTTTAAACGGCATCAACAACACTACAAGAACCCCAAGCTGACCCGGATGCTGACCTT
CAAGTTCTACATGCCCAAGAAGGCCACCGAGCTGAAGCATTACAGTGTCTGGAGGAAAGGCT
GAAGCCTTTAGAGGAAGTGCTCAATTTAGCCAGTCCAAGAAGCTTCCATTTAAGGCCACGTGAT
TTAATCTCCAACATCAACGTGATCGTGCTGGAGCTGAAGGGCTCCGAGACCACCTTCATGTGCG

AGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGATCACCTTCTGCCAGAG
CATCATCTCCACTTTAACCTGA

[000163] Protein sequence (SEQ ID NO:29)

GAPTSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEERLKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

[000164] IL2M4: R38A, F42A

[000165] DNA sequence (SEQ ID NO:30)

GGCGCCCCACATCCTCCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCATCTGCTGCTGGATC
TGCAGATGATTTTAAACGGCATCAACAATAACAAGACCCCAAGCTGACCGCCATGCTGACCGC
CAAGTTCTACATGCCCAAGAAGGCCACCGAGCTGAAGCATTACAGTGTCTGGAGGAAGAGCT
GAAGCCTTTAGAGGAAGTGCTCAATTTAGCCCAGTCCAAGAAGCTTCCATTTAAGGCCACGTGAT
TTAATCTCCAACATCAACGTGATCGTGCTGGAGCTGAAGGGCTCCGAGACCACCTTCATGTGCG
AGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGATCACCTTCTGCCAGAG
CATCATCTCCACTTTAACCTGA

[000166] Protein sequence (SEQ ID NO:31)

GAPTSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

[000167] IL2M5: R38A, F42A, Y45A, E62A, C125S

[000168] DNA sequence (SEQ ID NO:32)

GGAGCTCCTACCTCCTCCTCCACCAAGAAGACCCAGCTCCAGCTGGAACATTTACTGCTGGATTT
ACAGATGATTTTAAACGGCATCAACAATTACAAGAACCCCAAGCTGACCGCCATGCTGACCGCC
AAGTTTGCCATGCCCAAGAAGGCCACCGAGCTGAAGCATTACAGTGTGTTAGAGGAGGCTTTAA
AGCCTTTAGAGGAGGTGCTGAATTTAGCCCAGTCCAAGAAGCTTCCATTTAAGGCCCCGGGATTT
AATCTCCAACATCAACGTGATCGTGCTGGAAGTGAAGGGCAGCGAGACCACCTTTATGAGCGAA
TACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGATCACCTTCTGCCAGTCCAT
CATCTCCACTTTAACCTGA

[000169] Protein sequence (SEQ ID NO:33)

GAPTSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMSEYADETATIVEFLNRWITFSQSIISTLT

[000170] IL2M6: R38A, F42K

[000171] DNA sequence (SEQ ID NO:34)

GGCGCCCCACATCCTCCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCATCTGCTGCTGGATC
TGCAGATGATTTTAAACGGCATCAACAATAACAAGACCCCAAGCTGACCGCCATGCTGACCAA
GAAGTTCTACATGCCCAAGAAGGCCACCGAGCTGAAGCATTACAGTGTCTGGAGGAAGAGCT

GAAGCCTTTAGAGGAAGTGCTCAATTTAGCCCAGTCCAAGAAGTCCATTTAAGGCCACGTGAT
TTAATCTCCAACATCAACGTGATCGTGCTGGAGCTGAAGGGCTCCGAGACCACCTTCATGTGCG
AGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGATCACCTTCTGCCAGAG
CATCATCTCCACTTTAACCTGA

[000172] Protein sequence (SEQ ID NO:35)

GAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTKKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

[000173] IL2M7: R38A, F42A, E62R

[000174] DNA sequence (SEQ ID NO:36)

GGCGCCCCCACATCCTCCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCATCTGCTGCTGGATC
TGCAGATGATTTTAAACGGCATCAACAACACTACAAGAACCCCAAGCTGACCGCCATGCTGACCGC
CAAGTTCTACATGCCCAAGAAGGCCACCGAGCTGAAGCATTACAGTGTCTGGAGGAAAGGCT
GAAGCCTTTAGAGGAAGTGCTCAATTTAGCCCAGTCCAAGAAGTCCATTTAAGGCCACGTGAT
TTAATCTCCAACATCAACGTGATCGTGCTGGAGCTGAAGGGCTCCGAGACCACCTTCATGTGCG
AGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGATCACCTTCTGCCAGAG
CATCATCTCCACTTTAACCTGA

[000175] Protein sequence (SEQ ID NO:37)

GAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFYMPKKATELKHLQCLEERLKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

[000176] IL2M8: F42K, Y45R, E62R

[000177] DNA sequence (SEQ ID NO:38)

GGCGCCCCCACATCCTCCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCATCTGCTGCTGGATC
TGCAGATGATTTTAAACGGCATCAACAACACTACAAGAACCCCAAGCTGACCCGGATGCTGACCAA
GAAGTTCCGCATGCCCAAGAAGGCCACCGAGCTGAAGCATTACAGTGTCTGGAGGAAAGGCT
GAAGCCTTTAGAGGAAGTGCTCAATTTAGCCCAGTCCAAGAAGTCCATTTAAGGCCACGTGAT
TTAATCTCCAACATCAACGTGATCGTGCTGGAGCTGAAGGGCTCCGAGACCACCTTCATGTGCG
AGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGATCACCTTCTGCCAGAG
CATCATCTCCACTTTAACCTGA

[000178] Protein sequence (SEQ ID NO:39)

GAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTKKFRMPKKATELKHLQCLEERLKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

[000179] IL2M9: F42A, Y45R, E62R

[000180] DNA sequence (SEQ ID NO:40)

GGCGCCCCACATCCTCCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCATCTGCTGCTGGATC
TGCAGATGATTTTAAACGGCATCAACAACACAAGAACCCCAAGCTGACCCGGATGCTGACCGC
CAAGTTCCGCATGCCCAAGAAGGCCACCGAGCTGAAGCATTTACAGTGTCTGGAGGAAAGGCT
GAAGCCTTTAGAGGAAGTGCTCAATTTAGCCCAGTCCAAGAAGTCCATTTAAGGCCACGTGAT
TTAATCTCCAACATCAACGTGATCGTGCTGGAGCTGAAGGGCTCCGAGACCACCTTCATGTGCG
AGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGATCACCTTCTGCCAGAG
CATCATCTCCACTTTAACCTGA

[000181] Protein sequence (SEQ ID NO:41)

GAPTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLTAKFRMPKKATELKHLQCLEERLKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

[000182] IL2M10: R38A, F42K, E62R

[000183] DNA sequence (SEQ ID NO:42)

GGCGCCCCACATCCTCCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCATCTGCTGCTGGATC
TGCAGATGATTTTAAACGGCATCAACAACACAAGAACCCCAAGCTGACCGCCATGCTGACCAA
GAAGTTCTACATGCCCAAGAAGGCCACCGAGCTGAAGCATTTACAGTGTCTGGAGGAAAGGCT
GAAGCCTTTAGAGGAAGTGCTCAATTTAGCCCAGTCCAAGAAGTCCATTTAAGGCCACGTGAT
TTAATCTCCAACATCAACGTGATCGTGCTGGAGCTGAAGGGCTCCGAGACCACCTTCATGTGCG
AGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGATCACCTTCTGCCAGAG
CATCATCTCCACTTTAACCTGA

[000184] Protein sequence (SEQ ID NO:43)

GAPTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTAMLTKKFYMPKKATELKHLQCLEERLKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

[000185] IL2DelM3: Del. E62R

[000186] DNA sequence (SEQ ID NO:44)

GGCGCCCCACATCCTCCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCATCTGCTGCTGGATC
TGCAGATGATTTTAAACGGCATCAACGCCACCGAGCTGAAGCATTTACAGTGTCTGGAGGAAAG
GCTGAAGCCTTTAGAGGAAGTGCTCAATTTAGCCCAGTCCAAGAAGTCCATTTAAGGCCACGT
GATTTAATCTCCAACATCAACGTGATCGTGCTGGAGCTGAAGGGCTCCGAGACCACCTTCATGT
GCGAGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGATCACCTTCTGCCA
GAGCATCATCTCCACTTTAACCTGA

[000187] Protein sequence (SEQ ID NO:45)

GAPTSSSTKKTQLQLEHLLDLQMILNGINATELKHLQCLEERLKPLEEVLNLAQSKNFHLRPRDLIS
NINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

[000188] IL2M3K: E62K

[000189] DNA sequence (SEQ ID NO:46)

GGCGCCCCACATCCTCCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCATCTGCTGCTGGATC
TGCAGATGATTTTAAACGGCATCAACAACCTACAAGAACCCCAAGCTGACCCGGATGCTGACCTT
CAAGTTCTACATGCCCAAGAAGGCCACCGAGCTGAAGCATTACAGTGTCTGGAGGAAAAGCT
GAAGCCTTTAGAGGAAGTGCTCAATTTAGCCCAGTCCAAGAAGCTTCCATTTAAGGCCACGTGAT
TTAATCTCCAACATCAACGTGATCGTGCTGGAGCTGAAGGGCTCCGAGACCACCTTCATGTGCG
AGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGATCACCTTCTGCCAGAG
CATCATCTCCACTTTAACCTGA

[000190] Protein sequence (SEQ ID NO:47)

GAPTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEKLP
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

[000191] IL2M6K: R38A, F42K, E62K

[000192] DNA sequence (SEQ ID NO:48)

GGCGCCCCACATCCTCCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCATCTGCTGCTGGATC
TGCAGATGATTTTAAACGGCATCAACAACCTACAAGAACCCCAAGCTGACCGCCATGCTGACCAA
GAAGTTCTACATGCCCAAGAAGGCCACCGAGCTGAAGCATTACAGTGTCTGGAGGAAAAGCT
GAAGCCTTTAGAGGAAGTGCTCAATTTAGCCCAGTCCAAGAAGCTTCCATTTAAGGCCACGTGAT
TTAATCTCCAACATCAACGTGATCGTGCTGGAGCTGAAGGGCTCCGAGACCACCTTCATGTGCG
AGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGATCACCTTCTGCCAGAG
CATCATCTCCACTTTAACCTGA

[000193] Protein sequence (SEQ ID NO:49)

GAPTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTAMLTKKFYMPKKATELKHLQCLEEKLP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

[000194] IL2M7K: R38A, F42A, E62K

[000195] DNA sequence (SEQ ID NO:50)

GGCGCCCCACATCCTCCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCATCTGCTGCTGGATC
TGCAGATGATTTTAAACGGCATCAACAACCTACAAGAACCCCAAGCTGACCGCCATGCTGACCGC
CAAGTTCTACATGCCCAAGAAGGCCACCGAGCTGAAGCATTACAGTGTCTGGAGGAAAAGCT
GAAGCCTTTAGAGGAAGTGCTCAATTTAGCCCAGTCCAAGAAGCTTCCATTTAAGGCCACGTGAT
TTAATCTCCAACATCAACGTGATCGTGCTGGAGCTGAAGGGCTCCGAGACCACCTTCATGTGCG
AGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGATCACCTTCTGCCAGAG
CATCATCTCCACTTTAACCTGA

[000196] Protein sequence (SEQ ID NO:51)

GAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFYMPKKATELKHLQCLEEKLP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

[000197] IL2M8K: F42K, Y45R, E62K

[000198] DNA sequence (SEQ ID NO:52)

GGCGCCCCACATCCTCCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCATCTGCTGCTGGATC
TGCAGATGATTTTAAACGGCATCAACAACACTACAAGAACCCCAAGCTGACCCGGATGCTGACCAA
GAAGTTCCGCATGCCCAAGAAGGCCACCGAGCTGAAGCATTACAGTGTCTGGAGGAAAAGCT
GAAGCCTTTAGAGGAAGTGCTCAATTTAGCCAGTCCAAGAAGCTTCCATTTAAGGCCACGTGAT
TTAATCTCCAACATCAACGTGATCGTGCTGGAGCTGAAGGGCTCCGAGACCACCTTCATGTGCG
AGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGATCACCTTCTGCCAGAG
CATCATCTCCACTTTAACCTGA

[000199] Protein sequence (SEQ ID NO:53)

GAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTKKFRMPKKATELKHLQCLEEKLP
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

[000200] IL2M9K: F42A, Y45R, E62K

[000201] DNA sequence (SEQ ID NO:54)

GGCGCCCCACATCCTCCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCATCTGCTGCTGGATC
TGCAGATGATTTTAAACGGCATCAACAACACTACAAGAACCCCAAGCTGACCCGGATGCTGACCGC
CAAGTTCCGCATGCCCAAGAAGGCCACCGAGCTGAAGCATTACAGTGTCTGGAGGAAAAGCT
GAAGCCTTTAGAGGAAGTGCTCAATTTAGCCAGTCCAAGAAGCTTCCATTTAAGGCCACGTGAT
TTAATCTCCAACATCAACGTGATCGTGCTGGAGCTGAAGGGCTCCGAGACCACCTTCATGTGCG
AGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGATCACCTTCTGCCAGAG
CATCATCTCCACTTTAACCTGA

[000202] Protein sequence (SEQ ID NO:55)

GAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFRMPKKATELKHLQCLEEKLP
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

[000203] IL2MNew: K35E R38A F42A E61K L72G

[000204] DNA sequence (SEQ ID NO:56)

GGCGCCCCACATCCTCCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCATCTGCTGCTGGATC
TGCAGATGATTTTAAACGGCATCAACAACACTACAAGAACCCCGAGCTGACCGCCATGCTGACCGC
CAAGTTCTACATGCCCAAGAAGGCCACCGAGCTGAAGCATTACAGTGTCTGGAGAAAGAGCT
GAAGCCTTTAGAGGAAGTGCTCAATGGCGCCAGTCCAAGAAGCTTCCATTTAAGGCCACGTGAT
TTAATCTCCAACATCAACGTGATCGTGCTGGAGCTGAAGGGCTCCGAGACCACCTTCATGTGCG

AGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGATCACCTTCTGCCAGAG
CATCATCTCCACTTTAACCTGA

[000205] Protein sequence (SEQ ID NO:57)

GAPTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPELTAMLTAKFYMPKKATELKHLQCLEKELKPL
EEVLNGAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

[000206] It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

[000207] Throughout this application, various publications, patents, and/or patent applications are referenced in order to more fully describe the state of the art to which this invention pertains. The disclosures of these publications, patents, and/or patent applications are herein incorporated by reference in their entireties to the same extent as if each independent publication, patent, and/or patent application was specifically and individually indicated to be incorporated by reference.

CLAIMS

1. A fusion protein, comprising a therapeutic protein connected to a mutant Interleukin-2 (IL2) polypeptide.
2. The fusion protein of claim 1, wherein the therapeutic protein is a therapeutic antibody, or a tumor antigen-binding polypeptide.
3. The fusion protein of claim 1, wherein the mutant IL2 polypeptide is less selective towards a high-affinity receptor of a wild type IL2.
4. The fusion protein of claim 1, wherein the fusion protein comprises a portion of or an entire immune checkpoint antibody, and a mutant Interleukin-2 (IL2) polypeptide.
5. The fusion protein of claim 4, wherein the portion of or the entire immune checkpoint antibody comprises a pair of heavy chains and a pair of light chains, wherein the mutant IL2 polypeptide is connected to the C-terminals of the heavy chains.
6. The fusion protein of claim 5, wherein the mutant IL2 polypeptide is connected to the C-terminals of the heavy chains through a linker sequence.
7. The fusion protein of claim 4, wherein the fusion protein comprises an antibody analog with a bispecific design with a first arm corresponding to that of an immune checkpoint antibody and a second arm comprising a mutant IL2 polypeptide fused to the Fc portion of the antibody analog.
8. The fusion protein of claim 1, wherein the fusion protein consists of an antigen-binding polypeptide connected to a mutant IL2 polypeptide.
9. The fusion protein of claim 8, wherein the antigen-binding polypeptide and a mutant IL2 polypeptide are connected through a linker sequence.
10. The fusion protein of claim 8, wherein the antigen-binding polypeptide is selected from the group consisting of an anti-checkpoint scFv, a ligand and a portion of the ligand.
11. The fusion protein of claim 10, wherein the anti-checkpoint scFv is an anti-PDL1 scFv, and the ligand is PD1 or CTLA4.
12. The fusion protein of any of claims 1, 2, 3, 4 or 8, wherein the mutant IL2 polypeptide has one or both of the following features in comparison to a wild type IL2: (a) it has a reduced capability for activating CTLL2 and human and mouse T_{reg} while it has retained a comparable capability for activating effector T cells or NK cells; and (b) it exhibits a significantly more selective capability for activating T_{eff} over T_{reg}.
13. The fusion protein of any of claims 1, 2, 3, 4 or 8, wherein the mutant IL2 polypeptide comprises an amino acid sequence substantially identical to the amino acid sequences selected from

the group consisting of SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57 and combinations thereof.

14. The fusion protein of any of the claims 4, 5 or 7, wherein the immune checkpoint antibody is an anti-PDL1 antibody.

15. The fusion protein of claim 14, wherein the anti-PDL1 antibody has one or more of the following features: (a) it binds PDL1 with $K_d < 100$ nM to both purified protein as well as protein-expressed on cell surface; (b) it blocks PDL1-PD1 interaction in vitro; and/or (c) it de-represses PDL1-mediated repression of effector T cells in activated PBMC cultures.

16. The fusion protein of any of claims 4, 5 or 7, wherein the immune checkpoint antibody is an antibody of any available isotype or any known mutant forms of Fc region that mediates or enhances ADCC and/or CDC function.

17. The fusion protein of any of claims 4, 5 or 7, wherein the immune checkpoint antibody is an antibody of IgG1 isotype.

18. The fusion protein of claim 5, wherein the immune checkpoint antibody further comprises a second pair of heavy and light chains variable regions substantially identical to those of the first pair.

19. The fusion protein of claim 14, wherein the anti-PDL1 antibody comprises a pair of heavy chain variable region and light chain variable region where their respective sequences consist essentially of the following pairings: (a) SEQ ID NO: 3 and SEQ ID NO: 6; (b) SEQ ID NO: 9 and SEQ ID NO: 12; or (c) SEQ ID NO: 15 and SEQ ID NO: 18.

20. The fusion protein of claim 14, wherein the anti-PDL1 antibody comprises a pair of heavy chain and light chain where their respective sequences consist essentially of the following pairings: (a) SEQ ID NO: 2 and SEQ ID NO: 5; (b) SEQ ID NO: 8 and SEQ ID NO: 11; or (c) SEQ ID NO: 14 and SEQ ID NO: 17.

21. The fusion protein of claim 6 or 9, wherein the linker sequence is a serine-glycine linker.

22. The fusion protein of claim 21, wherein the linker sequence is SEQ ID NO: 19.

23. The fusion protein of any of claims 1, 2, 3, 4 or 8, wherein mutation has been introduced at one or more amino acid positions in the fusion protein to disrupt at least one proteolytic site.

24. The fusion protein of any of claims 1, 2, 3, 4 or 8, wherein mutation has been introduced at one or more amino acid positions in the fusion protein to disrupt at least one glycosylation site.

25. The fusion protein of any of claims 1, 2, 3, 4 or 8, wherein an alternate secretory sequence has been introduced into the fusion protein to replace an innate secretory sequence.
26. A pharmaceutical composition comprising the fusion protein of any of claims 1-25, and a pharmaceutically acceptable excipient, carrier or diluent.
27. A nucleic acid molecule that encodes the fusion protein of any of claims 1-25, wherein the nucleic acid molecule is a DNA molecule or RNA molecule.
28. A method of therapeutically treating a subject in need thereof for a condition, said method comprising administering to said subject a therapeutically effective amount of the pharmaceutical composition of claim 26.
29. A method of therapeutically treating a subject in need thereof for a condition, said method comprising administering to said subject a therapeutically effective amount of the pharmaceutical composition of claim 26 in combination with (a) antibodies targeting at least another immunosuppressive pathways; (b) chemotherapy, targeted therapy or radiation therapy; (c) another mechanism of blocking an immunosuppressive pathway; or (d) another immunotherapy agent.
30. The method of any of claims 28-29, wherein said condition is a mammalian cancer.
31. The method of any of claims 28-29, wherein said cancer is selected from the group consisting of melanoma, lung cancer, renal cancer, head neck cancer, gastric cancer, lymphoma, ovarian cancer, colon cancer, breast cancer, lung cancer, myelomas, brain tumors, leukemias, lymphomas, bladder cancer, liver cancer, urothelial carcinoma, cutaneous carcinoma, pancreatic cancer, and combinations thereof.
32. A mammalian expression system that produces fusion protein of any of claims 1-25.
33. A mutant IL2 polypeptide that is less selective towards a high-affinity receptor of a wild type IL2.
34. The mutant IL2 polypeptide of claim 33, wherein the mutant IL2 polypeptide comprises an amino acid sequence substantially identical to a sequence selected from the group consisting of SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57 and combinations thereof.
35. A fusion protein comprising:
an antigen-binding part comprising a portion of or an entire anti-PDL1 antibody connected to a mutant Interleukin-2 (IL2) polypeptide;

wherein the antigen-binding part comprises a pair of heavy chain and light chain where their respective sequences consist essentially of the following pairings: (a) SEQ ID NO: 2 and SEQ ID NO: 5; (b) SEQ ID NO: 8 and SEQ ID NO: 11;

wherein the mutant IL2 polypeptide comprises an amino acid sequence substantially identical to a sequence selected from the group consisting of SEQ ID NO: 25, SEQ ID NO: 27; and

wherein the mutant IL2 polypeptide is connected to the C-terminals of the heavy chains of the antigen-binding part.

36. The fusion protein of claim 35, wherein the mutant IL2 polypeptide is connected to the C-terminals of the heavy chains through a linker sequence comprising SEQ ID NO: 19.