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(56) Related Art
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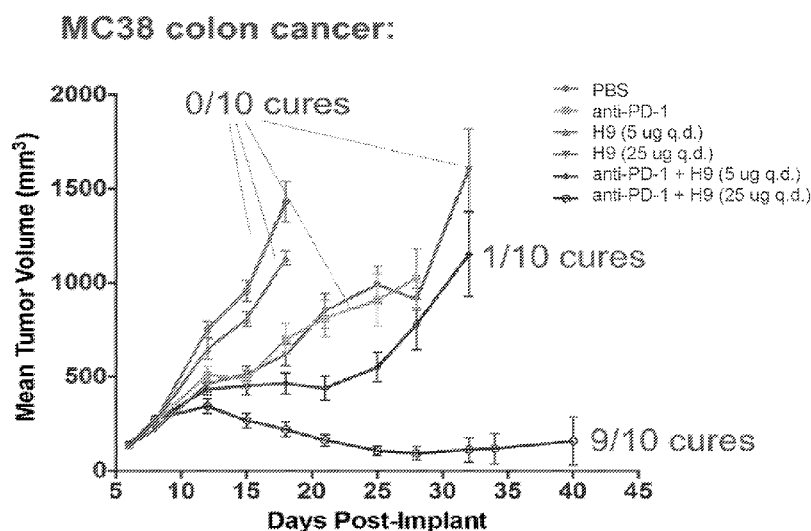
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Figure 1



* 10 mg/kg IV q4dx3

(57) Abstract: Human interleukin-2 (IL-2) muteins or variants thereof are provided. In particular, provided are IL-2 muteins that have an increased binding capacity for IL-2R β receptor as compared to wild-type IL-2 for use in combination therapies with anti-PD-1 antibodies for the treatment of cancer. Also provided are pharmaceutical compositions that include such anti-PD-1 antibodies and the disclosed IL-2 muteins.



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USES AND METHODS FOR IL-2 SUPERAGONISTS, AGONISTS, AND FUSIONS THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. §119 to U.S. Patent Application Nos. 62/521,957, filed on June 19, 2017, and 62/679,687, filed on June 01, 2018, all of which are expressly incorporated herein by reference in their entireties.

BACKGROUND

[0002] Interleukin 2 (IL-2) is a pluripotent cytokine produced primarily by activated CD4⁺ T cells, which plays a crucial role in producing a normal immune response. IL-2 promotes proliferation and expansion of activated T lymphocytes, potentiates B cell growth, and activates monocytes and natural killer cells. It was by virtue of these activities that IL-2 was tested and is used as an approved treatment of cancer (aldesleukin, Proleukin®). In eukaryotic cells, human IL-2 is synthesized as a precursor polypeptide of 153 amino acids, from which 20 amino acids are removed to generate mature secreted IL-2 (Taniguchi 1983). Recombinant human IL-2 has been produced in *E. coli* (Rosenberg 1984), in insect cells (Smith 1985) and in mammalian COS cells (Taniguchi 1983).

[0003] Interleukin-2 (IL-2) is a four α -helical bundle type I cytokine first identified as a T cell growth factor (Morgan et al., *Science* 193: 1007 (1976)) but subsequently shown to have broad actions. IL-2 promotes T helper differentiation (Zhu et al., *Annual review of immunology* 28: 445 (2010); Liao et al., *Nat Immunol* 9: 1288 (2008); and Liao et al., *Nat Immunol* 12: 551 (2011)) and the development of regulatory T (Treg) cells (Cheng et al., *Immunol Rev* 241: 63 (2011)), induces natural killer and lymphokine activated killer activity (Liao et al., *Immunity* 38: 13 (2013)), and mediates activation-induced cell death (AICD) (Lenardo et al., *Nature* 353: 858 (1991)).

[0004] IL-2 works by interacting with three different receptors: the interleukin 2 receptor alpha (IL-2R α ; CD25), the interleukin 2 receptor beta (IL-2R β ;CD122), and the interleukin 2 receptor gamma (IL-2R γ ;CD132; common gamma chain). The first receptor to be identified was the IL-2R α , which is a 55 kD polypeptide (p55) that appears upon T cell activation and was originally

called Tac (for T activation) antigen. The IL-2R α binds IL-2 with a K_d of approximately 10^{-8} M, and is also known as the “low affinity” IL-2 receptor. Binding of IL-2 to cells expressing only the IL-2R α does not lead to any detectable biologic response. In most circumstances, IL-2 works through three different receptors: the IL-2R α , the IL-2R β , and the IL-2R γ . Most cells, such as resting T cells, are not responsive to IL-2 since they only express the IL-2R β , and the IL-2R γ , which have low affinity for IL-2. Upon stimulation, resting T cells express the relatively high affinity IL-2 receptor IL-2R α . Binding of IL-2 to the IL-2R α causes this receptor to sequentially engage the IL-2R β , and the IL-2R γ , bringing about T cell activation. IL-2 “superkines” with augmented action due to enhanced binding affinity for IL-2R β were previously developed (Levin et al., *Nature* 484: 529 (2012)).

[0005] Despite the wealth of knowledge around IL-2, including IL-2 superagonists, there remains a need in the art for better combination therapies for the treatment of cancer, including combination therapies with anti-PD-1 antibodies as well as combinations with oncolytic viruses or CAR-T cells. The present invention meets this need, providing combination therapies of IL-2 superagonists or agonists for the treatment of cancer, in particular combinations of anti-PD-1 antibodies with IL-2 muteins comprising substitutions L80F, R81D, L85V, I86V and I92F, numbered in accordance with wild-type IL-2.

BRIEF SUMMARY

[0006] IL-2 exerts a wide spectrum of effects on the immune system, and it plays crucial roles in regulating both immune activation and homeostasis. As an immune system stimulator, IL-2 muteins of the present invention have found use in combination with anti-PD-1 antibodies for the treatment of cancer.

[0007] In another aspect, provided herein is a method of treating a subject having cancer comprising administering an IL-2 mutein in combination with an anti-PD-1 antibody or inhibitor. In some embodiments, the method comprises administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising any one of the IL-2 muteins disclosed herein. In some embodiments, the pharmaceutical composition comprises an IL-2 mutein having the amino acid substitutions L80F, R81D, L85V, I86V, and I92F.

[0008] As such, in some embodiments, the present invention provides a method of treating cancer comprising administering a combination treatment comprising: (i) an anti-PD-1 antibody

or inhibitor and (ii) an IL-2 mutein comprising the following amino acid substitutions L80F, R81D, L85V, I86V and I92F, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2.

[0009] In some embodiments, the anti-PD-1 antibody or inhibitor is selected from the group consisting of nivolumab, BMS-936558, MDX-1106, ONO-4538, AMP224, CT-011, and MK-3475 (pembrolizumab), cemiplimab (REGN2810), SHR-1210 (CTR20160175 and CTR20170090), SHR-1210 (CTR20170299 and CTR20170322), JS-001 (CTR20160274), IBI308 (CTR20160735), BGB-A317 (CTR20160872) and a PD-1 antibody as recited in U.S. Patent Publication No. 2017/0081409. In some embodiments, the anti-PD-L1 antibody or inhibitor is selected from the group consisting of atezolizumab, avelumab, and Durvalumab.

[0010] In some embodiments, the IL-2 mutein further comprises F42A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprising a F42A substitution exhibits reduced binding affinity for CD25 as compared to wild-type human IL-2.

[0011] In some embodiments, the IL-2 mutein further comprises K43N substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprising a K43N substitution exhibits reduced binding affinity for CD25 as compared to wild-type human IL-2.

[0012] In some embodiments, the IL-2 mutein further comprises Y45A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprising a Y45A substitution exhibits reduced binding affinity for CD25 as compared to wild-type human IL-2.

[0013] In some embodiments, the IL-2 mutein further comprises E62A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprising a E62A substitution exhibits reduced binding affinity for CD25 as compared to wild-type human IL-2.

[0014] In some embodiments, the IL-2 mutein is a fusion protein. In some embodiments, the fusion protein comprises said IL-2 linked to an albumin. In some embodiments, the fusion protein comprises said IL-2 linked to an Fc antibody fragment. In some embodiments, the Fc

antibody fragment is a human Fc antibody fragment. In some embodiments, the Fc antibody fragment comprises a N297A substitution.

[0015] In some embodiments, the cancer is selected from the group consisting of prostate cancer, ovarian cancer, breast cancer, endometrial cancer, multiple myeloma, melanoma, lymphomas, lung cancers including small cell lung cancer, kidney cancer, liver cancer, colon cancer, colorectal cancer, pancreatic cancer, gastric cancer, and brain cancer. In some embodiments, the cancer is colon cancer.

[0016] In some embodiments, the IL-2 mutein exhibits increased binding capacity for IL-2R β as compared to wild-type human IL-2. In some embodiments, the IL-2 mutein exhibits a greater binding affinity for IL-2R β as compared to wild-type human IL-2.

[0017] The method of any one of claims 3 to 7, wherein said IL-2 mutein exhibits decreased binding affinity for CD25 as compared to wild-type human IL-2.

[0018] In another aspect, provided herein is a pharmaceutical composition comprising any one of the IL-2 muteins or the IL-2 mutein fusion protein described herein and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition comprises an IL-2 mutein having the amino acid substitutions L80F, R81D, L85V, I86V, and I92F.

[0019] In some embodiments, the pharmaceutical composition comprises an anti-PD-1 antibody or inhibitor, any of the IL-2 muteins as described herein, and a pharmaceutically acceptable carrier.

[0020] The present invention also provides for an immune cell targeting or expression construct comprising: an interleukin-2 receptor β (IL-2R β) binding protein, wherein the equilibrium dissociation constant for the IL-2R β of said binding protein is less than that of wild-type human IL-2 (hIL-2); linked to an immune cell targeting or expression construct comprising at least one other targeting moiety.

[0021] In some embodiments, the immune cell targeting or expression construct exhibits a cytotoxic effect on a T-cell, for example a CD8⁺ T-cell or a CD4⁺ T-cell.

[0022] In some embodiments, the construct is a chimeric antigen receptor (CAR) and wherein the IL-2 mutein is fused to a transmembrane domain; linked to an intracellular signaling region. In some embodiments, the intracellular signaling region comprises a CD3 signaling domain. In

some embodiments, the intracellular signaling region comprises one or more of a CD28 signaling domain, a CD137 signaling domain, an OX-40 signaling domain, an ICOS signaling domain, a DAP10 signaling domain.

[0023] In some embodiments, the IL-2 mutein or other targeting moiety is fused to a ligand that binds a protein associated with the TCR complex; fused to a T cell receptor signaling domain polypeptide.

[0024] In some embodiments, the protein associated with the TCR complex is CD3.

[0025] In some embodiments, the T cell receptor signaling domain polypeptide comprises CD4 cytosolic domain and CD4 transmembrane domain.

[0026] In some embodiments, the construct is an antibody coupled T cell receptors (ACTR), comprising a chimeric antigen receptor component that binds to the IL-2 or other targeting moiety mutein at a high affinity.

[0027] In some embodiments, the CAR component comprises CD16, and the IL-2 mutein or other targeting moiety is fused to an Fc sequence.

[0028] In some embodiments, the construct is a bispecific T cell exchanger (BiTE) comprising an IL-2 mutein fused to a variable region of an antibody that binds to a component of a T cell receptor.

[0029] In some embodiments, the BiTE component of a T cell receptor is CD3.

[0030] In some embodiments, the IL-2 mutein comprises the following amino acid substitutions: L80F, R81D, L85V, I86V, and I92F, numbered in accordance with wild-type hIL-2.

[0031] In some embodiments, a nucleic acid encoding a construct according to the above is provided.

[0032] In some embodiments, a vector comprising the nucleic acid is provided.

[0033] In some embodiments, a T cell comprising a construct or a vector according to the above is provided. In some embodiments, the the T cell is a CD4⁺ T cell. In some embodiments, the T cell is a CD8⁺ T cell.

[0034] In some embodiments, an NK cell comprising a construct or a vector according to the above is provided

[0035] Also provided is an isolated population of immune cells according to the above.

[0036] Also provided is a pharmaceutical formulation comprising the immune cell population of according to the above.

[0037] In some embodiments, a method of targeting a cell expressing an IL-2 receptor, including a cell expressing an IL-2 receptor, is provided, wherein the method comprising contacting a cell with a formulation comprising the immune cell population of according to the above. In some embodiments, the contacting is *in vitro*. In some embodiments, the contacting is *in vivo*.

[0038] The present invention also provides a method of treating cancer, the method comprising contacting an individual having cancer with an effective dose of a formulation comprising the immune cell population of according to the above.

[0039] In some embodiments, the cancer is a leukemia, lymphoma, glioblastoma, medulloblastoma, breast cancer, head and neck cancer, kidney cancer, ovarian cancer, Kaposi's sarcoma, acute myelogenous leukemia, B-lineage malignancies, colorectal, pancreatic, kidney, or mesothelioma.

[0040] The present invention also provides methods for targeting an IL-2 mutein protein to a cancer cell comprising contacting said cancer cell with an IL-2 mutein oncolytic virus combination, wherein said combination comprises an IL-2 mutein conjugated to or expressed by an oncolytic virus, and wherein said oncolytic virus is capable of targeting a cancer cell.

[0041] In some embodiments, the contacting occurs *in vitro*. In some embodiments, the contacting occurs *in vivo*.

[0042] In some embodiments, the oncolytic virus is selected from the group consisting of an adenovirus, a self-replicating alphavirus, a vaccinia virus, a Seneca Valley Virus, a Newcastle disease Virus, a Maraba virus, vesicular stomatitis virus (VSV), a Herpes virus (including HSV-1 and HSV-2), a measles virus, a poliovirus, a reovirus, a coxsackie virus, a lentivirus, a morbillivirus, an influenza virus, Sinbis virus, myxoma virus, and a retrovirus.

[0043] In some embodiments, the vaccinia virus genome comprises thymidine kinase gene is inactivated by a substitution in the thymidine kinase (TK) gene and/or an open reading frame ablating deletion of at least one nucleotide providing a partially deleted thymidine kinase gene,

the vaccinia growth factor gene is deleted, and the modified vaccinia virus vector comprises at least one nucleic acid sequence encoding an IL-2 mutein as described herein.

[0044] In some embodiments, the in vivo contacting results in an increased concentration of the IL-2 mutein protein in the tumor microenvironment as compared to the concentration of an IL-2 mutein protein not conjugated to an oncolytic virus.

[0045] In some embodiments, the modified oncolytic virus targets the IL-2 mutein to the immunosuppressive cells of the tumor microenvironment (TME), such as tumor associated macrophages and MDSCs (myeloid-derived suppressor cells) in order to have an improved therapeutic benefit.

[0046] In some embodiments, modified oncolytic virus targets the IL-2 mutein to one or more immunosuppressive cells expressing one or more tumor antigens.

[0047] In some embodiments, the modified oncolytic virus targets the IL-2 mutein to the TME.

[0048] In some embodiments, the IL-2 mutein protein enhances effector T cells and/or NK cells.

[0049] In some embodiments, the IL-2 mutein suppresses Treg activity.

[0050] In some embodiments, the IL-2 comprises the following amino acid substitutions L80F, R81D, L85V, I86V, and I92F, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2.

[0051] According to the present invention, also provided is a modified vaccinia virus vector, characterized in that the vector comprises vaccinia virus genome wherein the thymidine kinase gene is inactivated by a substitution in the thymidine kinase (TK) gene and/or an open reading frame ablating deletion of at least one nucleotide providing a partially deleted thymidine kinase gene, the vaccinia growth factor gene is deleted, and the modified vaccinia virus vector comprises at least one nucleic acid sequence encoding an IL-2 mutein as described herein.

[0052] According to the present invention, also provided is a modified oncolytic adenovirus comprising (i) a modified nucleic acid, wherein optionally the nucleotides encoding amino acids 122-129 of the encoded E1A polypeptide are deleted, and (ii) an expression cassette comprising a polynucleotide encoding an IL-2 mutein as described herein.

[0053] In some embodiments, the IL-2 mutein directs the modified oncolytic virus to the immunosuppressive cells of the tumor microenvironment (TME), such as tumor associated macrophages and MDSCs (myeloid-derived suppressor cells) in order to have an improved therapeutic benefit.

[0054] In some embodiments, the IL-2 mutein protein directs the modified oncolytic virus to one or more tumor antigens.

[0055] In some embodiments, the IL-2 mutein protein directs modified oncolytic virus to the TME.

[0056] In some embodiments, the IL-2 mutein protein enhances effector T cells and NK cells.

[0057] In some embodiments, the IL-2 mutein suppresses Treg activity.

[0058] The present invention also provides for a method of treating cancer comprising administering and oncolytic virus capable of expressing an IL-2 mutein to a subject in need thereof. In some embodiments, the IL-2 mutein comprises the following amino acid substitutions L80F, R81D, L85V, I86V, and I92F, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 the oncolytic virus is selected from the group consisting of an adenovirus, a self-replicating alphavirus, a vaccinia virus, a Seneca Valley Virus, a Newcastle disease Virus, a Maraba virus, vesicular stomatitis virus (VSV), a Herpes virus (including HSV-1 and HSV-2), a measles virus, a poliovirus, a reovirus, a coxsackie virus, a lentivirus, a morbillivirus, an influenza virus, Sinbis virus, myxoma virus, and a retrovirus.

BRIEF DESCRIPTION OF THE DRAWINGS

[0059] **Figure 1. H9 Synergizes with Anti-PD-1 Immunotherapy.** Combination Therapy Produces Robust Responses in a Dose-Dependent Fashion. Anti-PD-1 antibody was administered at 10 mg/kg intravenously with 3 doses administered every 4 days (10 mg/kg IV q4dx3). H9 (IL-2 mutein having the amino acid substitutions L80F, R81D, L85V, I86V, and I92F, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2) was administered at the indicated dosage of 5 µg q.d. or 25 µg q.d. (dosing was µg/mouse), according to the same dosing regimen. MC38 colon cancer model mice were then monitored for up to 40

days post-tumor implant. The combination of anti-PD-1 antibody plus H9 resulted in an increase in the number of cures at both the low and high dose, with a substantial increase at the 25 ug q.d, dose of H9.

[0060] Figure 2. Provides examples of IgG1, IgG2, IgG3, and IgG4 sequences.

[0061] Figure 3. Provides exemplary H9-Fc fusion sequences.

[0062] Figure 4. Comparative analysis of the IL-13R α 1- and the IL-13R α 2-selective IL-13 variants Human IL-13 and IL-13R α 1 and IL-13R α 2 selective variants sequences are given for the indicated residue numbers. Kinetic and affinity parameters were determined by surface plasmon resonance.

[0063] Figure 5. H9-Fc has improved potency towards key immune cells H9 and H9-Fc have largely improved potency towards key effector T cells, particularly CD8⁺ T cells responsible for tumor cell killing. H9 and its Fc-variant do not lose potency towards Tregs, but enable a much increased relative activation of anti-tumor effector CD8⁺ T cells.

[0064] Figure 6. H9-Fc has similar in vivo potency and extended PK profile vs H9. An optimized dose and schedule for the extended PK variant of H9 has been identified. H9-Fc enables effective B16F10 tumor control with a biweekly schedule, a similar schedule as anti-PD-1 antibodies used in mice. Accordingly, we predict weekly or once every two week administration of H9-Fc. Subcutaneous administration: Subcutaneous H9-Fc is an advantageous administration approach for a future immunotherapy drug. Checkpoint inhibitors, Proleukin and competitor IL-2 therapies (NKTR-214, ALKS 4230) all require IV infusion, with lengthy administration and monitoring time in the clinic. Subcutaneous administration offers fast and convenient administration that is typically preferred by patients for common targeted cancer therapies.

[0065] Figure 7. Exemplary anti-PD-1 antibodies for use with the combinations of the invention.

[0066] Figure 8. Exemplary anti-PD-L1 antibodies for use with the combinations of the invention.

[0067] Figure 9: Exemplary oncolytic viruses.

DETAILED DESCRIPTION

[0068] In order for the present disclosure to be more readily understood, certain terms and phrases are defined below as well as throughout the specification.

Definitions

[0069] All references cited herein are incorporated by reference in their entirety as though fully set forth. Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton *et al.*, *Dictionary of Microbiology and Molecular Biology 3rd ed.*, J. Wiley & Sons (New York, NY 2001); March, *Advanced Organic Chemistry Reactions, Mechanisms and Structure 5th ed.*, J. Wiley & Sons (New York, NY 2001); and Sambrook and Russell, *Molecular Cloning: A Laboratory Manual 3rd ed.*, Cold Spring harbor Laboratory Press (Cold Spring Harbor, NY 2001), provide one skilled in the art with a general guide to many terms used in the present disclosure. As appropriate, procedures involving the use of commercially available kits and reagents are generally carried out in accordance with manufacturer defined protocols and/or parameters unless otherwise noted.

[0070] As used herein, "IL-2" means wild-type IL-2, whether native or recombinant. Mature human IL-2 occurs as a 133 amino acid sequence (less the signal peptide, consisting of an additional 20 N-terminal amino acids), as described in Fujita, *et. al.*, PNAS USA, 80, 7437-7441 (1983). The amino acid sequence of human IL-2 (SEQ ID NO:1; full length) is found in Genbank under accession locator NP_000577.2. The amino acid sequence of mature human IL-2 is depicted in SEQ ID NO:2 (human wild-type mature; position numbering of the substitutions is based on this sequence). The murine (*Mus musculus*) IL-2 amino acid sequence is found in Genbank under accession locator (SEQ ID NO:3). The amino acid sequence of mature murine IL-2 is depicted in SEQ ID NO:4.

SEQ ID NO:1

MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRM
LTFKFYMPKKATELKHLCLEELKPLEEVNLNAQSKNFHLRPRDLISNINVIVLELKGSE
TTFMCEYADETATIVEFLNRWITFCQSIISTLT

SEQ ID NO:2

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCL
 EEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNR
 WITFCQSIISTLT

SEQ ID NO:3

MYSMQLASCVTLLVLLVNSAPTSSSTSSSTAEAQQQQQQQQQQQHLEQLLMDLQEL
 LSRMENYRNLKLPRLTFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQSKSFQLEDA
 ENFISNIRVTVVKLKGSNTFECQFDDESATVVDFLRRWIAFCQSIISTSPQ

SEQ ID NO:4

APTSSSTSSSTAEAQQQQQQQQQQQHLEQLLMDLQELLSRMENYRNLKLPRLTFKF
 YLPKQATELKDLQCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTVVKLKGSNTF
 ECQFDDESATVVDFLRRWIAFCQSIISTSPQ

[0071] As used herein, “IL-2 mutein” means an IL-2 polypeptide wherein specific substitutions to the interleukin-2 protein have been made. The IL-2 muteins are characterized by amino acid insertions, deletions, substitutions and modifications at one or more sites in or at the other residues of the native IL-2 polypeptide chain. In accordance with this disclosure, any such insertions, deletions, substitutions and modifications result in an IL-2 mutein that retains the IL-2R β binding activity. Exemplary muteins can include substitutions of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids.

[0072] Muteins also include conservative modifications and substitutions at other positions of IL-2 (i.e., those that have a minimal effect on the secondary or tertiary structure of the mutein). Such conservative substitutions include those described by Dayhoff in *The Atlas of Protein Sequence and Structure* 5 (1978), and by Argos in *EMBO J.*, 8:779-785 (1989). For example, amino acids belonging to one of the following groups represent conservative changes: Group I: ala, pro, gly, gln, asn, ser, thr; Group II: cys, ser, tyr, thr; Group III: val, ile, leu, met, ala, phe; Group IV: lys, arg, his; Group V: phe, tyr, trp, his; and Group VI: asp, glu.

[0073] “Numbered in accordance with IL-2” means identifying a chosen amino acid with reference to the position at which that amino acid normally occurs in the mature sequence of wild type IL-2, for example R81 refers to the eighty-first amino acid, arginine, that occurs in SEQ ID NO:2. L80 refers to the eightieth amino acid, leucine, that occurs in SEQ ID NO:2. L85 refers to the eighty-fifth amino acid, leucine, that occurs in SEQ ID NO:2. I86 refers to the eighty-sixth amino acid, isoleucine, that occurs in SEQ ID NO:2. I92 refers to the ninety-second amino acid, isoleucine, that occurs in SEQ ID NO:2. F42 refers to the forty-second amino acid, phenylalanine, that occurs in SEQ ID NO:2. K43 refers to the forty-third amino acid, lysine, that occurs in SEQ ID NO:2.

[0074] As used herein, the abbreviations for the genetically encoded L-enantiomeric amino acids used in the disclosure methods are conventional and are as follows in Table 1.

Table 1: Amino acid abbreviations

Amino Acid	One-Letter Symbol	Common Abbreviation
Alanine	A	Ala
Arginine	R	Arg
Asparagine	N	Asn
Aspartic acid	D	Asp
Cysteine	C	Cys
Glutamine	Q	Gln
Glutamic acid	E	Glu
Glycine	G	Gly
Histidine	H	His
Isoleucine	I	Ile
Leucine	L	Leu
Lysine	K	Lys
Methionine	M	Met
Phenylalanine	F	Phe
Proline	P	Pro
Serine	S	Ser

Amino Acid	One-Letter Symbol	Common Abbreviation
Threonine	T	Thr
Tryptophan	W	Trp
Tyrosine	Y	Tyr
Valine	V	Val

[0075] “Hydrophilic Amino Acid” refers to an amino acid exhibiting a hydrophobicity of less than zero according to the normalized consensus hydrophobicity scale of Eisenberg *et al.*, 1984, *J. Mol. Biol.* 179: 125-142. Genetically encoded hydrophilic amino acids include Thr (T), Ser (S), His (H), Glu (E), Asn (N), Gln (Q), Asp (D), Lys (K) and Arg (R).

[0076] The term “cell types having the IL-2R $\alpha\beta\gamma$ receptor” means the cells known to have this receptor type, *i.e.*, T cells, activated T cells, B cells, activated monocytes, and activated NK cells. The term “cell types having the IL-2R $\beta\gamma$ receptor” means the cells known to have that receptor type, *i.e.*, B cells, resting monocytes, and resting NK cells.

[0077] The term “identity,” as used herein in reference to polypeptide or DNA sequences, refers to the subunit sequence identity between two molecules. When a subunit position in both of the molecules is occupied by the same monomeric subunit (*i.e.*, the same amino acid residue or nucleotide), then the molecules are identical at that position. The similarity between two amino acid or two nucleotide sequences is a direct function of the number of identical positions. In general, the sequences are aligned so that the highest order match is obtained. If necessary, identity can be calculated using published techniques and widely available computer programs, such as the GCS program package (Devereux *et al.*, *Nucleic Acids Res.* 12:387, 1984), BLASTP, BLASTN, FASTA (Atschul *et al.*, *J. Molecular Biol.* 215:403, 1990). Sequence identity can be measured using sequence analysis software such as the Sequence Analysis Software Package of the Genetics Computer Group at the University of Wisconsin Biotechnology Center (1710 University Avenue, Madison, Wis. 53705), with the default parameters thereof.

[0078] The terms “polypeptide,” “protein” or “peptide” refer to any chain of amino acid residues, regardless of its length or post-translational modification (*e.g.*, glycosylation or phosphorylation).

[0079] In the event the mutant IL-2 polypeptides of the disclosure are “substantially pure,” they can be at least about 60% by weight (dry weight) the polypeptide of interest, for example, a polypeptide containing the mutant IL-2 amino acid sequence. For example, the polypeptide can be at least about 75%, about 80%, about 85%, about 90%, about 95% or about 99%, by weight, the polypeptide of interest. Purity can be measured by any appropriate standard method, for example, column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

[0080] An “agonist” is a compound that interacts with a target to cause or promote an increase in the activation of the target.

[0081] A “partial agonist” is a compound that interacts with the same target as an agonist but does not produce as great a magnitude of a biochemical and/or physiological effect as the agonist, even by increasing the dosage of the partial agonist.

[0082] A “superagonist” (also referred to as a “superkine”) is a type of agonist that is capable of producing a maximal response greater than the endogenous agonist for the target receptor, and thus has an efficacy of more than 100%.

[0083] “Operably linked” is intended to mean that the nucleotide sequence of interest (i.e., a sequence encoding an IL-2 mutein) is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). “Regulatory sequences” include promoters, enhancers, and other expression control elements (e.g., polyadenylation signals). *See*, for example, Goeddel (1990) in *Gene Expression Technology: Methods in Enzymology* 185 (Academic Press, San Diego, Calif.). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cells and those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression constructs of the invention can be introduced into host cells to thereby produce the human IL-2 muteins disclosed herein or to produce biologically active variants thereof.

[0084] The terms “host cell” and “recombinant host cell” are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or

potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell but are still included within the scope of the term as used herein.

[0085] As used herein, the terms “transformation” and “transfection” refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, particle gun, or electroporation.

[0086] As used herein, the term “pharmaceutically acceptable carrier” includes, but is not limited to, saline, solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds (e.g., antibiotics) can also be incorporated into the compositions.

[0087] As used herein, the term “anti-PD-1 antibody” refers to any antibody that binds to PD-1, including inhibitory antibodies. An “anti-PD-1 inhibitor” refers to an inhibitor that binds to and inhibits PD-1. Such anti-PD-1 antibodies and/or inhibitors include but are not limited to nivolumab, BMS-936558, MDX-1106, ONO-4538, AMP224, CT-011, and MK-3475, among others.

[0088] As used herein, the terms “cancer” (or “cancerous”), “hyperproliferative,” and “neoplastic” to refer to cells having the capacity for autonomous growth (i.e., an abnormal state or condition characterized by rapidly proliferating cell growth). Hyperproliferative and neoplastic disease states may be categorized as pathologic (i.e., characterizing or constituting a disease state), or they may be categorized as non-pathologic (i.e., as a deviation from normal but not associated with a disease state). The terms are meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. “Pathologic hyperproliferative” cells occur in disease states characterized by malignant tumor growth. Examples of non-pathologic hyperproliferative cells include proliferation of cells associated with wound repair. The terms “cancer” or “neoplasm” are used to refer to malignancies of the various organ systems, including those affecting the lung, breast, thyroid, lymph glands and lymphoid tissue, reproductive systems, gastrointestinal organs, and the genitourinary tract, as well as to

adenocarcinomas which are generally considered to include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus. Cancers generally can include prostate cancer, ovarian cancer, breast cancer, endometrial cancer, multiple myeloma, melanoma, lymphomas, lung cancers including small cell lung cancer, kidney cancer, colorectal cancer, pancreatic cancer, gastric cancer, and brain cancer.

[0089] The term “carcinoma” is art-recognized and refers to malignancies of epithelial or endocrine tissues including respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, breast carcinomas, prostatic carcinomas, endocrine system carcinomas, and melanomas. An “adenocarcinoma” refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures.

[0090] As used herein, the term “hematopoietic neoplastic disorders” refers to diseases involving hyperplastic/neoplastic cells of hematopoietic origin, e.g., arising from myeloid, lymphoid or erythroid lineages, or precursor cells thereof. Preferably, the diseases arise from poorly differentiated acute leukemias (e.g., erythroblastic leukemia and acute megakaryoblastic leukemia). Additional exemplary myeloid disorders include, but are not limited to, acute promyeloid leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) (reviewed in Vaickus, L. (1991) Crit Rev. in Oncol./Hematol. 11:267-97); lymphoid malignancies include, but are not limited to acute lymphoblastic leukemia (ALL) which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HLL) and Waldenstrom's macroglobulinemia (WM). Additional forms of malignant lymphomas include, but are not limited to non-Hodgkin lymphoma and variants thereof, peripheral T cell lymphomas, adult T cell leukemia/lymphoma (ATL), cutaneous T cell lymphoma (CTCL), large granular lymphocytic leukemia (LGF), Hodgkin's disease and Reed-Stemberg disease.

[0091] As used herein, the terms “treatment,” “treating,” and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease.

“Treatment,” as used herein, covers any treatment of a disease in a mammal, particularly in a

human, and includes: (a) preventing the disease from occurring in a subject predisposed to the disease or at risk of acquiring the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, *i.e.*, arresting its development; and (c) relieving the disease, *i.e.*, causing regression of the disease. A therapeutically effective amount can be an amount that reduces tumor number, tumor size, and/or increases survival.

[0092] The terms “individual,” “subject,” and “patient” are used interchangeably herein, and refer to a mammal, including, but not limited to, human and non-human primates, including simians and humans; mammalian sport animals (*e.g.*, horses); mammalian farm animals (*e.g.*, sheep, goats, etc.); mammalian pets (dogs, cats, etc.); and rodents (*e.g.*, mice, rats, etc.).

[0093] The terms “pharmaceutically acceptable” and “physiologically acceptable” mean a biologically acceptable formulation, gaseous, liquid or solid, or mixture thereof, suitable for one or more routes of administration, *in vivo* delivery or contact. A “pharmaceutically acceptable” or “physiologically acceptable” composition is a material that is not biologically or otherwise undesirable, *e.g.*, the material may be administered to a subject without causing substantial undesirable biological effects. Thus, such a pharmaceutical composition may be used, for example in administering an IL-2 mutein to a subject. In particular, an IL-2 mutein comprising the substitutions L80F, R81D, L85V, I86V, and I92F is administered in combination with anti-PD-1 to a subject with cancer. In some embodiments, the IL-2 mutein administered further comprises a substitution at position F42A. In some embodiments, the IL-2 administered mutein further comprises a substitution at position K43N.

[0094] The phrase a “unit dosage form” as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity optionally in association with a pharmaceutical carrier (excipient, diluent, vehicle or filling agent) which, when administered in one or more doses, produces a desired effect (*e.g.*, prophylactic or therapeutic effect). In some embodiments, the therapeutic effect is to reduce tumor number. In some embodiments, the therapeutic effect is to reduce tumor size. In some embodiments, the therapeutic effect is to increase survival.

[0095] In some embodiments, unit dosage forms may be within, for example, ampules and vials, including a liquid composition, or a composition in a freeze-dried or lyophilized state; a sterile liquid carrier, for example, can be added prior to administration or delivery *in vivo*. Individual

unit dosage forms can be included in multi-dose kits or containers. IL-2 muteins in combination with anti-PD-1 antibodies, and pharmaceutical compositions thereof can be packaged in a single or multiple unit dosage form for ease of administration and uniformity of dosage.

[0096] A “therapeutically effective amount” will fall in a relatively broad range determinable through experimentation and/or clinical trials. For example, for *in vivo* injection, *e.g.*, injection directly into the tissue or vasculature of a subject (for example, liver tissue or veins). Other effective dosages can be readily established by one of ordinary skill in the art through routine trials establishing dose response curves.

[0097] An “effective amount” or “sufficient amount” refers to an amount providing, in single or multiple doses, alone or in combination, with one or more other compositions (therapeutic agents such as a drug), treatments, protocols, or therapeutic regimens agents (including, for example, vaccine regimens), a detectable response of any duration of time (long or short term), an expected or desired outcome in or a benefit to a subject of any measurable or detectable degree or for any duration of time (*e.g.*, for minutes, hours, days, months, years, or cured).

[0098] The doses of an “effective amount” or “sufficient amount” for treatment (*e.g.*, to ameliorate or to provide a therapeutic benefit or improvement) typically are effective to provide a response to one, multiple or all adverse symptoms, consequences or complications of the disease, one or more adverse symptoms, disorders, illnesses, pathologies, or complications, for example, caused by or associated with the disease, to a measurable extent, although decreasing, reducing, inhibiting, suppressing, limiting or controlling progression or worsening of the disease is also a satisfactory outcome. In some embodiments, the effective amount is an amount sufficient to reduce tumor number. In some embodiments, the effective amount is an amount sufficient to reduce tumor size. In some embodiments, the effective amount is an amount sufficient to increase survival.

[0099] “Prophylaxis” and grammatical variations thereof mean a method in which contact, administration or *in vivo* delivery to a subject is prior to disease. Administration or *in vivo* delivery to a subject can be performed prior to development of an adverse symptom, condition, complication, etc. caused by or associated with the disease. For example, a screen (*e.g.*, genetic) can be used to identify such subjects as candidates for the described methods and uses, but the subject may not manifest the disease. Such subjects therefore include those screened positive for

an insufficient amount or a deficiency in a functional gene product (protein), or producing an aberrant, partially functional or non-functional gene product (protein), leading to disease; and subjects screening positive for an aberrant, or defective (mutant) gene product (protein) leading to disease, even though such subjects do not manifest symptoms of the disease.

I. DETAILED DESCRIPTION

[00100] Described herein IL-2 muteins comprising the substitutions L80F, R81D, L85V, I86V, and I92F, which have an increased binding capacity for IL-2R β receptor and that find use in combination treatments with anti-PD-1 antibodies. In some embodiments, the IL-2 mutein comprising L80F, R81D, L85V, I86V and I92F, numbered in accordance with wild-type human IL-2 (SEQ ID NO:2; wild-type hIL-2) is referred to as H9. Such IL-2 muteins find use, for example, when combined with anti-PD-1 antibodies for the treatment of cancer. Also provided are nucleic acids encoding such IL-2 muteins, methods of making such IL-2 muteins, pharmaceutical compositions that include such IL-2 muteins and methods of treatment using such IL-2 muteins.

A. IL-2 MUTEINS

[00101] The substituted amino acid residue(s) can be, but are not necessarily, conservative substitutions, which typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. These mutations can be at amino acid residues that contact the IL-2R β and/or the IL-2R γ .

[00102] More specifically, a mutation (whether conservative or non-conservative, by way of addition(s) or deletion(s)) can be made at one or more of positions. For example, the mutation can be: I24V, P65H, Q74R, Q74 H, Q74N, Q74S, L80F, L80V, R81I, R81T, R81D, L85V, I86V, I89V, I92F, V93I. The sequences of exemplary IL-2 muteins are as follows: 5-1 SEQ ID NO:5; 5-2 SEQ ID NO:6; 6-6 SEQ ID NO:7; A2 SEQ ID NO:8; B1 SEQ ID NO:9; B11 SEQ ID NO:10; C5 SEQ ID NO:11; D10 SEQ ID NO:12; E10 SEQ ID NO:13; G8 SEQ ID NO:14; H4 SEQ ID NO:15; and H9 SEQ ID NO:5.

[00103] In some embodiments, the substitutions in the IL-2 mutein comprise L80F, R81D, L85V, I86V, and I92F, numbered in accordance with wild-type human IL-2 of SEQ ID NO:2. In

some embodiments, the IL-2 mutein further comprises F42A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises Y45A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises E62A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the substitutions in the IL-2 mutein comprise F42A, L80F, R81D, L85V, I86V, and I92F, numbered in accordance with wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the substitutions in the IL-2 mutein comprise F42A, Y45A, L80F, R81D, L85V, I86V, and I92F, numbered in accordance with wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the substitutions in the IL-2 mutein comprise F42A, E62A, L80F, R81D, L85V, I86V, and I92F, numbered in accordance with wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the substitutions in the IL-2 mutein comprise F42A, Y45A, E62A, L80F, R81D, L85V, I86V, and I92F, numbered in accordance with wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the substitutions in the IL-2 mutein comprise E62A, L80F, R81D, L85V, I86V, and I92F, numbered in accordance with wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the substitutions in the IL-2 mutein comprise Y45A, E62A, L80F, R81D, L85V, I86V, and I92F, numbered in accordance with wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the substitutions in the IL-2 mutein comprise Y45A and E62A, numbered in accordance with wild-type human IL-2 of SEQ ID NO:2.

[00104] In some embodiments, the substitutions in the IL-2 mutein that lead to increased and/or enhanced IL-2R β binding include L80F, R81D, L85V, I86V, and I92F, numbered in accordance with wild-type human IL-2 of SEQ ID NO:2. In some embodiments, an IL-2 mutein for use in the invention comprises L80F, R81D, L85V, I86V, and I92F and exhibits increased IL-2R β binding. In some embodiments, an IL-2 mutein for use in the invention further comprises a substitution at position F42A. In some embodiments, the IL-2 mutein for use in the invention further comprises a substitution at position K43N. In some embodiments, the mutein comprises substitutions L80F, R81D, L85V, I86V, and I92F, and one or more substitutions selected from the group consisting of F42A, Y45A, and E62A, all as compared to wild-type human IL-2 (SEQ ID NO:2).

[00105] In some embodiments, the amino acid substitutions increasing IL-2R β binding affinity include: L80F, R81D, L85V, I86V, and I92F. In some embodiments, the amino acid substitutions that increase IL-2R β binding affinity include: L80F, R81D, L85V, I86V, and I92F.

[00106] In some embodiments, the subject IL-2 mutein having a greater binding affinity for IL-2R β as compared to wild-type human IL-2, includes the amino acid substitutions L80F, R81D, L85V, I86V, and I92F. In some embodiments, the IL-2 mutein has the amino acid sequence:

APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFKYMPKKATELKHLQCL
EEELKPLEEVLNLAQSKNFHFDPRDVVSNINVFVLEELKGSETTFMCEYADETATIVEFLN
RWITFCQSIISTLT (SEQ ID NO:5; H9 as used in Example 1).

[00107] In some embodiments, the IL-2 mutein has increased capabilities to stimulate one or more signaling pathways that are dependent on IL-2R β /IL-2R γ_c heterodimerization. In some embodiments, the subject IL-2 mutein has an enhanced capability to stimulate STAT5 phosphorylation in an IL-2R β^+ cell as compared to wild-type human IL-2. In some embodiments, the IL-2 mutein stimulates STAT5 phosphorylation in an IL-2R β^+ cell at a level that is 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of the level that wild-type IL-2 stimulates STAT5 phosphorylation in the same cell. In some embodiments, the IL-2 mutein stimulates STAT5 phosphorylation in an IL-2R β^+ cell at a level that is 105%, 110%, 115%, 120%, 125%, 130%, 135%, 140%, 145%, 150%, 155%, 160%, 165%, 170%, 175%, 180%, 185%, 190%, 195% or more as compared to the level that wild-type IL-2 stimulates STAT5 phosphorylation in the same cell. In some embodiments, the IL-2R β^+ cell is a T cell. In particular embodiments, the T cell is a CD8 $^+$ T cell. In some embodiments, the CD8 $^+$ T cell is a freshly isolated CD8 $^+$ T cell. In other embodiments, the CD8 $^+$ T cell T cell is an activated CD8 $^+$ T cell. In other embodiments, the IL-2R β^+ cell is a natural killer (NK) cell. In some embodiments, the IL-2 mutein comprises substitutions L80F, R81D, L85V, I86V, and I92F, as compared to wild-type human IL-2 (SEQ ID NO:2).

[00108] In some embodiments, the mutein has an enhanced capability to stimulate ERK1/ERK2 signaling in an IL-2R β^+ cell as compared to wild-type human IL-2. In some embodiments, the IL-2 mutein stimulates pERK1/ERK2 signaling in an IL-2R β^+ cell at a level

that is 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of the level that wild-type IL-2 stimulates pERK1/ERK2 signaling in the same cell. In some embodiments, the IL-2 mutein stimulates pERK1/ERK2 phosphorylation in an IL-2R β + cell at a level that is 105%, 110%, 115%, 120%, 125%, 130%, 135%, 140%, 145%, 150%, 155%, 160%, 165%, 170%, 175%, 180%, 185%, 190%, 195% or more as compared to the level that wild-type IL-2 stimulates pERK1/ERK2 phosphorylation in the same cell. In some embodiments, the IL-2R β + cell is a T cell. In particular embodiments, the T cell is a CD8+ T cell. In some embodiments, the CD8+ T cell is a freshly isolated CD8+ T cell. In other embodiments, the CD8+ T cell T cell is an activated CD8+ T cell. In other embodiments, the IL-2R β + cell is a natural killer (NK) cell. In some embodiments, the IL-2 mutein comprises substitutions L80F, R81D, L85V, I86V, and I92F, as compared to wild-type human IL-2 (SEQ ID NO:2).

[00109] STAT5 and ERK1/2 signaling can be measured, for example, by phosphorylation of STAT5 and ERK1/2 using any suitable method known in the art. For example, STAT5 and ERK1/2 phosphorylation can be measured using antibodies specific for the phosphorylated version of these molecules in combination with flow cytometry analysis as described herein. In some embodiments, the mutein has an enhanced capability to stimulate PI 3-kinase signaling in an IL-2R β + cell as compared to wild-type human IL-2. In some embodiments, the IL-2 mutein stimulates PI 3-kinase signaling in an IL-2R β + cell at a level that is 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or less of the level that wild-type IL-2 stimulates PI 3-kinase signaling in the same cell. In some embodiments, the IL-2 mutein stimulates PI 3-kinase signaling in an IL-2R β + cell at a level that is 105%, 110%, 115%, 120%, 125%, 130%, 135%, 140%, 145%, 150%, 155%, 160%, 165%, 170%, 175%, 180%, 185%, 190%, 195% or more as compared to the level that wild-type IL-2 stimulates PI 3-kinase signaling phosphorylation in the same cell. In some embodiments, the IL-2R β + cell is a T cell. In particular embodiments, the T cell is a CD8+ T cell. In some embodiments, the CD8+ T cell T cell is an activated CD8+ T cell. In other embodiments, the IL-2R β + cell is a natural killer (NK) cell. In some embodiments, the IL-2 mutein comprises substitutions L80F, R81D, L85V, I86V, and I92F, as compared to wild-type human IL-2 (SEQ ID NO:2). PI3-kinase signaling can be measured using any suitable method known in the art. For

example, PI 3-kinase signaling can be measured using antibodies that are specific for phospho-S6 ribosomal protein in conjunction with flow cytometry analysis as described herein.

[00110] In some embodiments the IL-2 mutein is a stimulator of IL-2 and/or IL-15 STAT5 phosphorylation in CD8+ T cells. In some embodiments, the mutein is a promoter of IL-2 and/or IL-15 induced proliferation of CD8+ T cells. In some embodiments, the mutein is a stimulator of IL-2 dependent, TCR-induced cell proliferation. In some embodiments, the IL-2 mutein comprises substitutions L80F, R81D, L85V, I86V, and I92F, as compared to wild-type human IL-2 (SEQ ID NO:2).

[00111] IL-2 promotes Th1, Th9, and Treg T cell differentiation and inhibits Th17 differentiation. Therefore, without being bound by any particular theory of operation, it is believed that IL-2 muteins that function as IL-2 superagonists are capable of promoting Th1, Th9, and/or Treg cell differentiation or inhibiting Th17 cell differentiation. In some embodiments, the IL-2 mutein is a promoter of IL-2 dependent Th1, Th9 and/or Treg differentiation. In some embodiments, the mutein is an inhibitor of Th17 differentiation. In some embodiments, the IL-2 mutein comprises substitutions L80F, R81D, L85V, I86V, and I92F, as compared to wild-type human IL-2 (SEQ ID NO:2).

[00112] In some embodiments, the IL-2 mutein signals less and/or independently of CD25 (for example, has reduced or ablated CD25 binding) as compared to wild-type human IL-2. In some embodiments the reduced and/or independent signaling with regard to CD25 allows for preferential activation of effector T-cells while limiting the stimulation of Tregs. In some embodiments the reduced and/or independent signaling with regard to CD25 allows for reduced toxicity. In some embodiments, the mutein comprises substitutions L80F, R81D, L85V, I86V, and I92F, and one or more substitutions selected from the group consisting of F42A, Y45A, and E62A, all as compared to wild-type human IL-2 (SEQ ID NO:2).

[00113] In some embodiments, the IL-2 mutein is capable of increasing and/or restoring responsiveness to anergic NK cells. In some embodiments, the IL-2 mutein is capable of increasing and/or restoring responsiveness to anergic NK cells in the tumor microenvironment. In some embodiments, the IL-2 mutein comprises substitutions L80F, R81D, L85V, I86V, and I92F, as compared to wild-type human IL-2 (SEQ ID NO:2).

[00114] In some embodiments the mutein is an inhibitor an inhibitor of IL-2 dependent activation of natural killer (NK) cells. IL-2 activation of NK cells can be measured by any suitable method known in the art, for example, by measuring IL-2 induced CD69 expression and/or cytotoxicity, as described herein.

[00115] In some embodiments, an increase in IL-2R β binding affinity is any binding affinity for IL-2R β that is greater than the wild-type human IL-2 binding affinity for IL-2R β . In some embodiments, the binding affinity is a 2-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 120-fold, 150-fold, 170-fold, 190-fold, 200-fold, 220-fold, 240-fold or more increase in binding affinity for IL-2R β as compared to the wild-type human IL-2 binding affinity for IL-2R β .

[00116] In some embodiments, an increase in binding capacity for IL-2R β is any binding capacity for IL-2R β that is greater than the wild-type human IL-2 binding capacity for IL-2R β . In some embodiments, the binding capacity is a 2-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 120-fold, 150-fold, 170-fold, 190-fold, 200-fold, 220-fold, 240-fold or more increase in binding capacity for IL-2R β as compared to the wild-type human IL-2 binding capacity for IL-2R β .

[00117] In some embodiments, the subject IL-2 mutein having a greater binding affinity for IL-2R β as compared to wild-type human IL-2 also exhibits reduced binding to CD25 and includes the amino acid substitutions F42A, L80F, R81D, L85V, I86V, and I92F. In some embodiments, the reduce binding affinity is about 220-fold, *i.e.*, from about K_d of 6.6 nM for wild-type human IL-2 to about 1.4 μ M for the mutein comprising F42A, L80F, R81D, L85V, I86V, and I92F. In some embodiments, the IL-2 mutein has the amino acid sequence:

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHFDPRDVVSNINVFVLEELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT (SEQ ID NO:6; also referred to as H9-F42A).

[00118] In some embodiments, the subject IL-2 mutein having a greater binding affinity for IL-2R β as compared to wild-type human IL-2 also exhibits reduced binding to CD25 and includes the amino acid substitutions K43N, L80F, R81D, L85V, I86V, and I92F. In some embodiments, the reduce binding affinity is due to allowing for glycosylation at position 43 with the K43N substitution. By substituting lysine for asparagine (K43N), CD25 binding is reduced

and/or ablated in the IL-2 mutein comprising the amino acid substitutions K43N, L80F, R81D, L85V, I86V, and I92F. In some embodiments, the IL-2 mutein has the amino acid sequence:

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFNFYMPKKATELKHLQCL
EEELKPLEEVLNLAQSKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLN
RWITFCQSIISTLT (SEQ ID NO:7; also referred to as H9-K43N).

[00119] In some embodiments, a reduction in binding affinity for CD25 is any binding affinity for CD25 that is less than the wild-type human IL-2 binding affinity. In some embodiments, the binding affinity is a 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 120-fold, 150-fold, 170-fold, 190-fold, 200-fold, 220-fold, 240-fold or more decrease in binding affinity for CD25 as compared to the wild-type human IL-2 binding affinity for CD25.

[00120] In some embodiments, the subject IL-2 mutein having a greater binding affinity for IL-2R β and a reduced binding affinity for CD25 as compared to wild-type human IL-2 includes the amino acid substitutions F42A, Y45A L80F, R81D, L85V, I86V, and I92F. In some embodiments, the IL-2 mutein has the amino acid sequence:

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFAMPKKATELKHLQCL
EEELKPLEEVLNLAQSKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLN
RWITFCQSIISTLT (SEQ ID NO:8; H9-F42A/Y45A; H9-FYAA).

[00121] In some embodiments, the subject IL-2 mutein having a greater binding affinity for IL-2R β and a reduced binding affinity for CD25 as compared to wild-type human IL-2 includes the amino acid substitutions F42A, E62A L80F, R81D, L85V, I86V, and I92F. In some embodiments, the IL-2 mutein has the amino acid sequence:

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCL
EEALKPLEEVLNLAQSKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLN
RWITFCQSIISTLT (SEQ ID NO:9; H9-F42A/E62A; H9-FEAA).

[00122] In some embodiments, the subject IL-2 mutein having a greater binding affinity for IL-2R β and a reduced binding affinity for CD25 as compared to wild-type human IL-2 includes the amino acid substitutions F42A, Y45A, E62A, L80F, R81D, L85V, I86V, and I92F. In some embodiments, the IL-2 mutein has the amino acid sequence:

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT (SEQ ID NO:10; H9-F42A/Y45A/E62A; H9-FYEAAA).

[00123] In some embodiments, the IL-2 mutein sequence is 90% identical to any one of SEQ ID NO:2 or SEQ ID NO:6 through SEQ ID NO:10 or SEQ ID NO:16. In some embodiments, the IL-2 mutein sequence is 95% identical to any one of SEQ ID NO:2 or SEQ ID NO:6 through SEQ ID NO:10. In some embodiments, the IL-2 mutein sequence is 98% identical to any one of SEQ ID NO:2 or SEQ ID NO:6 through SEQ ID NO:10. In some embodiments, the IL-2 mutein sequence is 99% identical to any one of SEQ ID NO:2 or SEQ ID NO:6 through SEQ ID NO:10.

[00124] Further exemplary IL-2 sequences are provided in the table below.

Table 2: List of Exemplary IL-2 Muteins

Amino Acid Sequences SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:6 (also referred to as H9-F42A)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLT <u>A</u> K <u>F</u> YMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFH <u>F</u> D <u>P</u> R <u>D</u> <u>V</u> V <u>S</u> NIN <u>V</u> <u>F</u> VLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT (
(SEQ ID NO:7 (also referred to as H9-K43N)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLT <u>F</u> <u>N</u> YMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFH <u>F</u> D <u>P</u> R <u>D</u> <u>V</u> V <u>S</u> NIN <u>V</u> <u>F</u> VLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
SEQ ID NO:8 (H9-F42A/Y45A; H9-FYAA)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLT <u>A</u> K <u>F</u> A <u>M</u> PKKATELKHLQCLEEELKPLEEVLNLAQSKNFH <u>F</u> D <u>P</u> R <u>D</u> <u>V</u> V <u>S</u> NIN <u>V</u> <u>F</u> VLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

Amino Acid Sequences SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:9) (H9-F42A/E62A; H9-FEAA)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TRMLT <u>A</u> KFYMPKKATELKHLQCLEE <u>A</u> LKPLEEVLN LAQSKNFH <u>FD</u> PRD <u>VV</u> SNINV <u>F</u> VLELKGSETTFMCE YADETATIVEFLNRWITFCQSIISTLT
SEQ ID NO:10; H9- F42A/Y45A/E62A; H9-FYEAAA).	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TRMLT <u>A</u> K <u>F</u> AMPKKATELKHLQCLEE <u>A</u> LKPLEEVLN LAQSKNFH <u>FD</u> PRD <u>VV</u> SNINV <u>F</u> VLELKGSETTFMCE YADETATIVEFLNRWITFCQSIISTLT
SEQ ID NO:20	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTF KFYMPKKATELKHLQCLEEELKPLEEVLNLA <u>R</u> SKNFHLRPRD LISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLT
SEQ ID NO:21	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTF KFYMPKKATELKHLQCLEEELKPLEEVLNLA <u>R</u> SKNFHLRPRD <u>V</u> ISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLT
SEQ ID NO:22	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTF KFYMPKKATELKHLQCLEEELKPLEEVLNLA <u>R</u> SKNFHL <u>I</u> PRD <u>V</u> ISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLT
SEQ ID NO:23	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTF KFYMPKKATELKHLQCLEEELKPLEEVLNLA <u>H</u> SKNFHL <u>T</u> PRD <u>VV</u> SNINV <u>F</u> I <u>L</u> LELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLT
SEQ ID NO:24	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTF KFYMPKKATELKHLQCLEEELKPLEEVLNLA <u>N</u> SKNFH <u>FD</u> PRD <u>VV</u> SNVNV <u>F</u> VLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLT
SEQ ID NO:25	APTSSSTKKTQLQLEHLLLDLQM <u>V</u> LNGINNYKNPKLTRMLTF KFYMPKKATELKHLQCLEEELKPLEEVLNLA <u>S</u> SKNFH <u>FD</u> PRD <u>VV</u> SNINV <u>F</u> VLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLT
SEQ ID NO:26	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTF KFYMPKKATELKHLQCLEEELK <u>H</u> LEEVLNLA <u>N</u> SKNFH <u>VT</u> PRD <u>VV</u> SNINV <u>F</u> VLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLT
SEQ ID NO:27	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTF KFYMPKKATELKHLQCLEEELKPLEEVLNLA <u>H</u> SKNFH <u>FD</u> PRD

Amino Acid Sequences	
SEQ ID NO: (Information)	Amino acid sequence
	<u>V</u> VSNINV <u>F</u> VLEELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLT
SEQ ID NO:28	APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTF KFYMPKKATELKHLQCLEEELKPLEEVLNLA <u>S</u> SKNFH <u>FD</u> PRD <u>V</u> VSNINV <u>F</u> VLEELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLT
SEQ ID NO:29	APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTF KFYMPKKATELKHLQCLEEELKPLEEVLNLA <u>N</u> SKNFH <u>FD</u> PRD <u>V</u> VSNINV <u>F</u> VLEELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLT
SEQ ID NO:30	APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTF KFYMPKKATELKHLQCLEEELKPLEEVLNLA <u>S</u> SKNFH <u>L</u> T <u>PRD</u> <u>V</u> ISNINV <u>F</u> VLEELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLT
SEQ ID NO:31	APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTF KFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFH <u>FD</u> PRD <u>V</u> VSNINV <u>F</u> VLEELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLT
SEQ ID NO:32 IL-2 agonist	H9D10 APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFK FYMPKKATELKHLQCLEEELKPLEEVLNLAHSKNFHFDPRDVVSNIN NVFVLEELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
SEQ ID NO:33 IL-2 agonist	H9E10 APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFY MPKKATELKHLQCLEEELKPLEEVLNLAASSKNFHFDPDRDVVSNIN VLEELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
SEQ ID NO:34 IL-2 agonist	H9G8 APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFY MPKKATELKHLQCLEEELKPLEEVLNLANSKNFHFDPDRDVVSNIN VLEELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
SEQ ID NO:35 IL-2 agonist	H9B1 APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFY MPKKATELKHLQCLEEELKPLEEVLNLANSKNFHFDPDRDVVSNIN VLEELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

B. IL-2 MUTEIN FUSION PROTEINS

[00125] The IL-2 muteins can be prepared as fusion or chimeric polypeptides that include a subject IL-2 mutein and a heterologous polypeptide (i.e., a polypeptide that is not IL-2 or a mutant thereof) (*see, e.g.*, U.S. Pat. No. 6,451,308). Exemplary heterologous polypeptides can increase the circulating half-life of the chimeric polypeptide *in vivo*, and may, therefore, further enhance the properties of the mutant IL-2 polypeptides. In various embodiments, the polypeptide that increases the circulating half-life may be a serum albumin, such as human serum albumin,

PEG, PEG-derivatives, or the Fc region of the IgG subclass of antibodies that lacks the IgG heavy chain variable region. Exemplary Fc regions can include a mutation that inhibits complement fixation and Fc receptor binding, or it may be lytic, i.e., able to bind complement or to lyse cells via another mechanism, such as antibody-dependent complement lysis (ADCC; USSN 08/355,502 filed Dec. 12, 1994).

[00126] The “Fc region” can be a naturally occurring or synthetic polypeptide that is homologous to the IgG C-terminal domain produced by digestion of IgG with papain. IgG Fc has a molecular weight of approximately 50 kDa. The mutant IL-2 polypeptides can include the entire Fc region, or a smaller portion that retains the ability to extend the circulating half-life of a chimeric polypeptide of which it is a part. In addition, full-length or fragmented Fc regions can be variants of the wild-type molecule. In some embodiments, the IL-2 mutein fusion protein (*e.g.*, an IL-2 mutein as described herein) includes an IgG1, IgG2, IgG3, or IgG4 Fc region (see, for example, sequences in Figure 2A-2B). In some embodiments, the Fc region comprises the substitution N297A.

[00127] In some embodiments, the IL-2 mutein is linked directly or indirectly to the heterologous fusion polypeptide.

[00128] In some embodiments, the IL-2 mutein is linked directly to the Fc region. In some embodiments, the IL-2 mutein is linked to the Fc region via a linker peptide, such as GGGGS. In some embodiments, the linker is (GGGGS)*n*, wherein *n* is an integer between 1 and 10. In some embodiments, the linker is GGGGS. In some embodiments, the linker is GGGGSGGGGS (SEQ ID NO:16). In some embodiments, the linker is GGGGSGGGGSGGGGS (SEQ ID NO:17). In some embodiments, the linker is GGGGSGGGGSGGGGSGGGGS (SEQ ID NO:18). In some embodiments, the linker is GGGGSGGGGSGGGGSGGGGS (SEQ ID NO:19).

[00129] The Fc region can be “lytic” or “non-lytic,” but is typically non-lytic. A non-lytic Fc region typically lacks a high affinity Fc receptor binding site and a C'1q binding site. The high affinity Fc receptor binding site of murine IgG Fc includes the Leu residue at position 235 of IgG Fc. Thus, the Fc receptor binding site can be destroyed by mutating or deleting Leu 235. For example, substitution of Glu for Leu 235 inhibits the ability of the Fc region to bind the high affinity Fc receptor. The murine C'1q binding site can be functionally destroyed by mutating or deleting the Glu 318, Lys 320, and Lys 322 residues of IgG. For example,

substitution of Ala residues for Glu 318, Lys 320, and Lys 322 renders IgG1 Fc unable to direct antibody-dependent complement lysis. In contrast, a lytic IgG Fc region has a high affinity Fc receptor binding site and a C'1q binding site. The high affinity Fc receptor binding site includes the Leu residue at position 235 of IgG Fc, and the C'1q binding site includes the Glu 318, Lys 320, and Lys 322 residues of IgG1. Lytic IgG Fc has wild-type residues or conservative amino acid substitutions at these sites. Lytic IgG Fc can target cells for antibody dependent cellular cytotoxicity or complement directed cytolysis (CDC). Appropriate mutations for human IgG are also known (see, e.g., Morrison et al., *The Immunologist* 2:119-124, 1994; and Brekke et al., *The Immunologist* 2: 125, 1994).

[00130] In other embodiments, the chimeric polypeptide can include a subject IL-2 mutein and a polypeptide that functions as an antigenic tag, such as a FLAG sequence. FLAG sequences are recognized by biotinylated, highly specific, anti-FLAG antibodies, as described herein (see also Blonar et al., *Science* 256:1014, 1992; LeClair et al., *Proc. Natl. Acad. Sci. USA* 89:8145, 1992). In some embodiments, the chimeric polypeptide further comprises a C-terminal c-myc epitope tag.

[00131] In other embodiments, the chimeric polypeptide includes the mutant IL-2 polypeptide and a heterologous polypeptide that functions to enhance expression or direct cellular localization of the mutant IL-2 polypeptide, such as the Aga2p agglutinin subunit (see, e.g., Boder and Wittrup, *Nature Biotechnol.* 15:553-7, 1997).

[00132] In other embodiments, a chimeric polypeptide including a mutant IL-2 and an antibody or antigen-binding portion thereof can be generated. The antibody or antigen-binding component of the chimeric protein can serve as a targeting moiety. For example, it can be used to localize the chimeric protein to a particular subset of cells or target molecule. Methods of generating cytokine-antibody chimeric polypeptides are described, for example, in U.S. Pat. No. 6,617,135.

[00133] In some embodiments, the chimeric polypeptide comprises a fusion to an antibody or an antigen-binding portion thereof that disrupts the interaction between the PD-1 receptor and its ligand, PD-L1, and/or is an antibody to a component of the PD-1/PD-L1 signaling pathway. Antibodies known in the art which bind to PD-1 and disrupt the interaction between the PD-1 and its ligand, PD-L1, and stimulate an anti-tumor immune response, are suitable for use in the

chimeric polypeptides disclosed herein. In some embodiments, the antibody or antigen-binding portion thereof binds specifically to PD-1. For example, antibodies that target PD-1 and which can find used in the present invention include, e.g., but are not limited to nivolumab (BMS-936558, Bristol-Myers Squibb), pembrolizumab (lambrolizumab, MK03475 or MK-3475, Merck), humanized anti-PD-1 antibody JS001 (ShangHai JunShi), monoclonal anti-PD-1 antibody TSR-042 (Tesar, Inc.), Pidilizumab (anti-PD-1 mAb CT-011, Medivation), anti-PD-1 monoclonal Antibody BGB-A317 (BeiGene), and/or anti-PD-1 antibody SHR-1210 (ShangHai HengRui), human monoclonal antibody REGN2810 (cemiplimab, Regeneron), human monoclonal antibody MDX-1106 (Bristol-Myers Squibb), and/or humanized anti-PD-1 IgG4 antibody PDR001 (Novartis). In some embodiments, the PD-1 antibody is from clone: RMP1-14 (rat IgG) - BioXcell cat# BP0146. Other suitable antibodies include anti-PD-1 antibodies disclosed in U.S. Patent No. 8,008,449, herein incorporated by reference. In some embodiments, the antibody or antigen-binding portion thereof binds specifically to PD-L1 and inhibits its interaction with PD-1, thereby increasing immune activity. Any antibodies known in the art which bind to PD-L1 and disrupt the interaction between the PD-1 and PD-L1, and stimulates an anti-tumor immune response, are suitable for use in the chimeric polypeptides disclosed herein. For example, antibodies that target PD-L1 and are in clinical trials, include BMS-936559 (Bristol-Myers Squibb) and MPDL3280A (Genetech). Other suitable antibodies that target PD-L1 are disclosed in U.S. Patent No. 7,943,743, herein incorporated by reference. It will be understood by one of ordinary skill that any antibody which binds to PD-1 or PD-L1, disrupts the PD-1/PD-L1 interaction, and stimulates an anti-tumor immune response, is suitable for use in the chimeric polypeptides disclosed herein. In some embodiments, the chimeric polypeptide comprises a fusion to an anti-PD-1 antibody. In some embodiments, the chimeric polypeptide comprises a fusion to an anti-PD-L1 antibody.

[00134] In some embodiments, the chimeric polypeptide comprises a fusion to an antibody or an antigen-binding portion thereof that targets CTLA-4 and disrupts its interaction with CD80 and CD86. Exemplary antibodies that target CTLA-4 include ipilimumab (MDX-010, MDX-101, Bristol-Myers Squibb), which is FDA approved, and tremelimumab (ticilimumab, CP-675, 206, Pfizer), currently undergoing human trials. Other suitable antibodies that target CTLA-4 are disclosed in WO 2012/120125, U.S. Patents No. 6,984720, No. 6,682,7368, and U.S. Patent Applications 2002/0039581, 2002/0086014, and 2005/0201994, herein incorporated by

reference. It will be understood by one of ordinary skill that any antibody which binds to CTLA-4, disrupts its interaction with CD80 and CD86, and stimulates an anti-tumor immune response, is suitable for use in the chimeric polypeptides disclosed herein. In some embodiments, the chimeric polypeptide comprises a fusion to an anti-CTLA-4 antibody.

[00135] In some embodiments, the chimeric polypeptide comprises a fusion to an antibody or an antigen-binding portion thereof that targets LAG-3 and disrupts its interaction with MHC class II molecules. An exemplary antibody that targets LAG-3 is IMP321 (Immutep), currently undergoing human trials. Other suitable antibodies that target LAG-3 are disclosed in U.S. Patent Application 2011/0150892, herein incorporated by reference. It will be understood by one of ordinary skill that any antibody which binds to LAG-3, disrupts its interaction with MHC class II molecules, and stimulates an anti-tumor immune response, is suitable for use in the chimeric polypeptides disclosed herein. In some embodiments, the chimeric polypeptide comprises a fusion to an anti-LAG-3 antibody.

[00136] In some embodiments, the chimeric polypeptide comprises a fusion to an antibody or an antigen-binding portion thereof that targets B7-H3 or B7-H4. The B7 family does not have any defined receptors but these ligands are upregulated on tumor cells or tumor-infiltrating cells. An exemplary antibody that targets B7-H3 is MGA271 (Macrogenics) is currently undergoing human trials. Other suitable antibodies that target B7 family members are disclosed in U.S. Patent Application 2013/0149236, herein incorporated by reference. It will be understood by one of ordinary skill that any antibody which binds to B7-H3 or H4, and stimulates an anti-tumor immune response, is suitable for use in the chimeric polypeptides disclosed herein. In some embodiments, the chimeric polypeptide comprises a fusion to an anti- B7-H3 or B7-H4 antibody.

[00137] In some embodiments, the chimeric polypeptide comprises a fusion to an antibody or an antigen-binding portion thereof that targets TIM-3 and disrupts its interaction with galectin 9. Suitable antibodies that target TIM-3 are disclosed in U.S. Patent Application 2013/0022623, herein incorporated by reference. It will be understood by one of ordinary skill that any antibody which binds to TIM-3, disrupts its interaction with galectin 9, and stimulates an anti-tumor immune response, is suitable for use in the chimeric polypeptides disclosed herein. In some embodiments, the chimeric polypeptide comprises a fusion to an anti- TIM-3 antibody.

[00138] In some embodiments, the chimeric polypeptide comprises a fusion to an antibody or an antigen-binding portion thereof that targets 4-1BB/CD137 and disrupts its interaction with CD137L. It will be understood by one of ordinary skill that any antibody which binds to 4-1BB/CD137, disrupts its interaction with CD137L or another ligand, and stimulates an anti-tumor immune response or an immune stimulatory response that results in anti-tumor activity overall, is suitable for use in the chimeric polypeptides disclosed herein. In some embodiments, the chimeric polypeptide comprises a fusion to an anti-4-1BB/CD137 antibody.

[00139] In some embodiments, the chimeric polypeptide comprises a fusion to an antibody or an antigen-binding portion thereof that targets GITR and disrupts its interaction with its ligand. It will be understood by one of ordinary skill that any antibody which binds to GITR, disrupts its interaction with GITRL or another ligand, and stimulates an anti-tumor immune response or an immune stimulatory response that results in anti-tumor activity overall, is suitable for use in the chimeric polypeptides disclosed herein. In some embodiments, the chimeric polypeptide comprises a fusion to an anti-GITR antibody.

[00140] In some embodiments, the chimeric polypeptide comprises a fusion to an antibody or an antigen-binding portion thereof that targets OX40 and disrupts its interaction with its ligand. It will be understood by one of ordinary skill that any antibody which binds to OX40, disrupts its interaction with OX40L or another ligand, and stimulates an anti-tumor immune response or an immune stimulatory response that results in anti-tumor activity overall, is suitable for use in the chimeric polypeptides disclosed herein. In some embodiments, the chimeric polypeptide comprises a fusion to an anti-OX40 antibody.

[00141] In some embodiments, the chimeric polypeptide comprises a fusion to an antibody or an antigen-binding portion thereof that targets CD40 and disrupts its interaction with its ligand. It will be understood by one of ordinary skill that any antibody which binds to CD40, disrupts its interaction with its ligand, and stimulates an anti-tumor immune response or an immune stimulatory response that results in anti-tumor activity overall, is suitable for use in the chimeric polypeptides disclosed herein. In some embodiments, the chimeric polypeptide comprises a fusion to an anti-CD40 antibody

[00142] In some embodiments, the chimeric polypeptide comprises a fusion to an antibody or an antigen-binding portion thereof that targets ICOS and disrupts its interaction with its

ligand. It will be understood by one of ordinary skill that any antibody which binds to ICOS, disrupts its interaction with its ligand, and stimulates an anti-tumor immune response or an immune stimulatory response that results in anti-tumor activity overall, is suitable for use in the chimeric polypeptides disclosed herein. In some embodiments, the chimeric polypeptide comprises a fusion to an anti-ICOS antibody.

[00143] In some embodiments, the chimeric polypeptide comprises a fusion to an antibody or an antigen-binding portion thereof that targets CD28 and disrupts its interaction with its ligand. It will be understood by one of ordinary skill that any antibody which binds to CD28, disrupts its interaction with its ligand, and stimulates an anti-tumor immune response or an immune stimulatory response that results in anti-tumor activity overall, is suitable for use in the chimeric polypeptides disclosed herein. In some embodiments, the chimeric polypeptide comprises a fusion to an anti-CD28 antibody.

[00144] In some embodiments, the chimeric polypeptide comprises a fusion to an antibody or an antigen-binding portion thereof that targets IFN α and disrupts its interaction with its ligand. It will be understood by one of ordinary skill that any antibody which binds to IFN α , disrupts its interaction with its ligand, and stimulates an anti-tumor immune response or an immune stimulatory response that results in anti-tumor activity overall, is suitable for use in the chimeric polypeptides disclosed herein. In some embodiments, the chimeric polypeptide comprises a fusion to an anti-IFN α antibody.

[00145] In some embodiments, the chimeric polypeptide comprises a fusion to a tumor antigen or polypeptide targeting a tumor antigen. Generally, tumor antigens allow for distinguishing the tumor cells from their normal cellular counterparts and can include, for example, tumor-specific antigens (TSA) as well as tumor-associated antigens (TAA). In some embodiments, a tumor antigen is a protooncogene and/or a tumor suppressor, as well as overexpressed or aberrantly expressed cellular proteins, tumor antigens produced by oncogenic viruses, oncofetal antigens, altered cell surface glycolipids and glycoproteins, and/or cell type-specific differentiation antigens. Such tumor antigens can include melanoma antigens, cancer-testis antigens, epithelial tumor antigens, cell cycle regulatory proteins, prostate specific antigens (including prostate carcinoma antigens, such as for example those disclosed in U.S. Pat. No. 5,538,866) lymphoma (U.S. Pat. Nos. 4,816,249; 5,068,177; and 5,227,159). Tumor antigens can include for example,

but are not limited to, HMW mucins bound by 2G3 and 369F10, c-erbB-2 related tumor antigen (an approximately 42 kD or 55 kD glycoprotein), the approximately 40, 60, 100 and 200 kD antigens bound by 113F1, 9-O-acetyl GD3, p97, alphafetoprotein (AFP) (for example, for germ cell tumors and/or hepatocellular carcinoma), carcinoembryonic antigen (CEA) (for example, for bowel cancers occasional lung or breast cancer), CA-125 (for example, for ovarian cancer), MUC-1 (for example, for breast cancer), epithelial tumor antigen (ETA) (for example, for breast cancer), tyrosinase (for example, for malignant melanoma), melanoma-associated antigen (MAGE) (for example, for malignant melanoma), cancer/testis antigen 1 (CTAG1B), melanoma-associated antigen 1 (MAGEA1), abnormal Ras products, abnormal p53 products, overexpression of cyclins (including, for example, cyclin B1), mutation in fibronectin, posttranslational alteration in the MUC1 glycoprotein, secreted tumor antigens (including, for example, gangliosides).

[00146] Other fusions can include fusions with pro-apoptotic payloads. Such exemplary sequences are provided in the table below. In some embodiments, and IL-2 mutein as described herein is fused to a pro-apoptotic payload, for example a BAD, BAX, BAK, BIK, and/or BID sequence. In some embodiments, the pro-apoptotic payload is a Bcl-2 domain containing peptide and/or a subsequence of a BAD, BAX, BAK, BIK, and/or BID sequence. Exemplary pro-apoptotic fusions are provided below, in Table 3.

Table 3: List of Selected Pro-Apoptotic Fusion Partners

SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO: 38 BAD amino acid sequence	MFQIPEFEPSEQEDSSSAERGLGSPAGDGPSPGSGKHHHRQ APGLLDASHQQEQPTSSSHHGGAGAVEIRSRHSAYPAGT EDDEGMGEEPS PFRGRSRAAPPNLWAAQRYGRELRRMSDE FVDSFKKGLPRPKSAGTATQMRQSSSWTRVFQSWWDRNLG RGSSAPSQ
SEQ ID NO: 39 >HsBAD_Q92934-1(UniProtKB)	MFQIPEFEPSEQEDSSSAERGLGSPAGDGPSPGSGKHHHRQ APGLLDASHQQEQPTSSSHHGGAGAVEIRSRHSYYPAGT EDDEGMGEEPS PFRGRSRSAPPNLWAAQRYGRELRRMSDE FVDSFKKGLPRPKSAGTATQMRQSSSWTRVFQSWWDRNLG RGSSAPSQ

SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:40 >HsBAX_Q07812-1(UniProtKB)	MDGSGEQPRGGGPTSSEQIMKTGALLLQGFIQDRAGRMGG EAPELALDPVPQDASTKKLSECLKRI GDELD SNMELQMI AAVDTDSPREVFFRVAADMFS DGNFNWGRVVALFYFASKL VLKALCTKVPELIRTIMGWTLDLFLRERLLGWIQDQGGWDG LLSYFGTPTWQTVTIFVAGVLTASLTIWKKMG
SEQ ID NO:41 >HsBAK1_Q16611-1(UniProtKB)	MASGQGP GPPRQECGEPALPSASEEQVAQDTEEVFRSYVF YRHQQEQEAE GVAAPADPEMVTLP LQPSSTMGQVGRQLAI IGDDINRRYDSEFQTMLQHLQPTAENAYEYFTKIATS LFE SGINWGRVVAL LGFGYRLALHVYQHGLTGF LGQVTRFVVD FMLHHC IARWIAQRGGWVAALNLGN GPI LN VLVVLGVVLL GQFVVRFFKS
SEQ ID NO:42 >HsBIK_Q13323-1(UniProtKB)	MSEVRPLSRD ILMETLLYEQLLEPPTMEVLGMTDSEEDLD PMEFDFSLEC MEGSDALALRLACIGDEM DVS LRAPRLAQLSEVAMHSLGL AFIYDQTEDI RDVLR SFMDGFTTLKENIMRFWRS PNP GSWVSCEQVLLAL LLLLALLLPL LSGGLHLLK
SEQ ID NO:43 >HsBID_P55957-1(UniProtKB)	MDCEVNNGSSLRDECITNLLVFGFLQSCSDNSFRRELDAL GHELPVLAPQ WEGYDELQTDGNRSSHSRLGRIEADSESQEDIIRNIARHL AQVGD SMDRS I PPGLVNGLALQLRNTSRSEEDRNRDLATALEQLLQAYPR DMEKEKTMLV LALLLAKKVASH TPSLLRDVFHTTVNF INQNLRTYVRS LA RNGMD

In some particular embodiments, an IL-2 antagonist can be fused to a pro-apoptotic payload for the treatment of cancer. An “antagonist” is a compound that opposes the actions of an agonist, e.g. by preventing, reducing, inhibiting, or neutralizing the activity of an agonist. An “antagonist” can also prevent, inhibit, or reduce constitutive activity of a target, e.g., a target receptor, even where there is no identified agonist. While typically IL-2 muteins with agonist or superagonist activity as compared to wild-type IL-2 are employed with the cancer treatment

methods of the present invention, IL-2 muteins with antagonistic properties can be employed when such antagonists are fused to a pro-apoptotic payload. In some embodiments, the IL-2 antagonist comprises the following amino acid substitutions L18R, Q22E, Q126T, and S130R as compared to the wild-type IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 antagonist comprises the following amino acid substitutions L18R, Q22E, L80F, R81D, L85V, I86V, and Q126T as compared to the wild-type IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 antagonist comprises the following amino acid substitutions L18R, Q22E, L80F, R81D, L85V, I86V, Q126T, and S130R as compared to the wild-type IL-2 of SEQ ID NO:2. Exemplary antagonists that can be fused with pro-apoptotic payloads, such as those provided above, are provided below in Table 4.

Table 4: IL-2 Antagonists for Fusion with Pro-Apoptotic Payloads

SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:36 IL-2 antagonist	APTSSSTKKTQLQLEHLRLDLEMILNGINNYKNPKLTRMLTFK FYM PKKATELKHLQCLEEEELKPLEEVLNLAQSKNFHFDPR DVVSNINVFV LELKGSETTF MCEYADETATIVEFLNRWIT FCTSIISTLT
SEQ ID NO:37 IL-2 VARIANT (antagonist)	APTSSSTKKTQLQLEHLRLDLEMILNGINNYKNPKLTRMLTFK FYM PKKATELKHLQCLEEEELKPLEEVLNLAQSKNFHFDPRDVVSNINVF VLELKGSETTFMCEYADETATIVEFLNRWITFCTSIIRTLT
SEQ ID NO:56 IL-2 extended half-life fusion (GS linker can be GGGSGGGSGGGGS as shown or anything other GS containing in linker)	H9RET-Fc APTSSSTKKTQLQLEHLRLDLEMILNGINNYKNPKLTRMLTFK FYM PKKATELKHLQCLEEEELKPLEEVLNLAQSKNFHFDPRDVVSNINVF VLELKGSETTFMCEYADETATIVEFLNRWITFCTSIISTLT GGGGS GGGSGGGGS DKTHTCPPCPAPELLGGPSVFLFPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNV FSC SVMHEALHNHYTQKS LSLSPGK*
SEQ ID NO:57 IL-2 extended half-life fusion (GS linker can be GGGSGGGSGGGGS as shown or anything other GS containing in linker)	IL-2 VARIANT-Fc APTSSSTKKTQLQLEHLRLDLEMILNGINNYKNPKLTRMLTFK FYM PKKATELKHLQCLEEEELKPLEEVLNLAQSKNFHFDPRDVVSNINVF VLELKGSETTFMCEYADETATIVEFLNRWITFCTSIIRTLT GGGGS GGGSGGGGS DKTHTCPPCPAPELLGGPSVFLFPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNV FSC SVMHEALHNHYTQKS LSLSPGK*
SEQ ID NO:58	H9RETFYAA-Fc APTSSSTKKTQLQLEHLRLDLEMILNGINNYKNPKLTRMLTAKFAM PKKATELKHLQCLEEEELKPLEEVLNLAQSKNFHFDPRDVVSNINVF

SEQ ID NO: (Information)	Amino acid sequence
<p>IL-2 extended half-life fusion (GS linker can be <u>GGGSGGGSGGGGS</u> as shown or anything other GS containing in linker)</p>	<p>VLELKGSETTFMCEYADETATIVEFLNRWITFCTSIISTLT<u>GGGGS</u> <u>GGGSGGGGS</u>DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNV FSC SVMHEALHNHYTQKS LSLSPGK*</p>
<p>SEQ ID NO:59 IL-2 extended half-life fusion (GS linker can be <u>GGGSGGGSGGGGS</u> as shown or any other GS containing linker)</p>	<p>IL-2 VARIANTFYAA-Fc APTSSSTKKTQLQLEHLRRLDLEMILNGINNYKNPKLTRMLTAKFAM PKKATELKHLLQCLEEELKPLEEVLNLAQSKNFHFDPDRVVSNINVF VLELKGSETTFMCEYADETATIVEFLNRWITFCTSIIRTLT<u>GGGGS</u> <u>GGGSGGGGS</u>DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNV FSC SVMHEALHNHYTQKS LSLSPGK*</p>
<p>SEQ ID NO:60 IL-2 extended half-life fusion (GS linker can be <u>GGGSGGGSGGGGS</u> as shown or any other GS containing linker)</p>	<p>H9RETFEAA-Fc APTSSSTKKTQLQLEHLRRLDLEMILNGINNYKNPKLTRMLTAKFYM PKKATELKHLLQCLEEALKPLEEVLNLAQSKNFHFDPDRVVSNINVF VLELKGSETTFMCEYADETATIVEFLNRWITFCTSIISTLT<u>GGGGS</u> <u>GGGSGGGGS</u>DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNV FSC SVMHEALHNHYTQKS LSLSPGK*</p>
<p>SEQ ID NO:61 IL-2 extended half-life fusion (GS linker can be <u>GGGSGGGSGGGGS</u> as shown or any other GS containing linker)</p>	<p>IL-2 VARIANTFEAA-Fc APTSSSTKKTQLQLEHLRRLDLEMILNGINNYKNPKLTRMLTAKFYM PKKATELKHLLQCLEEALKPLEEVLNLAQSKNFHFDPDRVVSNINVF VLELKGSETTFMCEYADETATIVEFLNRWITFCTSIIRTLT<u>GGGGS</u> <u>GGGSGGGGS</u>DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNV FSC SVMHEALHNHYTQKS LSLSPGK*</p>

[00147] Other fusions can include fusions with anti-apoptotic payloads for use in prolonging activation of CD8 cells, NK cells and anergic NK cells as well, and such exemplary sequences are provided in the table below. Such prolong activation of T-cells can prove beneficial in cancer therapy treatment methods.

Table 4: List of Exemplary IL-2 Anti-Apoptotic Fusion Amino Acid Sequences

SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:40	H9-BclxL APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFFKFY MPKKATELKHLLQCLEEEELKPLEEVLNLAQSKNFHFDPRDVVSNIN VVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGG GGSMSQSNRELVVDFLSYKLSQKGYSSWSQFSDVEENRTEAPEGTE SEMETPSAINGNPSWHLADSPAVNGATGHSSSLDAREVIMPAAVK QALREAGDEFELRYRRAFSDLTSQLHITPGTAYQSFEQVVNELFR DGVNWGRIVAFFSFGGALCVESVDKEMQVLVSRIAAMMATYLNH LEPWIQENGGWDTFVELYGNNAAAESRKGQERFNRWFLTGMTVAG VVLLGSLFSRK*
SEQ ID NO:41	H9FYAA-BclxL APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTAKFA MPKKATELKHLLQCLEEEELKPLEEVLNLAQSKNFHFDPRDVVSNIN VVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGG GGSMSQSNRELVVDFLSYKLSQKGYSSWSQFSDVEENRTEAPEGTE SEMETPSAINGNPSWHLADSPAVNGATGHSSSLDAREVIMPAAVK QALREAGDEFELRYRRAFSDLTSQLHITPGTAYQSFEQVVNELFR DGVNWGRIVAFFSFGGALCVESVDKEMQVLVSRIAAMMATYLNH LEPWIQENGGWDTFVELYGNNAAAESRKGQERFNRWFLTGMTVAG VVLLGSLFSRK*
SEQ ID NO:42	H9FEAA-BclxL APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTAKFY MPKKATELKHLLQCLEEALKPLEEVLNLAQSKNFHFDPRDVVSNIN VVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGG GGSMSQSNRELVVDFLSYKLSQKGYSSWSQFSDVEENRTEAPEGTE SEMETPSAINGNPSWHLADSPAVNGATGHSSSLDAREVIMPAAVK QALREAGDEFELRYRRAFSDLTSQLHITPGTAYQSFEQVVNELFR DGVNWGRIVAFFSFGGALCVESVDKEMQVLVSRIAAMMATYLNH LEPWIQENGGWDTFVELYGNNAAAESRKGQERFNRWFLTGMTVAG VVLLGSLFSRK*
SEQ ID NO:43	H9D10-BclxL APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFFKFY MPKKATELKHLLQCLEEEELKPLEEVLNLAHASKNFHFDPRDVVSNIN VVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGG GGSMSQSNRELVVDFLSYKLSQKGYSSWSQFSDVEENRTEAPEGTE SEMETPSAINGNPSWHLADSPAVNGATGHSSSLDAREVIMPAAVK QALREAGDEFELRYRRAFSDLTSQLHITPGTAYQSFEQVVNELFR DGVNWGRIVAFFSFGGALCVESVDKEMQVLVSRIAAMMATYLNH LEPWIQENGGWDTFVELYGNNAAAESRKGQERFNRWFLTGMTVAG VVLLGSLFSRK*
SEQ ID NO:44	H9E10-BclxL APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFFKFY MPKKATELKHLLQCLEEEELKPLEEVLNLASKNFHFDPRDVVSNIN VVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGG GGSMSQSNRELVVDFLSYKLSQKGYSSWSQFSDVEENRTEAPEGTE SEMETPSAINGNPSWHLADSPAVNGATGHSSSLDAREVIMPAAVK QALREAGDEFELRYRRAFSDLTSQLHITPGTAYQSFEQVVNELFR DGVNWGRIVAFFSFGGALCVESVDKEMQVLVSRIAAMMATYLNH LEPWIQENGGWDTFVELYGNNAAAESRKGQERFNRWFLTGMTVAG VVLLGSLFSRK*
SEQ ID NO:45	H9G8-Bclxl

SEQ ID NO: (Information)	Amino acid sequence
	APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFY MPKKATELKHLQCLEEELKPLEEVLNLAN SKNFHFDPRDVVSNIN VVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGG GGSMSQSNRELVVDFLSYKLSQKGY SWSQFSDVEENRTEAPEGTE SEMETPSAINGNPSWHLADSPAVNGATGHSSSLDAREVIPMAAVK QALREAGDEFELRYRRAFSDLTSQLHITPGTAYQSFEQVVNELFR DGVNWGRIVAFFSFGGALCVESVDKEMQVLVSRIAAWMATYLN DH LEPWIQENGGWDTFVELYGNNAAAESRKGQERFNRWFLTGMTVAG VVLLGSLFSRK*
SEQ ID NO:46	H9B1-BclxL APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFY MPKKATELKHLQCLEEELKPLEEVLNLAN SKNFHFDPRDVVSNVN VVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGG GGSMSQSNRELVVDFLSYKLSQKGY SWSQFSDVEENRTEAPEGTE SEMETPSAINGNPSWHLADSPAVNGATGHSSSLDAREVIPMAAVK QALREAGDEFELRYRRAFSDLTSQLHITPGTAYQSFEQVVNELFR DGVNWGRIVAFFSFGGALCVESVDKEMQVLVSRIAAWMATYLN DH LEPWIQENGGWDTFVELYGNNAAAESRKGQERFNRWFLTGMTVAG VVLLGSLFSRK*

[00148] Other exemplary IL-2 fusions include those listed in the table below:

Table 5: List of Exemplary IL-2 Extended Half-Life Fusion Amino Acid Sequences

SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:47 IL-2 extended half-life fusion	H9-Fc (H9 at N-terminal only shown) APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFY MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHFDPRDVVSNIN VVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGG <u>GGSGGGGSGGGGS</u> DKTHTCPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEAL HNHYTQKSLSLSPGK*
SEQ ID NO:48 IL-2 extended half-life fusion	H9-Fc ("Knob-in-hole" with H9 at N-terminus) APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFY MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHFDPRDVVSNIN VVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGG <u>GGSGGGGSGGGGS</u> DKTHTCPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEAL HNHYTQKSLSLSPGK - [FC sequence]

SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:49 IL-2 extended half-life fusion	H9-Fc ("Knob-in-hole" with H9 at C-terminus) [FC sequence] - APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFY MPKKATELKHLLQCLEEELKPLEEVLNLAQSKNFHFDPRDVVSNIN VVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT GG GGSGGGSGGGGS DKTHTCPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEAL HNHYTQKSLSLSPGK*
SEQ ID NO:50 IL-2 extended half-life fusion	H9FYAA-Fc APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTAKFA MPKKATELKHLLQCLEEELKPLEEVLNLAQSKNFHFDPRDVVSNIN VVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT GG GGSGGGSGGGGS DKTHTCPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEAL HNHYTQKSLSLSPGK*
SEQ ID NO:51 IL-2 extended half-life fusion (GS linker can be GGGGSGGGSGGGGS as shown or any other GS containing linker)	H9FEAA-Fc APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTAKFY MPKKATELKHLLQCLEEALKPLEEVLNLAQSKNFHFDPRDVVSNIN VVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT GG GGSGGGSGGGGS DKTHTCPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEAL HNHYTQKSLSLSPGK*
SEQ ID NO:52 IL-2 extended half-life fusion (GS linker can be GGGGSGGGSGGGGS as shown or any other GS containing linker)	H9D10-Fc APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFY MPKKATELKHLLQCLEEELKPLEEVLNLAHASKNFHFDPRDVVSNIN VVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT GG GGSGGGSGGGGS DKTHTCPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEAL HNHYTQKSLSLSPGK*

SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:53 IL-2 extended half-life fusion (GS linker can be <u>GGGGSGGGSGGGGS</u> as shown or any other GS containing linker)	H9E10-Fc APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFY MPKKATELKHLQCLEEELKPLEEVLN LASSKNFHFDP RDVVSNNIN VFVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT GG <u>GGSGGGSGGGGS</u> DKTHTCPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDS DGSFFLYSKLTVDKSRWQQGNV FSCSVMHEAL HNHYTQKSLSLSPGK*
SEQ ID NO:54 IL-2 extended half-life fusion (GS linker can be <u>GGGGSGGGSGGGGS</u> as shown or any other GS containing linker)	H9G8-Fc APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFY MPKKATELKHLQCLEEELKPLEEVLN LANSKNFHFDP RDVVSNNIN VFVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT GG <u>GGSGGGSGGGGS</u> DKTHTCPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDS DGSFFLYSKLTVDKSRWQQGNV FSCSVMHEAL HNHYTQKSLSLSPGK*
SEQ ID NO:55 IL-2 extended half-life fusion (GS linker can be <u>GGGGSGGGSGGGGS</u> as shown or any other GS containing linker)	H9B1-Fc APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFY MPKKATELKHLQCLEEELKPLEEVLN LANSKNFHFDP RDVVSNNIN VFVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT GG <u>GGSGGGSGGGGS</u> DKTHTCPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDS DGSFFLYSKLTVDKSRWQQGNV FSCSVMHEAL HNHYTQKSLSLSPGK*
SEQ ID NO:62 (GS linker can be <u>GGGGSGGGSGGGGS</u> as shown or any other GS containing linker)	H9-Albumin (H9 at C-terminal shown) DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQC PFEDHVKLVNEVTEFA KTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNE CFLQHKDDNPNLPRVLRPEVDVMCTAFHDNEETFLLKLYEIAARRHPYFY APELLFFAKRYKA AFTECCQAADKAACLLPKLDEL RDEGKASSAKQRLKC ASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLT KVHTECCHGDL LECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPA DLPSLAADFVESKDVCKNYAEAKDVFLGMFLY EYARRHPDYSVLLLRLA KTYETTLKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFELQGE YKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAE DYLSVVLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPK EFNAEFTTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDD FAAFVKECKKADDEKTCFAEEGKLVAA SQAALGL <u>GGGGSGGGSGGGG</u> <u>S</u> APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFY YMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHFDP RDVVSNNI NVFVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT*

SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:63 (GS linker can be <u>GGGSGGGSGGGGS</u> as shown or any other GS containing linker)	H9FYAA-Albumin (H9FYAA at C-terminal shown) DAHKSEVAHRFKDLGEEENFKALVLI AFAQYLQQCPFEDHVKLVNEVTEFA KTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNE CFLQHKDDNPNLPRLVREVDVMCTAFHDNEETFLKKYLYE IARRHPYFY APELLFFAKRYKAAFTTECCQAADKAAACLLPKLDELDRDEGKASSAKQRLKC ASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLT KVHTECCHGDL LECADDRADLAKYICENQDSISSKLEKCEKPLLEKSHCIAEVENDEMPA DLPSLAADFVESKDVCKNYAEAKDVFLGMFLY EYARRHPDYSVLLLRRLA KTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNL I KQNCLELFEQLGE YKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAE DYLSVVLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPK EFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDD FAAFVEKCKADDKETCFAEEGKKLVAASQAALGL <u>GGGSGGGSGGGGS</u> <u>SAPTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTAKFAMPKK</u> <u>ATELKHLQCLEEEALKPLEEVLNLAQSKNFHFDPRDVVSNINVFVLELKG</u> <u>ETTFMCEYADETATIVEFLNRWITFCQSIISTLT</u>
SEQ ID NO:64 (GS linker can be <u>GGGSGGGSGGGGS</u> as shown or any other GS containing linker)	H9FEAA-Albumin (H9FEAA at C-terminal shown) DAHKSEVAHRFKDLGEEENFKALVLI AFAQYLQQCPFEDHVKLVNEVTEFA KTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNE CFLQHKDDNPNLPRLVREVDVMCTAFHDNEETFLKKYLYE IARRHPYFY APELLFFAKRYKAAFTTECCQAADKAAACLLPKLDELDRDEGKASSAKQRLKC ASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLT KVHTECCHGDL LECADDRADLAKYICENQDSISSKLEKCEKPLLEKSHCIAEVENDEMPA DLPSLAADFVESKDVCKNYAEAKDVFLGMFLY EYARRHPDYSVLLLRRLA KTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNL I KQNCLELFEQLGE YKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAE DYLSVVLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPK EFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDD FAAFVEKCKADDKETCFAEEGKKLVAASQAALGL <u>GGGSGGGSGGGGS</u> <u>SAPTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTAKFYMPKK</u> <u>ATELKHLQCLEEEALKPLEEVLNLAQSKNFHFDPRDVVSNINVFVLELKG</u> <u>ETTFMCEYADETATIVEFLNRWITFCQSIISTLT</u>
SEQ ID NO:65 (GS linker can be <u>GGGSGGGSGGGGS</u> as shown or any other GS containing linker)	H9D10-Albumin (H9D10 shown at N-terminal) APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFFKFMPPKA TELKHLQCLEEEALKPLEEVLNLAH SKNFHFDPRDVVSNINVFVLELKGSE TTFMCEYADETATIVEFLNRWITFCQSIISTLT <u>GGGSGGGSGGGGS</u> AHKSEVAHRFKDLGEEENFKALVLI AFAQYLQQCPFEDHVKLVNEVTEFAK TCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNEC FLQHKDDNPNLPRLVREVDVMCTAFHDNEETFLKKYLYE IARRHPYFYA PELLFFAKRYKAAFTTECCQAADKAAACLLPKLDELDRDEGKASSAKQRLKCA SLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLT KVHTECCHGDL ECADDRADLAKYICENQDSISSKLEKCEKPLLEKSHCIAEVENDEMPAD LPSLAADFVESKDVCKNYAEAKDVFLGMFLY EYARRHPDYSVLLLRRLAK TYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNL I KQNCLELFEQLGEY KFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAED YLSVVLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDF AAFVEKCKADDKETCFAEEGKKLVAASQAALGL

SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:66 (GS linker can be <u>GGGSGGGSGGGGS</u> as shown or any other GS containing linker)	H9D10FEAA-Albumin APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTAKFYMPKKA TELKHLQCLEEALKPLEEVLNLAHSKNFHFDPDRDVSNI NVFVLELKGSE TTFMCEYADETATIVEFLNRWITFCQSIISTLT <u>GGGSGGGSGGGGS</u> ^D AHKSEVAHRFKDLGEEENFKALVLI AFAQYLQCCPFEDHVKLVNEVTEFAK TCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNEC FLQHKDDNPNL PRLVRPEVDVMCTAFHDNEETFLKKYLYE IARRHPYFYA PELLFFAKRYKAAFTECCQAADKAACLLPKLDLDELREDEGKASSAKQRLKCA SLQKFGERAFAKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDL ECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPAD LPSLAADFVESKDVCKNYAEAKDVFLGMFLY EYARRHPDYSVVLRLAK TYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNL I KQNCELFEQLGEY KFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAED YLSVVLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKE FNAETTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDF AAFVEKCKADDKETCFAEEGKKLVAASQAALGL
SEQ ID NO:67 (GS linker can be <u>GGGSGGGSGGGGS</u> as shown or anything other GS containingin linker)	H9E10-Albumin (H9E10 shown at N-terminal) APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEVLNLASSKNFHFDPDRDVSNI NVFVLELKGSE TTFMCEYADETATIVEFLNRWITFCQSIISTLT <u>GGGSGGGSGGGGS</u> ^D AHKSEVAHRFKDLGEEENFKALVLI AFAQYLQCCPFEDHVKLVNEVTEFAK TCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNEC FLQHKDDNPNL PRLVRPEVDVMCTAFHDNEETFLKKYLYE IARRHPYFYA PELLFFAKRYKAAFTECCQAADKAACLLPKLDLDELREDEGKASSAKQRLKCA SLQKFGERAFAKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDL ECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPAD LPSLAADFVESKDVCKNYAEAKDVFLGMFLY EYARRHPDYSVVLRLAK TYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNL I KQNCELFEQLGEY KFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAED YLSVVLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKE FNAETTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDF AAFVEKCKADDKETCFAEEGKKLVAASQAALGL
SEQ ID NO:68 (GS linker can be <u>GGGSGGGSGGGGS</u> as shown or anything other GS containingin linker)	H9G8-Albumin (H9G8 shown at N-terminal) APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEVLNLANSKNFHFDPDRDVSNI NVFVLELKGSE TTFMCEYADETATIVEFLNRWITFCQSIISTLT <u>GGGSGGGSGGGGS</u> ^D AHKSEVAHRFKDLGEEENFKALVLI AFAQYLQCCPFEDHVKLVNEVTEFAK TCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNEC FLQHKDDNPNL PRLVRPEVDVMCTAFHDNEETFLKKYLYE IARRHPYFYA PELLFFAKRYKAAFTECCQAADKAACLLPKLDLDELREDEGKASSAKQRLKCA SLQKFGERAFAKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDL ECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPAD LPSLAADFVESKDVCKNYAEAKDVFLGMFLY EYARRHPDYSVVLRLAK TYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNL I KQNCELFEQLGEY KFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAED YLSVVLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKE FNAETTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDF AAFVEKCKADDKETCFAEEGKKLVAASQAALGL

SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:69 (GS linker can be <u>GGGSGGGSGGGGS</u> as shown or anything other GS containing in linker)	H9B1-Albumin (H9B1 shown at N-terminal) APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLT FKFYMPKKA TELKHLQCLEELKPLEEVLNLANSKNFHFDPRDVSNVNVFVLELKGSE TTFMCEYADETATIVEFLNRWITFCQSIISTLT <u>GGGSGGGSGGGGS</u> DA HKSEVAHRFKDLGEENFKALVLI AFAQYLQCCPFEDHV KLVNEVTEFAKT CVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECF LQHKKDDNPNL PRLVRPEVDVMCTAFHDNEETFLK KYLYEIARRHPYFYA ELLFFAKRYKAAFTECCQAADKAACLLPKLDELDRDEGKASSAKQRLKCA LQKFGGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLE CADDRADLAKYICENQDSISSKLEKCEKPLLEKSHCIAEVENDEMPADL PSLAADFVESKDVCKNYAEAKDVFLGMFLY EYARRHPDYSV VLLLLRLAKT YETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQELGEYK FQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDY LSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEF NAETTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFA AFVEKCKADDKETCFAE EGKKLVAASQAALGL
SEQ ID NO:70 (GS linker can be <u>GGGSGGGSGGGGS</u> as shown or any other GS containing linker)	H9FEAA-Albumin (H9FEAA at N-terminal shown) APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTAKFYMPKKA TELKHLQCLEELKPLEEVLNLAQSKNFHFDPRDVSNVNVFVLELKGSE TTFMCEYADETATIVEFLNRWITFCQSIISTLT <u>GGGSGGGSGGGGS</u> D AHKSEVAHRFKDLGEENFKALVLI AFAQYLQCCPFEDHV KLVNEVTEFAK TCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNEC FLQHKKDDNPNL PRLVRPEVDVMCTAFHDNEETFLK KYLYEIARRHPYFYA PELLFFAKRYKAAFTECCQAADKAACLLPKLDELDRDEGKASSAKQRLKCA SLQKFGGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLE ECADDRADLAKYICENQDSISSKLEKCEKPLLEKSHCIAEVENDEMPAD YETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQELGEY KFNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAED YLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDF AAFVEKCKADDKETCFAE EGKKLVAASQAALGL
SEQ ID NO:71 (GS linker can be <u>GGGSGGGSGGGGS</u> as shown or any other GS containing linker)	H9D10-Albumin (H9D10 shown at C-terminal) DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQCCPFEDHV KLVNEVTEFA KTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNE CFLQHKKDDNPNL PRLVRPEVDVMCTAFHDNEETFLK KYLYEIARRHPYFY APELLFFAKRYKAAFTECCQAADKAACLLPKLDELDRDEGKASSAKQRLKCA ASLQKFGGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLE CADDRADLAKYICENQDSISSKLEKCEKPLLEKSHCIAEVENDEMPADL PSLAADFVESKDVCKNYAEAKDVFLGMFLY EYARRHPDYSV VLLLLRLA KYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQELGE YKFNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAE DYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE EFNAETTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDD FAAFVEKCKADDKETCFAE EGKKLVAASQAALGL <u>GGGSGGGSGGGGS</u> <u>S</u> APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLT FKFYMPKKA ATELKHLQCLEELKPLEEVLNLAH SKNFHFDPRDVSNVNVFVLELKGSE ETTFMCEYADETATIVEFLNRWITFCQSIISTLT

SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:72 (GS linker can be <u>GGGSGGGSGGGGS</u> as shown or any other GS containing linker)	H9D10FEAA-Albumin (H9FEAA shown at C-terminal) DAHKSEVAHRFKDLGGEENFKALVLI AFAQYLQQCP FEDHVKLVNEVTEFA KTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNE CFLQHKDDNPNLPRLVREVDVMCTAFHDNEETFLKKYLYE IARRHPYFY APELLFFAKRYKAAFTECCQAADKAACLLPKLDELREDEGKASSAKQRLKC ASLQKFGERAFAKAWAVARLSQRFPKAEFAEVSKLVTDLT KVHTECCHGDL LECADDRADLAKYICENQDSISSKLEKCEKPLLEKSHCIAEVENDEMPA DLPSLAADFVESKDVCKNYAEAKDVFLGMFLY EYARRHPDYSVLLLRRLA KTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQIGE YKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAE DYLSVVLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPK EFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDD FAAFVEKCKADDKETCFAEEGKKLVAASQAALGL <u>GGGSGGGSGGGG</u> <u>SAPTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTAKFYMPKK</u> ATELKHLQCLEEALKPLEEVLNLAH SKNFHFDPRDVVSNINVFVLELKGS ETTFMCEYADETATIVEFLNRWITFCQSIISTLT
SEQ ID NO:73 (GS linker can be <u>GGGSGGGSGGGGS</u> as shown or anything other GS containingin linker)	H9E10-Albumin (H9E10 shown at C-terminal) DAHKSEVAHRFKDLGGEENFKALVLI AFAQYLQQCP FEDHVKLVNEVTEFA KTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNE CFLQHKDDNPNLPRLVREVDVMCTAFHDNEETFLKKYLYE IARRHPYFY APELLFFAKRYKAAFTECCQAADKAACLLPKLDELREDEGKASSAKQRLKC ASLQKFGERAFAKAWAVARLSQRFPKAEFAEVSKLVTDLT KVHTECCHGDL LECADDRADLAKYICENQDSISSKLEKCEKPLLEKSHCIAEVENDEMPA DLPSLAADFVESKDVCKNYAEAKDVFLGMFLY EYARRHPDYSVLLLRRLA KTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQIGE YKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAE DYLSVVLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPK EFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDD FAAFVEKCKADDKETCFAEEGKKLVAASQAALGL <u>GGGSGGGSGGGG</u> <u>SAPTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFFKYMPKK</u> ATELKHLQCLEEELKPLEEVLNLASSKNFHF DPRDVVSNINVFVLELKGS ETTFMCEYADETATIVEFLNRWITFCQSIISTLT
SEQ ID NO:74 (GS linker can be <u>GGGSGGGSGGGGS</u> as shown or anything other GS containingin linker)	H9G8-Albumin (H9G8 shown at C-terminal) DAHKSEVAHRFKDLGGEENFKALVLI AFAQYLQQCP FEDHVKLVNEVTEFA KTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNE CFLQHKDDNPNLPRLVREVDVMCTAFHDNEETFLKKYLYE IARRHPYFY APELLFFAKRYKAAFTECCQAADKAACLLPKLDELREDEGKASSAKQRLKC ASLQKFGERAFAKAWAVARLSQRFPKAEFAEVSKLVTDLT KVHTECCHGDL LECADDRADLAKYICENQDSISSKLEKCEKPLLEKSHCIAEVENDEMPA DLPSLAADFVESKDVCKNYAEAKDVFLGMFLY EYARRHPDYSVLLLRRLA KTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQIGE YKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAE DYLSVVLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPK EFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDD FAAFVEKCKADDKETCFAEEGKKLVAASQAALGL <u>GGGSGGGSGGGG</u> <u>SAPTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFFKYMPKK</u> ATELKHLQCLEEELKPLEEVLNLANSKNFHF DPRDVVSNINVFVLELKGS ETTFMCEYADETATIVEFLNRWITFCQSIISTLT

SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:75 (GS linker can be <u>GGGSGGGSGGGGS</u> as shown or anything other GS containingin linker)	H9B1-Albumin (H9B1 shown at C-terminal) DAHKSEVAHRFKDLGGEENFKALVLI AFAQYLQCCPFEDHVKLVNEVTEFA KTCVADESAENCCKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNE CFLQHKDDNPNLPRLVREVDVMCTAFHDNEETFLKKYLYE IARRHPYFY APELLFFAKRYKAAFTECCQAADKAACLLPKLDELDRDEGKASSAKQRLKC ASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVDTLTKVHTECCHGDL LECADDRADLAKYICENQDSISSKLECECEKPLEKSHCIAEVENDEMPA DLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVLLLRLA KTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCLELFEQLGE YKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAE DYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPK EFNAETFTFHADICTLSEKERQIKKQTLVELVKHKPKATKEQLKAVMDD FAAFVEKCKKADDKETCFABEGKKLVAASQAALGL <u>GGGSGGGSGGGGS</u> APTSSSTKKTQLQLEHLLLDLQMI LINGINNYKNPKLTRMLTFFKPYMPKKA TELKHLQCLEEELKPLEEVLNLANSKNFHFDPRDVVSNVNVFVLELKGSE TTFMCEYADETATIVEFLNRWITFCQSIISTLT

[00149] In some embodiments, the IL-2 mutein-Fc fusion comprises one of the following sequences:

Table 6: List of Amino Acid Sequences

SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:76 (also listed herein as SEQ ID NO:11)	<u>MYRMQLLSCIALSLALVTNS</u> APTSSSTKKTQLQLEHLLLDLQMI LINGINNYKNPK LTRMLTFFKPYMPKKA TELKHLQCLEE <u>ELKPLEEVLNLAQSKNFHLRPRDLISNINIVLELKGSETTFMCEYADETATIVE</u> FLNRWITFCQSIISTLTGGGSGGGG SGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQ YASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPDSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSDGSAFLYSLKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK*
SEQ ID NO:77 (also listed herein as SEQ ID NO:12)	<u>MYRMQLLSCIALSLALVTNS</u> APTSSSTKKTQLQLEHLLLDLQMI LINGINNYKNPK LTRMLTFFKPYMPKKA TELKHLQCLEE <u>ELKPLEEVLNLAQSKNFHFDPRDVVSNINIVFVLELKGSETTFMCEYADETATIVE</u> FLNRWITFCQSIISTLTGGGSGGGG SGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQ YASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPDSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSDGSAFLYSLKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK*
SEQ ID NO:78 (also listed herein as SEQ ID NO:13)	<u>MYRMQLLSCIALSLALVTNS</u> APTSSSTKKTQLQLEHLLLDLQMI LINGINNYKNPK LTRMLTAKFAMPKKA TELKHLQCLEE <u>ELKPLEEVLNLAQSKNFHFDPRDVVSNINIVFVLELKGSETTFMCEYADETATIVE</u> FLNRWITFCQSIISTLTGGGSGGGG SGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQ YASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPDSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSDGSAFLYSLKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK*

SEQ ID NO:79 (also listed herein as SEQ ID NO:14)	MYRMQLLSCIALSLALVTNS APTSSSTKKTQLQLEHLLLDLQMI LINGINNYKNPK LTRMLTAKFYMPKKATELKHLCLEE <u>ALKPLEEVLNLAQSKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVE</u> FLNRWITFCQSIISTLTGGGGSGGGG SGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQ YASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY LPPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSDGSAFLYSLKLTVDKSRWQQGNVFCSCVMHEA LHNHYTQKSLSLSPGK*
SEQ ID NO:80 (also listed herein as SEQ ID NO:15)	MYRMQLLSCIALSLALVTNS APTSSSTKKTQLQLEHLLLDLQMI LINGINNYKNPK LTRMLTAKFAMPKKATELKHLCLEE <u>ALKPLEEVLNLAQSKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVE</u> FLNRWITFCQSIISTLTGGGGSGGGG SGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQ YASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY LPPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSDGSAFLYSLKLTVDKSRWQQGNVFCSCVMHEA LHNHYTQKSLSLSPGK*

[00150] In some embodiments, the IL-2 mutein sequence is 90% identical to any one of SEQ ID NO:12 through SEQ ID NO:15 and/or SEQ ID NO:20 through SEQ ID NO:80 (for example, any of the IL-2 sequences provided herein). In some embodiments, the IL-2 mutein sequence is 95% identical to any one of SEQ ID NO:12 through SEQ ID NO:15 and/or SEQ ID NO:20 through SEQ ID NO:80 (for example, any of the IL-2 sequences provided herein). In some embodiments, the IL-2 mutein sequence is 98% identical to any one of SEQ ID NO:12 through SEQ ID NO:15 and/or SEQ ID NO:20 through SEQ ID NO:80 (for example, any of the IL-2 sequences provided herein). In some embodiments, the IL-2 mutein sequence is 99% identical to any one of SEQ ID NO:12 through SEQ ID NO:15 and/or SEQ ID NO:20 through SEQ ID NO:80 (for example, any of the IL-2 sequences provided herein).

C. IL-4, IL-13 IL-10, IL-12, IL15, AND IL-18 FOR FUSION WITH IL-2

[00151] In some embodiments, an IL-2 mutein can be fused to an IL-4 mutein as described herein. In some embodiments, an IL-2 mutein can be fused to an IL-13 mutein as described herein. In some embodiments, an IL-2 mutein can be fused to an IL-10. In some embodiments, an IL-2 mutein can be fused to an IL-12. In some embodiments, an IL-2 mutein can be fused to an IL-15. In some embodiments, an IL-2 mutein can be fused to an IL-18. In some embodiments, such fusions function to specifically target cancer cells and/or cancer stem cells and reduce or inhibit cancer stem cell growth, as well as targeting the immunosuppressive cells in the tumor microenvironment (TME).

[00152] Any IL-13 sequence or variant thereof can be used in a fusion with an IL-2 mutein as described herein. In some embodiments, the IL-2 mutein includes any one of 5-1 SEQ ID NO:5; 5-2 SEQ ID NO:6; 6-6 SEQ ID NO:7; A2 SEQ ID NO:8; B1 SEQ ID NO:9; B11 SEQ ID NO:10; C5 SEQ ID NO:11; D10 SEQ ID NO:12; E10 SEQ ID NO:13; G8 SEQ ID NO:14; H4 SEQ ID NO:15; and H9 SEQ ID NO:5. Exemplary IL-13 polypeptide sequences are provided in SEQ ID NO:81-SEQ ID NO:128, as well as the table below. In some embodiments, the IL-13 polypeptide sequence is as provided in any one of SEQ ID NO:81-SEQ ID NO:128. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:81. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:82. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:83. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:84. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:85. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:86. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:87. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:88. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:89. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:90. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:91. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:92. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:93. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:94. In some embodiments, the polypeptide sequence is SEQ ID NO:95. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:96. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:97. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:98. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:99. In some embodiments, the polypeptide sequence is SEQ ID NO:100. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:101. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:102. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:103. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:104. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:105. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:106. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:107. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:108. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:109. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:110. In some

embodiments, the polypeptide sequence is SEQ ID NO:111. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:112. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:113. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:114. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:115. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:116. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:117. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:118. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:119. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:120. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:121. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:122. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:123. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:124. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:125. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:126. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:127. In some embodiments, the IL-13 polypeptide sequence is SEQ ID NO:128. IL-13 In some embodiments, the IL-13 polypeptide sequence is 90% identical to any one of SEQ ID NO:81 through SEQ ID NO:128. In some embodiments, the IL-13 polypeptide sequence is 95% identical to any one of SEQ ID NO:81 through SEQ ID NO:128. In some embodiments, the IL-13 polypeptide sequence is 98% identical to any one of SEQ ID NO:81 through SEQ ID NO:128. In some embodiments, the IL-13 polypeptide sequence is 99% identical to any one of SEQ ID NO:81 through SEQ ID NO:128.

[00153] In some embodiments, any one of SEQ ID NO:81-SEQ ID NO:128 are linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:81 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:82 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:83 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:84 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:85 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:86 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:87 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:88 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:89 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:90 is linked to an IL-2 or IL-2

mutein as described herein. In some embodiments, SEQ ID NO:122 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:123 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:124 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:125 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:126 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:127 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:128 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, the IL-2 mutein includes any one of 5-1 SEQ ID NO:5; 5-2 SEQ ID NO:6; 6-6 SEQ ID NO:7; A2 SEQ ID NO:8; B1 SEQ ID NO:9; B11 SEQ ID NO:10; C5 SEQ ID NO:11; D10 SEQ ID NO:12; E10 SEQ ID NO:13; G8 SEQ ID NO:14; H4 SEQ ID NO:15; and H9 SEQ ID NO:5.

[00154] In some embodiments an IL-13 peptide of the invention comprises one or more of the amino acids substitutions: (1) L10F, L10I, L10V, L10A, L10D, L10T, L10H; (2) R11S, R11N, R11H, R11L, R11I; (3) I14L, I14F, I14V, I14M; (4) V18L, V18F, V18I; (5) E12A, (6) R65D, (7) R86K, R86T, R86M; (8) D87E, D87K, D87R, D87G, D87S; (9) T88I, T88K, T88R; (10) K89R, K89T, K89M; (11) L101 F, L101I, L101Y, L101H, L101N; (12) K104R, K104T, K104M; (13) K105T, K105A, K105R, K105E; (14) F107L, F107I, F107V, F107M; and (15) R108K, R108T, R108M, which substitutions cause an altered affinity for one or both of IL-13 α 1 and IL-13 α 2. In other embodiments, modified residues are at two or more, three or more, four or more, five or more, and not more than 14 amino acids within the combined set of contact residues defined above. As described in International Patent Publication WO 2013/112871, the disclosure of which is incorporated by reference herein in its entirety. In some embodiments, amino acid substitutions include without limitation those provided in Figure 4.

[00155] Sets of modifications may include the following specific changes: (1) L10H; L10A; (2) R11L; (4) V18I; (7) R86M; R86K; R86T; (8) D87K; D87G; (9) T88R, T88S; T88K; (10) K89R; (11) L101N; (12) K104R; (13) K105A; K105E; (14) R108K. In some embodiments, the modification includes any one of the recited specific changes. In some embodiments, the modification includes L10H. In some embodiments, the modification includes L10A. In some embodiments, the modification includes R11L. In some embodiments, the modification includes V18I. In some embodiments, the modification includes R86M. In some embodiments, the modification includes R86K. In some embodiments, the modification includes R86T. In some

embodiments, the modification includes D87K. In some embodiments, the modification includes D87G. In some embodiments, the modification includes T88R. In some embodiments, the modification includes T88S. In some embodiments, the modification includes T88K. In some embodiments, the modification includes K89R. In some embodiments, the modification includes L101N. In some embodiments, the modification includes K104R. In some embodiments, the modification includes K105A. In some embodiments, the modification includes K105E. In some embodiments, the modification includes R108K. In some embodiments, the polypeptide comprising the one or more modifications is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, amino acid substitutions include without limitation those provided in Figure 4. In some embodiments, the IL-2 mutein includes any one of 5-1 SEQ ID NO:5; 5-2 SEQ ID NO:6; 6-6 SEQ ID NO:7; A2 SEQ ID NO:8; B1 SEQ ID NO:9; B11 SEQ ID NO:10; C5 SEQ ID NO:11; D10 SEQ ID NO:12; E10 SEQ ID NO:13; G8 SEQ ID NO:14; H4 SEQ ID NO:15; and H9 SEQ ID NO:5.

[00156]

[00157] Specific sets of modifications that provide for greater selectivity in binding to IL-13R α 2 versus IL-13R α 1 relative to a native IL-13 sequence may include, without limitation:

- [L10D, R11I, V18I, R86K, D87K, K89R, R108K] (for example, C2, e.g. SEQ ID NO:31 or SEQ ID NO:49)
- [L10A, R86T, D87G, T88K, K89R, L101N, K104R, K105A, R108K] (for example, C3, e.g. SEQ ID NO:32 or SEQ ID NO:50)
- [L10V, K89R, L101N, K105E, R108T] (for example, C4, e.g. SEQ ID NO:33 or SEQ ID NO:31)
- [R11S, I14M, T88S, L101N, K105A, R108K] (for example, C7, e.g. SEQ ID NO:34 or SEQ ID NO:52)
- [L10H, R11L, V18I, R86K, D87E, K89R, L101N, K105T, R108K] (C9, e.g. SEQ ID NO:53)
- [L10H, R86T, D87G, T88R, R108K] (C11 e.g. SEQ ID NO:38 or SEQ ID NO:55)
- [L10A, V18F, R86K, D87K, K89R, L101I, K104R, R108K] (D7, e.g. SEQ ID NO:40 or SEQ ID NO:57)
- [L10T/D; R11I; V18I; R86K; D87K/G; T88S; K89R; L101Y; K104R; K105T; R108K]

- [L10A/V; R86T; D87G; T88K; K89R; L101N; K104R; K105A/E; R108K/T]

[00158] In some embodiments, the set of modifications comprises L10V, K89R, L101N, K105E, R108T. In some embodiments, the set of modifications comprises R11S, I14M, T88S, L101N, K105A, and R108K (C7, e.g. SEQ ID NO:35 or SEQ ID NO:52). In some embodiments, the set of modifications comprises L10H, R11L, V18I, R86K, D87E, K89R, L101N, K105T, and R108K (C9, e.g. SEQ ID NO:36 or SEQ ID NO:53). In some embodiments, the set of modifications comprises L10H, R86T, D87G, T88R, and R108K (C11 e.g. SEQ ID NO:38 or SEQ ID NO:55). In some embodiments, the set of modifications comprises L10A, V18F, R86K, D87K, K89R, L101I, K104R, and R108K (D7, e.g. SEQ ID NO:40 or SEQ ID NO:57). In some embodiments, the set of modifications comprises L10T/D, R11I, V18I, R86K, D87K/G, T88S, K89R, L101Y, K104R, K105T, and R108K. In some embodiments, the set of modifications comprises L10T, R11I, V18I, R86K, D87K, T88S, K89R, L101Y, K104R, K105T, and R108K. In some embodiments, the set of modifications comprises L10T, R11I, V18I, R86K, D87G, T88S, K89R, L101Y, K104R, K105T, and R108K. In some embodiments, the set of modifications comprises L10D, R11I, V18I, R86K, D87K, T88S, K89R, L101Y, K104R, K105T, and R108K. In some embodiments, the set of modifications comprises L10D, R11I, V18I, R86K, D87G, T88S, K89R, L101Y, K104R, K105T, R108K. In some embodiments, the set of modifications comprises L10A/V, R86T, D87G, T88K, K89R, L101N, K104R, K105A/E, and R108K/T. In some embodiments, the set of modifications comprises L10A, R86T, D87G, T88K, K89R, L101N, K104R, K105A, and R108K. In some embodiments, the set of modifications comprises L10A, R86T, D87G, T88K, K89R, L101N, K104R, K105E, and R108K. In some embodiments, the set of modifications comprises L10A, R86T, D87G, T88K, K89R, L101N, K104R, K105A, and R108T. In some embodiments, the set of modifications comprises L10A, R86T, D87G, T88K, K89R, L101N, K104R, K105E, and R108T. In some embodiments, the set of modifications comprises L10V, R86T, D87G, T88K, K89R, L101N, K104R, K105A, and R108K. In some embodiments, the set of modifications comprises L10V, R86T, D87G, T88K, K89R, L101N, K104R, K105E, and R108K. In some embodiments, the set of modifications comprises L10V, R86T, D87G, T88K, K89R, L101N, K104R, K105A, and R108T. In some embodiments, the set of modifications comprises L10V, R86T, D87G, T88K, K89R, L101N, K104R, K105E, and R108T. In some embodiments, the amino acid sequence is 90% identical. In some embodiments, the amino acid sequence is 95% identical. In some embodiments, the amino acid sequence is

98% identical. In some embodiments, the amino acid sequence is 99% identical. In some embodiments, the polypeptide comprising the one or more modifications is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, amino acid substitutions include without limitation those provided in Figure 4. In some embodiments, the IL-2 mutein includes any one of 5-1 SEQ ID NO:5; 5-2 SEQ ID NO:6; 6-6 SEQ ID NO:7; A2 SEQ ID NO:8; B1 SEQ ID NO:9; B11 SEQ ID NO:10; C5 SEQ ID NO:11; D10 SEQ ID NO:12; E10 SEQ ID NO:13; G8 SEQ ID NO:14; H4 SEQ ID NO:15; and H9 SEQ ID NO:5.

[00159] Specific sets of modifications that provide for greater selectivity in binding to IL-13R α 1 v IL-13R α 2 relative to a native IL-13 sequence may include, without limitation:

- [L10V, V18I, D87S, D88S, L101F, K104R, K105T]
- [R11S, V18I, R86K, D87G, T88S, K89M, L101Y, K104R, K105T]
- [L10V, V18I, D87S, T88S, L101F, K104R, K105T]
- [L10V/I; D87S; T88S; K89R; L101H/F; K104R; K105T]
- [L10I; V18I; R86T; D87G; T88S; K89R; L101Y/H; K104R; K105A]
- [L10V; V18I; D87S; T88S; L101F; K104R; K105T]
- [V18I, R86T, D87G, T88S, L101Y, K104R, K105A]
- [R11I, V18I, R86K, D87G, T88S, L101H, K104R, K105A, F107M]

which substitutions are optionally combined with the substitutions [E12A/G/S, R65D/E].

[00160] In some embodiments, the set of modifications comprises L10V, V18I, D87S, D88S, L101F, K104R, and K105T. In some embodiments, the set of modifications comprises R11S, V18I, R86K, D87G, T88S, K89M, L101Y, K104R, and K105T. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, and K105T. In some embodiments, the set of modifications comprises L10V/I, D87S, T88S, K89R, L101H/F, K104R, and K105T. In some embodiments, the set of modifications comprises L10I, V18I, R86T, D87G, T88S, K89R, L101Y/H, K104R, and K105A. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, and K105T. In some embodiments, the set of modifications comprises V18I, R86T, D87G, T88S, L101Y, K104R, and K105A. In some embodiments, the set of modifications comprises R11I, V18I, R86K, D87G, T88S, L101H, K104R, K105A, and F107M. In some embodiments, the set of modifications comprises L10V, V18I, D87S, D88S, L101F, K104R, K105T, E12A/G/S, and R65D/E. In some embodiments, the

set of modifications comprises R11S, V18I, R86K, D87G, T88S, K89M, L101Y, K104R, K105T, E12A/G/S, and R65D/E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12A/G/S, and R65D/E. In some embodiments, the set of modifications comprises L10V/I, D87S, T88S, K89R, L101H/F, K104R, K105T, E12A/G/S, and R65D/E. In some embodiments, the set of modifications comprises L10I, V18I, R86T, D87G, T88S, K89R, L101Y/H, K104R, K105A, E12A/G/S, and R65D/E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12A/G/S, and R65D/E. In some embodiments, the set of modifications comprises V18I, R86T, D87G, T88S, L101Y, K104R, K105A, E12A/G/S, and R65D/E. In some embodiments, the set of modifications comprises R11I, V18I, R86K, D87G, T88S, L101H, K104R, K105A, F107M, E12A/G/S, and R65D/E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, D88S, L101F, K104R, K105T, E12A, and R65D/E. In some embodiments, the set of modifications comprises R11S, V18I, R86K, D87G, T88S, K89M, L101Y, K104R, K105T, E12A, and R65D/E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12A, and R65D/E. In some embodiments, the set of modifications comprises L10V/I, D87S, T88S, K89R, L101H/F, K104R, K105T, E12A, and R65D/E. In some embodiments, the set of modifications comprises L10I, V18I, R86T, D87G, T88S, K89R, L101Y/H, K104R, K105A, E12A, and R65D/E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12A, and R65D/E. In some embodiments, the set of modifications comprises V18I, R86T, D87G, T88S, L101Y, K104R, K105A, E12A, and R65D/E. In some embodiments, the set of modifications comprises R11I, V18I, R86K, D87G, T88S, L101H, K104R, K105A, F107M, E12A, and R65D/E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, D88S, L101F, K104R, K105T, E12G, and R65D/E. In some embodiments, the set of modifications comprises R11S, V18I, R86K, D87G, T88S, K89M, L101Y, K104R, K105T, E12G, and R65D/E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12A/G/S, and R65D/E. In some embodiments, the set of modifications comprises L10V/I, D87S, T88S, K89R, L101H/F, K104R, K105T, E12G, and R65D/E. In some embodiments, the set of modifications comprises L10I, V18I, R86T, D87G, T88S, K89R, L101Y/H, K104R, K105A, E12G, and R65D/E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12G, and R65D/E. In some

embodiments, the set of modifications comprises V18I, R86T, D87G, T88S, L101Y, K104R, K105A, E12G, and R65D/E. In some embodiments, the set of modifications comprises R11I, V18I, R86K, D87G, T88S, L101H, K104R, K105A, F107M, E12G, and R65D/E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, D88S, L101F, K104R, K105T, E12S, and R65D/E. In some embodiments, the set of modifications comprises R11S, V18I, R86K, D87G, T88S, K89M, L101Y, K104R, K105T, E12A/G/S, and R65D/E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12S, and R65D/E. In some embodiments, the set of modifications comprises L10V/I, D87S, T88S, K89R, L101H/F, K104R, K105T, E12S, and R65D/E. In some embodiments, the set of modifications comprises L10I, V18I, R86T, D87G, T88S, K89R, L101Y/H, K104R, K105A, E12S, and R65D/E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12S, and R65D/E. In some embodiments, the set of modifications comprises V18I, R86T, D87G, T88S, L101Y, K104R, K105A, E12S, and R65D/E. In some embodiments, the set of modifications comprises R11I, V18I, R86K, D87G, T88S, L101H, K104R, K105A, F107M, E12S, and R65D/E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, D88S, L101F, K104R, K105T, E12A, and R65D. In some embodiments, the set of modifications comprises R11S, V18I, R86K, D87G, T88S, K89M, L101Y, K104R, K105T, E12A, and R65E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12A, and R65D. In some embodiments, the set of modifications comprises L10V/I, D87S, T88S, K89R, L101H/F, K104R, K105T, E12A, and R65D. In some embodiments, the set of modifications comprises L10I, V18I, R86T, D87G, T88S, K89R, L101Y/H, K104R, K105A, E12A, and R65D. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12A, and R65D. In some embodiments, the set of modifications comprises V18I, R86T, D87G, T88S, L101Y, K104R, K105A, E12A, and R65D. In some embodiments, the set of modifications comprises R11I, V18I, R86K, D87G, T88S, L101H, K104R, K105A, F107M, E12A, and R65D. In some embodiments, the set of modifications comprises L10V, V18I, D87S, D88S, L101F, K104R, K105T, E12G, and R65D. In some embodiments, the set of modifications comprises R11S, V18I, R86K, D87G, T88S, K89M, L101Y, K104R, K105T, E12G, and R65D. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12A/G/S, and R65D. In some embodiments, the set of modifications comprises L10V/I,

D87S, T88S, K89R, L101H/F, K104R, K105T, E12G, and R65D. In some embodiments, the set of modifications comprises L10I, V18I, R86T, D87G, T88S, K89R, L101Y/H, K104R, K105A, E12G, and R65D. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12G, and R65D. In some embodiments, the set of modifications comprises V18I, R86T, D87G, T88S, L101Y, K104R, K105A, E12G, and R65D. In some embodiments, the set of modifications comprises R11I, V18I, R86K, D87G, T88S, L101H, K104R, K105A, F107M, E12G, and R65D. In some embodiments, the set of modifications comprises L10V, V18I, D87S, D88S, L101F, K104R, K105T, E12S, and R65D. In some embodiments, the set of modifications comprises R11S, V18I, R86K, D87G, T88S, K89M, L101Y, K104R, K105T, E12S, and R65D. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12S, and R65D. In some embodiments, the set of modifications comprises L10V/I, D87S, T88S, K89R, L101H/F, K104R, K105T, E12S, and R65D. In some embodiments, the set of modifications comprises L10I, V18I, R86T, D87G, T88S, K89R, L101Y/H, K104R, K105A, E12S, and R65D. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12S, and R65D. In some embodiments, the set of modifications comprises V18I, R86T, D87G, T88S, L101Y, K104R, K105A, E12S, and R65D. In some embodiments, the set of modifications comprises R11I, V18I, R86K, D87G, T88S, L101H, K104R, K105A, F107M, E12S, and R65D. In some embodiments, the set of modifications comprises L10V, V18I, D87S, D88S, L101F, K104R, K105T, E12A, and R65E. In some embodiments, the set of modifications comprises R11S, V18I, R86K, D87G, T88S, K89M, L101Y, K104R, K105T, E12A, and R65E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12A, and R65E. In some embodiments, the set of modifications comprises L10V/I, D87S, T88S, K89R, L101H/F, K104R, K105T, E12A, and R65E. In some embodiments, the set of modifications comprises L10I, V18I, R86T, D87G, T88S, K89R, L101Y/H, K104R, K105A, E12A, and R65E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12A, and R65E. In some embodiments, the set of modifications comprises V18I, R86T, D87G, T88S, L101Y, K104R, K105A, E12A, and R65E. In some embodiments, the set of modifications comprises R11I, V18I, R86K, D87G, T88S, L101H, K104R, K105A, F107M, E12A, and R65E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, D88S, L101F, K104R, K105T, E12G, and R65E. In some

embodiments, the set of modifications comprises R11S, V18I, R86K, D87G, T88S, K89M, L101Y, K104R, K105T, E12G, and R65E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12A/G/S, and R65E. In some embodiments, the set of modifications comprises L10V/I, D87S, T88S, K89R, L101H/F, K104R, K105T, E12G, and R65E. In some embodiments, the set of modifications comprises L10I, V18I, R86T, D87G, T88S, K89R, L101Y/H, K104R, K105A, E12G, and R65E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12G, and R65E. In some embodiments, the set of modifications comprises V18I, R86T, D87G, T88S, L101Y, K104R, K105A, E12G, and R65E. In some embodiments, the set of modifications comprises R11I, V18I, R86K, D87G, T88S, L101H, K104R, K105A, F107M, E12G, and R65E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, D88S, L101F, K104R, K105T, E12S, and R65E. In some embodiments, the set of modifications comprises R11S, V18I, R86K, D87G, T88S, K89M, L101Y, K104R, K105T, E12A/G/S, and R65E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12S, and R65E. In some embodiments, the set of modifications comprises L10V/I, D87S, T88S, K89R, L101H/F, K104R, K105T, E12S, and R65E. In some embodiments, the set of modifications comprises L10I, V18I, R86T, D87G, T88S, K89R, L101Y/H, K104R, K105A, E12S, and R65E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12S, and R65E. In some embodiments, the set of modifications comprises L10V/I, D87S, T88S, K89R, L101H/F, K104R, K105T, E12S, and R65E. In some embodiments, the set of modifications comprises L10I, V18I, R86T, D87G, T88S, K89R, L101Y/H, K104R, K105A, E12S, and R65E. In some embodiments, the set of modifications comprises L10V, V18I, D87S, T88S, L101F, K104R, K105T, E12S, and R65E. In some embodiments, the set of modifications comprises V18I, R86T, D87G, T88S, L101Y, K104R, K105A, E12S, and R65E. In some embodiments, the set of modifications comprises R11I, V18I, R86K, D87G, T88S, L101H, K104R, K105A, F107M, E12S, and R65E. In some embodiments, the set of modifications comprises L10V, E12A, V18I, R65D, D87S, T88S, L101F, K104R, and K105T (see, for example, IL-13dn; SEQ ID NO:38). In some embodiments, the amino acid sequence is 90% identical. In some embodiments, the amino acid sequence is 95% identical. In some embodiments, the amino acid sequence is 98% identical. In some embodiments, the amino acid sequence is 99% identical. In some embodiments, the polypeptide comprising the one or more modifications is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, amino acid substitutions include without limitation those provided in Figure 4. In some embodiments, the IL-2 mutein includes any one of 5-1 SEQ ID NO:5; 5-2 SEQ ID NO:6; 6-6 SEQ ID NO:7; A2 SEQ ID NO:8; B1 SEQ ID NO:9; B11 SEQ ID NO:10; C5 SEQ ID NO:11;

D10 SEQ ID NO:12; E10 SEQ ID NO:13; G8 SEQ ID NO:14; H4 SEQ ID NO:15; and H9 SEQ ID NO:5.

[00161] Table of IL-13 sequences is provided below.

Table 7: List of IL-13 Amino Acid Sequences

SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:81 (IL-13 wildtype)	PGPVPPSTALRELIEELVNITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVRDTKIEVAQFVKDLLHLK LFREGQFN
SEQ ID NO:82	PGPVPPSTAVRALIEELINITQNQKAPLCNGSMVW SINRTAGMYCAALES LINVSGCSAIEKTQDMLSGF CPHKVSAGQFSSLHVRSSKIEVAQFVKDLLFHLRT LFREGQFN
SEQ ID NO:83	PGPVPPSTAIRELIEELINITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVRGSKIEVAQFVKDLLHLRA LFREGQFN
SEQ ID NO:84	PGPVPPSTAVRELIEELINITQNQKAPLCNGSMVW SINRTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVRSSKIEVAQFVKDLLFHLRT LFREGQFN
SEQ ID NO:85	PGPVPPSTALIELIEELINITQNQKAPLCNGSMVW SINLTAGIYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVKGSKIEVAQFVKDLLHLRA LMREGQFN
SEQ ID NO:86	PGPVPPSTAIRELIEELLNITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVMKSKIEVAQFVKDLLHLRA LFREGQFN
SEQ ID NO:87	PGPVPPSTAIRELIEELINITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVRSSRIEVAQFVKDLLHLRT LFREGQFN
SEQ ID NO:88	PGPVPPSTALRELIEELINITQNEKAPLCNGSMVW SINLTAGIYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVTGSKIEVAQFVKDLLYHLRA LFREGQFN

SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:89	PGPVPPSTALSELIEELINITQNQKAPLCNGSMVW SINPTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVAAGQFSSLHDKGSMIEVAQFVKDLLYHLRT LFREGQFN
SEQ ID NO:90	PGPVPPSTATRELIEELINITQNQKAPLCNGSMVW SINLTADMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSVGQFSSLHVGRGSKIEVAQFVKDLLYHLRT LFREGQFN
SEQ ID NO:91	PGPVPPSTADIELIAELINITQNQKAPLCNGSMVW SINLTADMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVKKTRIEVAQFVKDLLLHLKK LFKEGQFN
SEQ ID NO:92	PGPVPPSTAARELIEELVNITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQLSSLHVTGKRIEVAQFVKDLLNHLRA LFKEGQFN
SEQ ID NO:93	PGPVPPSTAVRELIEELVNITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVRDTRIEVAQFVKDLLNHLKE LFTEGQFN
SEQ ID NO:94	PGPVPPSTALSELMEELVNITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVRSKIEVAQFVKDLLNHLKA LFKEGQFN
SEQ ID NO:95	GPVPPSTAFRELIEELVNITQNQKAPLCNGSMVWS INLTAGMYCAALES LINVSGCSAIEKTQRMLSGFC PHKVS PGQFSSLHVTNSRIEVAQFVKDLLNHLKAL FKEGQYN
SEQ ID NO:96	GPVPPSTAHLELIEELINITQNQKAPLCNGSMVWS INLTAGMYCAALES LINVSGCSAIEKTQRMLSGFC PHKVSAGQFSSLHVKETRIEVAQFVKDLLNHLKTL FKEGQFN
SEQ ID NO:97	PGPVPPSTAHLELIEELINITQNQKAPLCNGSMVW SINPTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVMDTRIEVAQFVKDLLLHLKK LFKEGQFN
SEQ ID NO:98	PGPVPPSTAHRELIEELVNITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF

SEQ ID NO: (Information)	Amino acid sequence
	CPHKVSAGQFSSLHVTGRKIEVAQFVKDLLLHLKK LFKEGQFN
SEQ ID NO:99	PGPVPPSTAHRELIEELVNITQNQKAPLCNGSMVW RINRTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVMDSR IEVAQFVKDLLNHLRA LFKEGQFN
SEQ ID NO:2100	PGPVPPSTAARELIEELFNITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTKRMLSGF CPHKVSAGQFPSLVKKTRIEVAQFVKDLLIHLRK LFKEGQFN
SEQ ID NO:101 (Exemplary sequence comprising R11I, V18I, R86K, D87G, T88S, L101H, K104R, K105A, F107M, referred to herein as A5)	PGPVPPSTALIELIEELINITQNQKAPLCNGSMVWS INLTAGMYCAALES LINVSGCSAIEKTQRMLSGFCP HKVSAGQFSSLHVKGSKIEVAQFVKDLLHHLRALMR EGQFN
SEQ ID NO:102 (Exemplary sequence comprising L10I, V18L, R86M, D87K, T88S, L101H, K104R, K105A, referred to herein as A6)	PGPVPPSTAI RELIEELLNITQNQKAPLCNGSMVWS INLTAGMYCAALES LINVSGCSAIEKTQRMLSGFCP HKVSAGQFSSLHVMKSKIEVAQFVKDLLHHLRALFR EGQFN
SEQ ID NO:103 (Exemplary sequence comprising L10I, V18I, D87G, T88S, L101H, K104R, K105A, referred to herein as A7)	PGPVPPSTAI RELIEELINITQNQKAPLCNGSMVWS INLTAGMYCAALES LINVSGCSAIEKTQRMLSGFCP HKVSAGQFSSLHVRGSKIEVAQFVKDLLHHLRALFR EGQFN
SEQ ID NO:104 (Exemplary sequence comprising L10I, V18I, D87S, T88S, K89R, L101H, K104R, K105T; referred to herein as A8)	PGPVPPSTAI RELIEELINITQNQKAPLCNGSMVWS INLTAGMYCAALES LINVSGCSAIEKTQRMLSGFCP HKVSAGQFSSLHVRSSRIEVAQFVKDLLHHLRTLFR EGQFN

SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:105 (Exemplary sequence comprising L10V, V18I, D87S, T88S, L101F, K104R, K105T, referred to herein as A11 variant 1)	PGPVPPSTAVRELIEELINITQNQKAPLCNGSMVWS IN <u>L</u> TAGMYCAALES LINVSGCSAIEKTQRMLSGFCP HKVSAGQFSSLHVRSSKIEVAQFVKDLLFHLRTLFR EGQFN
SEQ ID NO:105 (Exemplary sequence comprising L10V, V18I, D87S, T88S, L101F, K104R, K105T, referred to herein as A11 variant 2)	PGPVPPSTAVRELIEELINITQNQKAPLCNGSMVWS IN <u>R</u> TAGMYCAALES LINVSGCSAIEKTQRMLSGFCP HKVSAGQFSSLHVRSSKIEVAQFVKDLLFHLRTLFR EGQFN
SEQ ID NO:106 (Exemplary sequence comprising V18I, R86T, D87G, T88S, L101Y, K104R, K105A, referred to herein as B2)	PGPVPPSTALRELIEELINITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVTGSKIEVAQFVKDLLYHLRA LFREGQFN
SEQ ID NO:107 (Exemplary sequence comprising R11S, V18I, R86K, D87G, T88S, K89M, L101Y, K104R, K105T, referred to herein as B4)	PGPVPPSTALSELIEELINITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVKGSMIEVAQFVKDLLYHLRT LFREGQFN
SEQ ID NO:108 (Exemplary sequence comprising L10T, V18I, D87G, T88S, K89K, L10Y1, K104R, K105T, referred to herein as B6)	PGPVPPSTATRELIEELINITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVRGSKIEVAQFVKDLLYHLRT LFREGQFN
SEQ ID NO:109 (Exemplary sequence	PGPVPPSTADIELIEELINITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF

SEQ ID NO: (Information)	Amino acid sequence
comprising L10D, R11I, V18I, R86K, D87K, K89R, R108K, referred to herein as C2)	CPHKVSAGQFSSLHVKKTRIEVAQFVKDLLLHLKK LFKEGQFN
SEQ ID NO:110 (Exemplary sequence comprising L10A, R86T, D87G, T88K, K89R, L101N, K104R, K105A, R108K, referred to herein as C3)	PGPVPPSTAARELIEELVNITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVTGKRIEVAQFVKDLLNHLRA LFKEGQFN
SEQ ID NO:111 (Exemplary sequence comprising L10V, K89R, L101N, K105E, R108T, referred to herein as C4)	PGPVPPSTAVRELIEELVNITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVRDTRIEVAQFVKDLLNHLKE LFTEGQFN
SEQ ID NO:112 (Exemplary sequence comprising R11S, I14M, T88S, L101N, K105A, R108K, referred to herein as C7)	PGPVPPSTALSELMEELVNITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVRDSKIEVAQFVKDLLNHLKA LFKEGQFN
SEQ ID NO:113 (Exemplary sequence comprising L10H, R11L, V18I, R86K, D87E, K89R, L101N, K105T, R108K, referred to herein as C9)	PGPVPPSTAHLELIEELINITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVKETRIEVAQFVKDLLNHLKT LFKEGQFN
SEQ ID NO:114 (Exemplary sequence comprising L10H, R11L, V18I, R86M, K89R, R108K, referred to herein as C10)	PGPVPPSTAHLELIEELINITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVMDTRIEVAQFVKDLLLHLKK LFKEGQFN

SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:115 (Exemplary sequence comprising L10H, R86T, D87G, T88R, R108K, referred to herein as C11)	PGPVPPSTAHRELIEELVNITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVTGRKIEVAQFVKDLLLHLKK LFKEGQFN
SEQ ID NO:116 (Exemplary sequence comprising L10H, R86M, T88S, K89R, L101N, K104R, K105A, R108K, referred to herein as C12)	PGPVPPSTAHRELIEELVNITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVMSR IEVAQFVKDLLNHLRA LFKEGQFN
SEQ ID NO:117 (Exemplary sequence comprising L10A, V18F, R86F, D87F, K89R, L101I, K104R, R108K, referred to herein as D7)	PGPVPPSTAARELIEELFNITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVKKTR IEVAQFVKDLLIHLRK LFKEGQFN
SEQ ID NO:118 (Exemplary sequence comprising L10V, E12A, V18I, R65D, D87S, T88S, L101F, K104R, K105T, referred to herein as IL-13dn)	PGPVPPSTAVRALIEELINITQNQKAPLCNGSMVW SINLTAGMYCAALES LINVSGCSAIEKTQDMLSGF CPHKVSAGQFSSLHVRSSK IEVAQFVKDLLFHLRT LFREGQFN
SEQ ID NO:119 <u>**signal peptide**</u>	<u>MHPLLNPLLLALGLMALLTTVIALTCLGGFAS</u> PG PVPSTAHRELIEELVNITQNQKAPLCNGSMVWSI NLTAGMYCAALES LINVSGCSAIEKTQRMLSGF CPHKVSAGQFSSLHVTGRKIEVAQFVKDLLLHLKKLF KEGQFN
SEQ ID NO:120 (Exemplary sequence comprising L10V, E12A, V18I, R65D, D87S, T88S, L101F,	PGPVPPSTAVRALIEELINITQNQKAPLCNGSMVW SIN R TAGMYCAALES LINVSGCSAIEKTQDMLSGF CPHKVSAGQFSSLHVRSSK IEVAQFVKDLLFHLRT LFREGQFN

SEQ ID NO: (Information)	Amino acid sequence
K104R, K105T, referred to herein as IL-13DN variant 1)	
SEQ ID NO:121 (Exemplary sequence comprising L10V, E12A, V18I, R65D, D87S, T88S, L101F, K104R, K105T, referred to herein as IL-13DN variant 2)	PGPVPPSTAVRALIEELINITQNQKAPLCNGSMVW SINLTAGMYCAALES L INVSGCSAIEKTQDMLSGF CPHKVSAGQFSSLHVRSSKIEVAQFVKDLLFHLRT LFREGQFN
SEQ ID NO:122 wild-type IL-13 including an additional methionine at the N-terminus	MPGPVPPSTALRELIEELVNITQNQKAPLCNGSMV WSINLTAGMYCAALES L INVSGCSAIEKTQRMLSG FCPHKVSAGQFSSLHVRD T KIEVAQFVKDLLLHLK KLFREGQFN
SEQ ID NO:123 circularly permuted IL-13	MYCAALES L INVSGCSAIEKTQRMLSGFCPHKVSA GQFSSLHVRD T KIEVAQFVKDLLLHLK K LFREGQF NGGSGPGPVPPSTALRELIEELVNITQNQKAPLCN GSMVWSINLTAG
SEQ ID NO:124 Circularly permuted IL-13	MYCAALES L INVSGCSAIEKTQRMLSGFCPHKVSA GQFSSLHVRD T KIEVAQFVKDLLLHLK K LFREGQF NGGSGMPGPVPPSTALRELIEELVNITQNQKAPLC NGSMVWSINLTAG
SEQ ID NO:125 circularly permuted IL-13 "A11" variant	MYCAALES L INVSGCSAIEKTQRMLSGFCPHKVSA GQFSSLHVRSSKIEVAQFVKDLLFHLRTL F REGQF NGGSGPGPVPPSTAVRELIEELINITQNQKAPLCN GSMVWSINRTAG
SEQ ID NO:126 circularly permuted IL-13	MYCAALES L INVSGCSAIEKTQRMLSGFCPHKVSA GQFSSLHVRSSKIEVAQFVKDLLFHLRTL F REGQF NGGSGMPGPVPPSTAVRELIEELINITQNQKAPLC NGSMVWSINRTAG
SEQ ID NO:127 circularly permuted IL-13 "DN" variant	MYCAALES L INVSGCSAIEKTQDMLSGFCPHKVSA GQFSSLHVRSSKIEVAQFVKDLLFHLRTL F REGQF NGGSGPGPVPPSTAVRALIEELINITQNQKAPLCN GSMVWSINLTAG
SEQ ID NO:128 circular permuted IL-13	MYCAALES L INVSGCSAIEKTQDMLSGFCPHKVSA GQFSSLHVRSSKIEVAQFVKDLLFHLRTL F REGQF

SEQ ID NO: (Information)	Amino acid sequence
	NGGSGMPGPVPPSTAVRALIEELINITQNQKAPLC NGSMVWSINLTAG

[00162] Any IL-4 sequence or variant thereof can be used in a fusion with an IL-2 mutein or variant, including those as described herein. In some embodiments, the IL-2 mutein includes any one of 5-1 SEQ ID NO:5; 5-2 SEQ ID NO:6; 6-6 SEQ ID NO:7; A2 SEQ ID NO:8; B1 SEQ ID NO:9; B11 SEQ ID NO:10; C5 SEQ ID NO:11; D10 SEQ ID NO:12; E10 SEQ ID NO:13; G8 SEQ ID NO:14; H4 SEQ ID NO:15; and H9 SEQ ID NO:5. Exemplary polypeptide sequences are provided in SEQ ID NO:130-SEQ ID NO:135, including any of those provided herein. In some embodiments, the IL-4 polypeptide sequence is as provided in any one of SEQ ID NO:130 through SEQ ID NO:135. In some embodiments, the IL-4 polypeptide sequence is SEQ ID NO:130. In some embodiments, the IL-4 polypeptide sequence is SEQ ID NO:131. In some embodiments, the IL-4 polypeptide sequence is SEQ ID NO:132. In some embodiments, the IL-4 polypeptide sequence is SEQ ID NO:133. In some embodiments, the IL-4 polypeptide sequence is SEQ ID NO:134. In some embodiments, the IL-4 polypeptide sequence is SEQ ID NO:135. In some embodiments, the IL-4 polypeptide sequence is 98% identical to any one of SEQ ID NO:130 through SEQ ID NO:135. In some embodiments, the IL-4 polypeptide sequence is 99% identical to any one of SEQ ID NO:130 through SEQ ID NO:135. In some embodiments, any one of SEQ ID NO:130-SEQ ID NO:135 are linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:130 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:131 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:132 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:133 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:134 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:135 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, the IL-2 mutein includes any one of 5-1 SEQ ID NO:5; 5-2 SEQ ID NO:6; 6-6 SEQ ID NO:7; A2 SEQ ID NO:8; B1 SEQ ID NO:9; B11 SEQ ID NO:10; C5 SEQ ID NO:11; D10 SEQ ID NO:12; E10 SEQ ID NO:13; G8 SEQ ID NO:14; H4 SEQ ID NO:15; and H9 SEQ ID NO:5.

[00163] Table of IL-4 sequences is provided below.

Table 8: List of IL-4 Amino Acid Sequences

SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:129 (IL-4 wildtype with signal peptide)	<u>MGLTSQLLPPLFFLLACAGNFVHGH</u> KCDITLQEI I KTLNSLTEQKTLCTELTVTDIFAASKNTEKETFC RAATVLRQFYSHHEKDTRCLGATAQQFHRHKQLIR FLKRLDRNLWGLAGLNSCPVKEANQSTLENFLERL KTIMREKYSKCSS
SEQ ID NO:130 IL-4 including an additional methionine at the N-terminus" starting	MHKCDITLQEI IKTLNSLTEQKTLCTELTVTDIFA ASKDTTEKETFCRAATVLRQFYSHHEKDTRCLGAT AQQFHRHKQLIRFLKRLDRNLWGLAGLNSCPVKEA NQSTLENFLERLKTIMREKYSKCSS
SEQ ID NO:131 KFR	KCDITLQEI IKTLNSLTEQKTLCTELTVTDIFAAS KNTTEKETFCRAATVLRQFYSHHEKDTRCLGATAQ QFHRHKQLIRFLKRLDRNLWGLAGLNSCPVKEANQ STLENFLERLKTIMKEKFRKCSS
SEQ ID NO:132 RGA	MDTTEKETFCRAATVLRQFYSHHEKDTRCLGATAQ QFHRHKQLIRFLKRLDRNLWGLAGLNSCPVKEANQ STLENFLERLRVIMQSKWFKCGAGGNGGHKCDITL QEI IKTLNSLTEQKTLCTELTVTDIFAAS
SEQ ID NO:133 circularly permuted wild-type IL-4	MDTTEKETFCRAATVLRQFYSHHEKDTRCLGATAQ QFHRHKQLIRFLKRLDRNLWGLAGLNSCPVKEANQ STLENFLERLKTIMREKYSKCSSGGNGGHKCDITL QEI IKTLNSLTEQKTLCTELTVTDIFAAS
SEQ ID NO:134 circularly permuted "KFR" IL-4 variant	MDTTEKETFCRAATVLRQFYSHHEKDTRCLGATAQ QFHRHKQLIRFLKRLDRNLWGLAGLNSCPVKEANQ STLENFLERLKTIMKEKFRKCSSGGNGGHKCDITL QEI IKTLNSLTEQKTLCTELTVTDIFAASRQFYSH HEKDTRCLGATAQQFHRHKQLIRFLKRLDRNLWGL AGLNSCPVKEANQSTLENFLERLRVIMQSKWFKCG AGGNGGHKCDITLQEI IKTLNSLTEQKTLCTELTV TDIFAAS
SEQ ID NO:135 circularly permuted "KF" IL-4 variant	MDTTEKETFCRAATVLRQFYSHHEKDTRCLGATAQ QFHRHKQLIRFLKRLDRNLWGLAGLNSCPVKEANQ STLENFLERLKTIMKEKFRKCSSGGNGGHKCDITLQ EIIKTLNSLTEQKTLCTELTVTDIFAAS

[00164] In some embodiments, an IL-2 mutein can be fused to an IL-10, IL-12, IL-15, and/or IL-18 sequence. In some embodiments, such fusions function to specifically target the fusion construct to NK cells and/or CD8+ cells. In some embodiments, the IL-2 mutein includes any one of 5-1 SEQ ID NO:5; 5-2 SEQ ID NO:6; 6-6 SEQ ID NO:7; A2 SEQ ID NO:8; B1 SEQ ID NO:9; B11 SEQ ID NO:10; C5 SEQ ID NO:11; D10 SEQ ID NO:12; E10 SEQ ID NO:13; G8 SEQ ID NO:14; H4 SEQ ID NO:15; and H9 SEQ ID NO:5. In some embodiments, SEQ ID NO:136 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:137 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:138 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:139 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:140 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, SEQ ID NO:141 is linked to an IL-2 or IL-2 mutein as described herein. In some embodiments, the IL-2 mutein can be fused to an IL- IL-10, IL-12, IL-15, and/or IL-18 sequence as provided in the table below, in SEQ ID NOs: 136-141.

Table 9: Exemplary IL-10, IL-12, IL-15, and/or IL-18 Sequences

SEQ ID NO:	Amino Acid Sequence
SEQ ID NO:136 IL-10 (Uniprot sp P22301)	MHSSALLCCLVLLTGVRASPGQGTQSENSCTHFPGNLPNMLRDLRDAFS RVKTFQMKDQLDNLLLKESLLEDFKGYLGCQALSEMIQFYLEEVMPOA ENQDPDIKAHVNSLGENLKTLLRRLRRCHRFLPCENKSKAVEQVKNAFN KLQEKGIYKAMSEFDIFINYIEAYMTMKIRN
SEQ ID NO:137 IL-12A (Uniprot sp P29459)	MCPARSLLLVATLVLLDHLNLPVATPDPGMFPCLLHHSQNLLRAVS NMLQKARQTFEYFCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLN SRETSFITNGSCLASRKTSMALCLSSIYEDLKMYQVEFKTMNAKLLM DPKRQIFLDQNLAVI DELMQALNFNSETVPPQKSSLEEDPFYKTKIKLC ILLHAFRIRAVTIDRVMSYLNAS
SEQ ID NO:138 IL-12B (Uniprot sp P29460)	MCHQQLVISWFSLVFLASPLVAIWELKDKDVYVVELDWYDPAPGEMVVLV CDTPEEDGITWTLTDSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLS HLLLLLHKKEDGIWSTDIKDKQKPKNKTFLRCEAKNYSGRFTCWWLTT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSVECCQED SACPAAEESLPIEVMVDAVHKLKYENYTSFFIRDIKPDPPKNLQKLP LKNSRQVEVSWEYPTWSTPHSYFSLTFCVQVQVQKSKREKKDRVFTDKT SATVICRKNASISVRAQDRYSSSWSEWASVPCS
SEQ ID NO:139 IL-15 (Uniprot sp P40933)	MRISKPHLRSSISIQCYLCLLLNSHFLTEAGIHVFIILGCF SAGLPKTEAN WVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCF LLELQVI SLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEF LQSFVHIVQMFINTS
SEQ ID NO:140	<u>MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENLES</u> DYFGKLESKLSVIR NLNDQVLFIDQGNRPLFEDMTSDCDRDNAPRTIFIIISMYKDSQPRGMVA

SEQ ID NO:	Amino Acid Sequence
IL-18 (Uniprot sp Q14116)	TISVKCEKISTLSCEKIIISFKEMNPPDNIKDTKSDIIFQRSVPGHDN KMQFESSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNE
SEQ ID NO:141 IL-18 (mature)	YFGKLESKLSVIRNLDQVLFIDQGNRPLFEDMTSDSCRDNAPRTIFII SMYKDSQPRGMAVTISVKCEKISTLSCEKIIISFKEMNPPDNIKDTKSD IIFQRSVPGHDNKMQFESSYEGYFLACEKERDLFKLILKKEDELGDR SIMFTVQNE

[00165] The sequences of exemplary IL-2 mutein includes any one of 5-1 SEQ ID NO:5; 5-2 SEQ ID NO:6; 6-6 SEQ ID NO:7; A2 SEQ ID NO:8; B1 SEQ ID NO:9; B11 SEQ ID NO:10; C5 SEQ ID NO:11; D10 SEQ ID NO:12; E10 SEQ ID NO:13; G8 SEQ ID NO:14; H4 SEQ ID NO:15; and H9 SEQ ID NO:5.

[00166] In some embodiments, the cytokine-cytokine fusion is one of those included in the table below.

Table 10: List of Exemplary IL-2 Fusion Amino Acid Sequences

SEQ ID NO: (Information)	Amino acid sequence
SEQ ID NO:142 IL-13 variant-H9 (linker in bold and underlined)	PGPVPPSTAVRALIEELINITQNQKAPLCNGSMVWSINRTAGMY CAALESLINVSGCSAIEKTQDMLSGFCPHKVSAGQFSSLHVRSS KIEVAQFVKDLLFHLRTLFREGQFN <u>GGGSGGGSGGGGS</u> APTS SSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFYMPK KATELKHLLQCLEEELKPLEEVLNLAQSKNFHFDPRDVVSNINVF VLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
SEQ ID NO:143 IL-13 variant-H9 (linker in bold and underlined)	PGPVPPSTAHRELIEELVNITQNQKAPLCNGSMVWSINLTAGMY CAALESLINVSGCSAIEKTQRMLSGFCPHKVSAGQFSSLHVTGR KIEVAQFVKDLLLHLKLFKEGQFN <u>GGGSGGGSGGGGS</u> APTS SSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFYMPK KATELKHLLQCLEEELKPLEEVLNLAQSKNFHFDPRDVVSNINVF VLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
SEQ ID NO:144 H9-IL-12 (linker in bold and underlined)	APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKF YMPKATELKHLLQCLEEELKPLEEVLNLAQSKNFHFDPRDVVSN INVFVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL <u>TGGGSGGGSGGGGS</u> RNLPVATPDGPMFPCLLHHSQNLRAVSN MLQKARQTFEYFCTSEEIDHEDITKDKTSTVEACLPLELTKNE SCLNSRETSFITNGSCLASRKTSMALCLSSIYEDLKMYQVEF KTMNAKLLMDPKRQIFLDQNMFLAVIDELMQALNFNSETVTPQKSS LEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNASIWELKGD VYVVELDWYDPDAGEMVVLTCDTPEEDGITWTLTDSSEVLGSGK TLTIQVKEFGDAGQYTCHKGGEVLSHLLLLLHKKEDGIWSTDIL KDQKEPKNKTFLRCEAKNYSGRFTCWLLTTISTDLTFSVKSSRG SSDPQGVTCGAATLSAERVRGDNKEYEYSVEQCEDSACPAEES

SEQ ID NO: (Information)	Amino acid sequence
	LP I E V M V D A V H K L K Y E N Y T S S F F I R D I I K P D P P K N L Q L K P L K N S R Q V E V S W E Y P D T W S T P H S Y F S L T F C V Q V Q G K S K R E K K D R V F T D K T S A T V I C R K N A S I S V R A Q D R Y Y S S S W S E W A S V P C S
SEQ ID NO:145 H9-IL18 (linker in bold and underlined)	A P T S S S T K K T Q L Q L E H L L L L D L Q M I L N G I N N Y K N P K L T R M L T F K F Y M P K K A T E L K H L Q C L E E E L K P L E E V L N L A Q S K N F H F D P R D V V S N I N V F V L E L K G S E T T F M C E Y A D E T A T I V E F L N R W I T F C Q S I I S T L T <u>GGGGSGGGSGGGGS</u> Y F G K L E S K L S V I R N L N D Q V L F I D Q G N R P L F E D M T D S D C R D N A P R T I F I I S M Y K D S Q P R G M A V T I S V K C E K I S T L S C E N K I I S F K E M N P P D N I K D T K S D I I F F Q R S V P G H D N K M Q F E S S S Y E G Y F L A C E K E R D L F K L I L K K E D E L G D R S I M F T V Q N E D

D. RECOMBINANT EXPRESSION OF IL-2 MUTEINS, EXPRESSION VECTORS AND HOST CELLS

[00167] In various embodiments, polypeptides used in the practice of the instant invention are synthetic, or are produced by expression of a recombinant nucleic acid molecule. In the event the polypeptide is a chimera (e.g., a fusion protein containing at least a mutant IL-2 polypeptide and a heterologous polypeptide), it can be encoded by a hybrid nucleic acid molecule containing one sequence that encodes all or part of the IL-2 mutein, and a second sequence that encodes all or part of the heterologous polypeptide. For example, subject IL-2 muteins described herein may be fused to a hexa-histidine tag to facilitate purification of bacterially expressed protein, or to a hemagglutinin tag to facilitate purification of protein expressed in eukaryotic cells.

[00168] Methods for constructing a DNA sequence encoding the IL-2 muteins and expressing those sequences in a suitably transformed host include, but are not limited to, using a PCR-assisted mutagenesis technique. Mutations that consist of deletions or additions of amino acid residues to an IL-2 polypeptide can also be made with standard recombinant techniques. In the event of a deletion or addition, the nucleic acid molecule encoding IL-2 is optionally digested with an appropriate restriction endonuclease. The resulting fragment can either be expressed directly or manipulated further by, for example, ligating it to a second fragment. The ligation may be facilitated if the two ends of the nucleic acid molecules contain complementary nucleotides that overlap one another, but blunt-ended fragments can also be ligated. PCR-generated nucleic acids can also be used to generate various mutant sequences.

[00169] The complete amino acid sequence can be used to construct a back-translated gene. A DNA oligomer containing a nucleotide sequence coding for IL-2 mutein can be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide can be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

[00170] In addition to generating mutant polypeptides via expression of nucleic acid molecules that have been altered by recombinant molecular biological techniques, subject IL-2 muteins can be chemically synthesized. Chemically synthesized polypeptides are routinely generated by those of skill in the art.

[00171] Once assembled (by synthesis, site-directed mutagenesis or another method), the DNA sequences encoding an IL-2 mutein will be inserted into an expression vector and operatively linked to an expression control sequence appropriate for expression of the IL-2 mutein in the desired transformed host. Proper assembly can be confirmed by nucleotide sequencing, restriction mapping, and expression of a biologically active polypeptide in a suitable host. As is well known in the art, in order to obtain high expression levels of a transfected gene in a host, the gene must be operatively linked to transcriptional and translational expression control sequences that are functional in the chosen expression host.

[00172] The DNA sequence encoding the IL-2 mutein, whether prepared by site directed mutagenesis, chemical synthesis or other methods, can also include DNA sequences that encode a signal sequence. Such signal sequence, if present, should be one recognized by the cell chosen for expression of the IL-2 mutein. It can be prokaryotic, eukaryotic or a combination of the two. It can also be the signal sequence of native IL-2. The inclusion of a signal sequence depends on whether it is desired to secrete the IL-2 mutein from the recombinant cells in which it is made. If the chosen cells are prokaryotic, it generally is preferred that the DNA sequence not encode a signal sequence. If the chosen cells are eukaryotic, it generally is preferred that a signal sequence be encoded and most preferably that the wild-type IL-2 signal sequence be used.

E. ONCOLYTIC VIRUSES TARGETING MOIETIES

[00173] In some examples, the IL-2 muteins described herein can be employed to target an oncolytic virus (*e.g.*, see Allen et al., *Mol. Ther.* 16:1556-64, 2008). In some examples, oncolytic virus can be used to target an IL-2 mutein to a tumor or TME. Numerous viruses can

be employed as the oncolytic virus, including adenoviruses as well as self-replicating alphavirus, as well as oncolytic vaccinia viruses (see, for example WO2013038066, incorporated herein by reference in its entirety; in particular Figure 17). Other oncolytic viruses can include Seneca Valley Virus, Newcastle disease Virus (also referred to as Newcastle virus), Maraba virus, vesicular stomatitis virus (VSV), Herpes virus (including HSV-1), Measles virus, poliovirus, reovirus, coxsackie virus, a lentivirus, a morbillivirus, an influenza virus, Sinbis virus, myxoma virus and/or retrovirus (see, for example, Twumasi-Boateng, et al., “Oncolytic viruses as engineering platforms for combination immunotherapy”, *Nature Reviews Cancer*, 2018), and Kaufman et al., *Cancer Immunotherapy*, 14:642-662 (2015), all of which are incorporated by reference herein their entireties). In some embodiments, the oncolytic virus includes but is not limited to an adenovirus, a self-replicating alphavirus, a vaccinia virus, a Seneca Valley Virus, a Newcastle disease Virus, a Maraba virus, vesicular stomatitis virus (VSV), a Herpes virus (including HSV-1 and HSV-2), a measles virus, a poliovirus, a reovirus, a coxsackie virus, a lentivirus, a morbillivirus, an influenza virus, Sinbis virus, myxoma virus and/or a retrovirus. The IL-2 superkines (H9 and IL-2 variants as described herein) also can be used to direct T cells/OVs to the TME. An IL-2 variant (such as H9) can boost effector T cells and NK cells while IL-2 variant can suppress T reg activity. Other oncolytic viruses include can include, for example, oncoVex/T-VEC, which involves the intratumoral injection of replication-conditional herpes simplex virus which preferentially infects cancer cells. The virus, which is also engineered to express GM-CSF, is able to replicate inside a cancer cell causing its lysis, releasing new viruses and an array of tumor antigens, and secreting GM-CSF in the process. Such oncolytic virus vaccines enhance DCs function in the tumor microenvironment to stimulate anti-tumor immune responses. These oncolytic viruses can be used to target or deliver the IL-2 muteins described herein to the tumor. In some embodiments, the IL-2 mutein is any IL-2 mutein or variant disclosed herein. In some embodiments, the IL-2 mutein sequence is 90% identical to any one of SEQ ID NO:2 or SEQ ID NO:6 through SEQ ID NO:10 or SEQ ID NO:16. In some embodiments, the IL-2 mutein includes any one of 5-1 SEQ ID NO:5; 5-2 SEQ ID NO:6; 6-6 SEQ ID NO:7; A2 SEQ ID NO:8; B1 SEQ ID NO:9; B11 SEQ ID NO:10; C5 SEQ ID NO:11; D10 SEQ ID NO:12; E10 SEQ ID NO:13; G8 SEQ ID NO:14; H4 SEQ ID NO:15; and H9 SEQ ID NO:5. In some embodiments, the substitutions in the IL-2 mutein comprise L80F, R81D, L85V, I86V, and I92F, numbered in accordance with wild-type human IL-2 of SEQ ID NO:2. In

some embodiments, the oncolytic virus comprises a transgene capable of expressing an IL-2 mutein as described herein. In some embodiments, the oncolytic virus comprises a transgene capable of expressing an IL-2 mutein comprising the following amino acid substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the oncolytic virus comprises a nucleic acid encoding an IL-2 mutein comprising the following amino acid substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the oncolytic virus comprises a transgene that is expressed as a therapeutic payload. In some embodiments, the therapeutic payload is an IL-2 as described herein. In some embodiments, the therapeutic payload is IL-2 mutein comprising the following amino acid substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with wild-type human IL-2 of SEQ ID NO:2.

[00174] In some embodiments, the oncolytic virus is an oncolytic vaccinia virus. In some embodiments, the oncolytic vaccinia virus vector is characterized in that the virus particle is of the type intracellular mature virus (IMV), intracellular enveloped virus (IEV), cell-associated enveloped virus (CEV), or extracellular enveloped virus (EEV). In some embodiments, the oncolytic vaccinia virus particle is of the type EEV or IMV. In some embodiments, the oncolytic vaccinia virus particle is of the type EEV.

[00175] Generally, construction of oncolytic vaccinia virus recombinants and cells and pharmaceutical compositions comprising said vectors which preferentially replicate in tumor cells and express at least one transgene (for example, and IL-2 mutein as described herein) to facilitate antitumor efficacy and apoptosis induction and to modulate host immune responses in a subject. According to the present invention, oncolytic adenoviruses and oncolytic vaccinia viruses can be combined with IL-2 expression or targeting moieties as described herein in order to target the oncolytic vaccinia virus or the oncolytic adenovirus and/or express the IL-2 mutein. Oncolysis releases tumor antigens and provides costimulatory danger signals. However, arming the virus can improve efficacy further. For example, CD40 ligand (CD40L, CD154) is known to induce apoptosis of tumor cells and it also triggers several immune mechanisms. One of these is a T-helper type 1 (Th1) response that leads to activation of cytotoxic T- cells and reduction of immune suppression. The present invention provides for oncolytic viruses that express the IL-2 muteins of the present invention. In some embodiments, the present invention provides for

oncolytic viruses that are targeted (for example, “armed”) with the IL-2 targeting moieties of the present invention.

[00176] In some embodiments, the oncolytic virus is a modified vaccinia virus vector, a virus particle, a host cell, a pharmaceutical composition and a kit comprising vaccinia virus genome wherein the thymidine kinase gene is inactivated by either a substitution in the thymidine kinase (TK) gene and/or an open reading frame ablating deletion of at least one nucleotide providing a partially deleted thymidine kinase gene, the vaccinia growth factor gene is deleted, and the modified vaccinia virus vector comprises at least one nucleic acid sequence encoding a non-viral protein (*e.g.*, an IL-2 mutein as described herein which is capable of being expressed). In another aspect is provided the modified vaccinia virus vector, the virus particle, the pharmaceutical composition or the kit can be used for cancer therapy, for eliciting immune response in a subject, for use in a method of inhibiting malignant cell proliferation in a mammal, for use in a therapy or prophylaxis of cancer, for detecting the presence of the modified vaccinia virus in a subject, and as an in situ cancer vaccine, optionally in combination with adenovirus. In some embodiments, the invention provides method of producing a modified vaccinia virus comprising vaccinia virus genome wherein the thymidine kinase gene is inactivated by a substitution in the thymidine kinase (TK) gene and/or an open reading frame ablating deletion of at least one nucleotide providing a partially deleted thymidine kinase gene, the vaccinia growth factor gene is deleted, and the modified vaccinia virus vector comprises at least one nucleic acid sequence encoding a non-viral protein (*e.g.*, an IL-2 mutein as described herein), comprising the steps of providing producer cells capable of sustaining production of vaccinia virus particles and carrying the modified vaccinia vector; culturing the producer cells in conditions suitable for virus replication and production; and harvesting the virus particles.

[00177] In some embodiments, the present invention provides methods of administering an oncolytic virus “armed” with or including an nucleic acid encoding an IL-2 mutein as described herein, wherein said IL-2 mutein is expressed at the tumor location or is expressed systemically in the subject. In some embodiments, the present invention also provides methods of administering an oncolytic virus “armed” or targeted with an IL-2 mutein as described herein. The routes of administration vary, naturally, with the location and nature of the tumor, and include, *e.g.*, intradermal, transdermal, parenteral, intravenous, intramuscular, intranasal, subcutaneous, regional (*e.g.*, in the proximity of a tumor, particularly with the vasculature or adjacent

vasculature of a tumor), percutaneous, intratracheal, intraperitoneal, intraarterial, intravesical, intratumoral, inhalation, perfusion, lavage, and oral administration. Compositions are formulated relative to the particular administration route.

1. ONCOLYTIC VACCINIA VIRUS

[00178] Vaccinia virus is a member of the Orthopoxvirus genus of the Poxviridae. It has large double-stranded DNA genome (~200 kb, ~200 genes) and a complex morphogenic pathway produces distinct forms of infectious virions from each infected cell. Viral particles contain lipid membranes(s) around a core. Virus core contains viral structural proteins, tightly compacted viral DNA genome, and transcriptional enzymes. Dimensions of vaccinia virus are ~ 360 x 270 x 250 nm, and weight of ~ 5-10 fg. Genes are tightly packed with little non-coding DNA and open-reading frames (ORFs) lack introns. Three classes of genes (early, intermediate, late) exists. Early genes (~ 100 genes; immediate and delayed) code for proteins mainly related to immune modulation and virus DNA replication. Intermediate genes code for regulatory proteins which are required for the expression of late genes (e.g. transcription factors) and late genes code for proteins required to make virus particles and enzymes that are packaged within new virions to initiate the next round of infection. Vaccinia virus replicates in the cell cytoplasm.

[00179] Different strains of vaccinia viruses have been identified (as an example: Copenhagen, modified virus Ankara (MVA), Lister, Tian Tan, Wyeth (= New York City Board of Health), Western Reserve (WR)). The genome of WR vaccinia has been sequenced (Accession number AY243312). In some embodiments, the oncolytic vaccinia virus is a Copenhagen, modified virus Ankara (MVA), Lister, Tian Tan, Wyeth, or Western Reserve (WR) vaccinia virus.

[00180] Different forms of viral particles have different roles in the virus life cycle Several forms of viral particles exist: intracellular mature virus (IMV), intracellular enveloped virus (IEV), cell-associated enveloped virus (CEV), extracellular enveloped virus (EEV). EEV particles have an extra membrane derived from the trans-Golgi network. This outer membrane has two important roles: a) it protects the internal IMV from immune aggression and, b) it mediates the binding of the virus onto the cell surface.

[00181] CEVs and EEVs help virus to evade host antibody and complement by being wrapped in a host-derived membrane. IMV and EEV particles have several differences in their biological properties and they play different roles in the virus life cycle. EEV and IMV bind to different

(unknown) receptors (1) and they enter cells by different mechanisms. EEV particles enter the cell via endocytosis and the process is pH sensitive. After internalization, the outer membrane of EEV is ruptured within an acidified endosome and the exposed IMV is fused with the endosomal membrane and the virus core is released into the cytoplasm. IMV, on the other hand, enters the cell by fusion of cell membrane and virus membrane and this process is pH-independent. In addition to this, CEV induces the formation of actin tails from the cell surface that drive virions towards uninfected neighboring cells.

[00182] Furthermore, EEV is resistant to neutralization by antibodies (NAb) and complement toxicity, while IMV is not. Therefore, EEV mediates long range dissemination in vitro and in vivo. Comet-inhibition test has become one way of measuring EEV-specific antibodies since even if free EEV cannot be neutralized by EEV NAb, the release of EEV from infected cells is blocked by EEV NAb and comet shaped plaques cannot be seen. EEV has higher specific infectivity in comparison to IMV particles (lower particle/pfu ratio) which makes EEV an interesting candidate for therapeutic use. However, the outer membrane of EEV is an extremely fragile structure and EEV particles need to be handled with caution which makes it difficult to obtain EEV particles in quantities required for therapeutic applications. EEV outer membrane is ruptured in low pH (pH ~6). Once EEV outer membrane is ruptured, the virus particles inside the envelope retain full infectivity as an IMV.

[00183] Some host-cell derived proteins co-localize with EEV preparations, but not with IMV, and the amount of cell-derived proteins is dependent on the host cell line and the virus strain. For instance, WR EEV contains more cell-derived proteins in comparison to VV IHD-J strain. Host cell derived proteins can modify biological effects of EEV particles. As an example, incorporation of the host membrane protein CD55 in the surface of EEV makes it resistance to complement toxicity. In the present invention it is shown that human A549 cell derived proteins in the surface of EEV particles may target virus towards human cancer cells. Similar phenomenon has been demonstrated in the study with human immunodeficiency virus type 1, where host- derived ICAM-1 glycoproteins increased viral infectivity. IEV membrane contains at least 9 proteins, two of those not existing in CEV/EEV. F12L and A36R proteins are involved in IEV transport to the cell surface where they are left behind and are not part of CEV/EEV (9, 11). 7 proteins are common in (IEV)/CEV/EEV: F13L, A33R, A34R, A56R, B5R, E2, (K2L). For Western Reserve strain of vaccinia virus, a maximum of 1% of virus particles are normally EEV

and released into the culture supernatant before oncolysis of the producer cell. 50-fold more EEV particles are released from International Health Department (IHD)-J strain of vaccinia. IHD has not been studied for use in cancer therapy of humans however. The IHD-W phenotype was attributed largely to a point mutation within the A34R EEV lectin-like protein. Also, deletion of A34R increases the number of EEVs released. EEV particles can be first detected on cell surface 6 hours post-infection (as CEV) and 5 hours later in the supernatant (IHD-J strain). Infection with a low multiplicity of infection (MOI) results in higher rate of EEV in comparison to high viral dose. The balance between CEV and EEV is influenced by the host cell and strain of virus.

[00184] Vaccinia has been used for eradication of smallpox and later, as an expression vector for foreign genes and as a live recombinant vaccine for infectious diseases and cancer. Vaccinia virus is the most widely used pox virus in humans and therefore safety data for human use is extensive. During worldwide smallpox vaccination programs, hundreds of thousands humans have been vaccinated safely with modified vaccinia virus strains and only very rare severe adverse events have been reported. Those are generalized vaccinia (systemic spread of vaccinia in the body), erythema multiforme (toxic/allergic reaction), eczema vaccinatum (widespread infection of the skin), progressive vaccinia (tissue destruction), and postvaccinia! encephalitis.

[00185] All together 44 melanoma patients have been treated in early clinical trials with wild type vaccinia virus in 1960s - 1990s and the overall objective response rate of injected tumors was 50%. Also some beneficial immunological responses were seen (36). Wild type vaccinia virus has been used also for treatment of bladder cancer, lung and kidney cancer, and myeloma and only mild adverse events were seen. JX-594, an oncolytic Wyeth strain vaccinia virus coding for GM-CSF, has been successfully evaluated in three phase I studies and preliminary results from randomized phase II trial has been presented in the scientific meeting.

[00186] Vaccinia virus is appealing for cancer gene therapy due to several characteristics. It has natural tropism towards cancer cells and the selectivity can be significantly enhanced by deleting some of the viral genes. The present invention relates to the use of double deleted vaccinia virus (vvdd) in which two viral genes, viral thymidine kinase (TK) and vaccinia growth factor (VGF), are at least partially deleted. TK and VGF genes are needed for virus to replicate in normal but not in cancer cells. The partial TK deletion may be engineered in the TK region conferring activity.

[00187] TK deleted vaccinia viruses are dependent on cellular nucleotide pool present in dividing cells for DNA synthesis and replication. IN some embodiments, the TK deletion limits virus replication significantly in resting cells allowing efficient virus replication to occur only in actively dividing cells (*e.g.*, cancer cells). VGF is secreted from infected cells and has a paracrine priming effect on surrounding cells by acting as a mitogen. Replication of VGF deleted vaccinia viruses is highly attenuated in resting (non- cancer) cells. The effects of TK and VGF deletions have been shown to be synergistic.

2. ONCOLYTIC ADENOVIRUS

[00188] Generally, adenovirus is a 36 kb, linear, double-stranded DNA virus (Grunhaus and Horwitz, 1992). The term “adenovirus” or “AAV” includes AAV type 1 (AAV1), AAV type 2 (AAV2), AAV type 3 (AAV3), AAV type 4 (AAV4), AAV type 5 (AAV5), AAV type 6 (AAV6), AAV type 7 (AAV7), AAV type 8 (AAV8), AAV type 9 (AAV9), AAV 9_hu14, avian AAV, bovine AAV, canine AAV, equine AAV, primate AAV, non-primate AAV, and ovine AAV. “Primate AAV” refers to AAV capable of infecting primates, “non-primate AAV” refers to AAV capable of infecting non-primate mammals, “bovine AAV” refers to AAV capable of infecting bovine mammals, etc.

[00189] Adenoviral infection of host cells results in adenoviral DNA being maintained episomally, which reduces the potential genotoxicity associated with integrating vectors. Also, adenoviruses are structurally stable, and no genome rearrangement has been detected after extensive amplification. Adenovirus can infect virtually all epithelial cells regardless of their cell cycle stage. (*See*, for example, US20060147420, incorporated by reference herein in its entirety.) Moreover, the E1a and E4 regions of adenovirus are essential for an efficient and productive infection of human cells. The E1a gene is the first viral gene to be transcribed in a productive infection, and its transcription is not dependent on the action of any other viral gene products. However, the transcription of the remaining early viral genes requires E1a gene expression. The E1a promoter, in addition to regulating the expression of the E1a gene, also integrates signals for packaging of the viral genome as well as sites required for the initiation of viral DNA replication. *See*, Schmid, S. I., and Hearing, P. in *Current Topics in Microbiology and Immunology*, vol. 199: pages 67–80 (1995).

[00190] In some embodiments, the oncolytic virus is an oncolytic adenovirus. It has been established that naturally occurring viruses can be engineered to produce an oncolytic effect in tumor cells (Wildner, 2001; Jacotat, 1967; Kim, 2001; Georger et al., 2002; Yan et al., 2003; Vile et al., 2002, each of which is incorporated herein by reference). In the case of adenoviruses, specific deletions within their adenoviral genome can attenuate their ability to replicate within normal quiescent cells, while they retain the ability to replicate in tumor cells. One such conditionally replicating adenovirus, $\Delta 24$, has been described by Fueyo et al. (2000), see also U.S. Patent Application No. 20030138405, each of which are incorporated herein by reference. The $\Delta 24$ adenovirus is derived from adenovirus type 5 (Ad-5) and contains a 24-base-pair deletion within the CR2 portion of the E1A gene. See, for example WO2001036650A2 (incorporated by reference herein in its entirety).

[00191] Oncolytic adenoviruses include conditionally replicating adenoviruses (CRADs), such as Delta 24, which have several properties that make them candidates for use as biotherapeutic agents. One such property is the ability to replicate in a permissive cell or tissue, which amplifies the original input dose of the oncolytic virus and helps the agent spread to adjacent tumor cells providing a direct antitumor effect.

[00192] In some embodiments, the oncolytic component of Delta 24 with a transgene expression approach to produce an armed Delta 24. Armed Delta 24 adenoviruses may be used for producing or enhancing bystander effects within a tumor and/or producing or enhancing detection/imaging of an oncolytic adenovirus in a patient, or tumor associated tissue and/or cell. In some embodiments, the combination of oncolytic adenovirus with various transgene strategies (e.g., expression of and IL-2 mutein) will improve the therapeutic potential, including for example, potential against a variety of refractory tumors, as well as provide for improved imaging capabilities. In certain embodiments, an oncolytic adenovirus may be administered with a replication defective adenovirus, another oncolytic virus, a replication competent adenovirus, and/or a wildtype adenovirus. Each of which may be administered concurrently, before or after the other adenoviruses.

[00193] In some embodiments, an E1a adenoviral vectors involves the replacement of the basic adenovirus E1a promoter, including the CAAT box, TATA box and start site for transcription initiation, with a basic promoter that exhibits tumor specificity, and preferably is E2F responsive,

and more preferably is the human E2F-1 promoter. Thus, this virus will be repressed in cells that lack molecules, or such molecules are non functional, that activate transcription from the E2F responsive promoter. Normal non dividing, or quiescent cells, fall in this class, as the transcription factor, E2F, is bound to pRb, or retinoblastoma protein, thus making E2F unavailable to bind to and activate the E2F responsive promoter. In contrast, cells that contain free E2F should support E2F based transcription. An example of such cells are neoplastic cells that lack pRb function, allowing for a productive viral infection to occur. In some embodiments, an E1a adenoviral vector is targeted use an IL-2 moiety as described herein.

[00194] Retention of the enhancer sequences, packaging signals, and DNA replication start sites which lie in the E1a promoter will ensure that the adenovirus infection proceeds to wild type levels in the neoplastic cells that lack pRb function. In essence, the modified E1a promoter confers tumor specific transcriptional activation resulting in substantial tumor specific killing, yet provides for enhanced safety in normal cells.

[00195] In some embodiments, an E1a adenoviral vector is prepared by substituting the endogenous E1a promoter with the E2F responsive promoter, the elements upstream of nucleotide 375 in the adenoviral 5 genome are kept intact. The nucleotide numbering is as described by See, Schmid, S. I., and Hearing, P. *Current Topics in Microbiology and Immunology*, vol. 199: pages 67–80 (1995). This includes all of the seven A repeat motifs identified for packaging of the viral genome. Sequences from nucleotide 375 to nucleotide 536 are deleted by a BsaAI to BsrBI restriction start site, while still retaining 23 base pairs upstream of the translational initiation codon for the E1A protein. An E2F responsive promoter, preferably human E2F-1 is substituted for the deleted endogenous E1a promoter sequences using known materials and methods. The E2F-1 promoter may be isolated as described in Example 1.

[00196] The E4 region has been implicated in many of the events that occur late in adenoviral infection, and is required for efficient viral DNA replication, late mRNA accumulation and protein synthesis, splicing, and the shutoff of host cell protein synthesis. Adenoviruses that are deficient for most of the E4 transcription unit are severely replication defective and, in general, must be propagated in E4 complementing cell lines to achieve high titers. The E4 promoter is positioned near the right end of the viral genome and governs the transcription of multiple open reading frames (ORF). A number of regulatory elements have been characterized in this

promoter that are critical for mediating maximal transcriptional activity. In addition to these sequences, the E4 promoter region contains regulatory sequences that are required for viral DNA replication. A depiction of the E4 promoter and the position of these regulatory sequences can be seen in FIGS. 2 and 3 of U.S. Patent No. 7,001,596, incorporated by reference herein in its entirety.

[00197] In some embodiments, the adenoviral vector that has the E4 basic promoter substituted with one that has been demonstrated to show tumor specificity, preferably an E2F responsive promoter, and more preferably the human E2F-1 promoter. The reasons for preferring an E2F responsive promoter to drive E4 expression are the same as were discussed above in the context of an E1a adenoviral vector having the E1a promoter substituted with an E2F responsive promoter. The tumor suppressor function of pRb correlates with its ability to repress E2F-responsive promoters such as the E2F-1 promoter (Adams, P. D., and W. G. Kaelin, Jr. 1995, *Cancer Biol.* 6:99–108; Sellers, W. R., and W. G. Kaelin. 1996, published erratum appears in *Biochim Biophys Acta* 1996 Dec. 9; 1288(3):E-1, *Biochim Biophys Acta.* 1288:M1–5. Sellers, W. R., J. W. Rodgers, and W. G. Kaelin, Jr. 1995, *Proc Natl Acad Sci USA.* 92:11544–8.) The human E2F-1 promoter has been extensively characterized and shown to be responsive to the pRb signaling pathway, including pRb/p107, E2F-1/-2/-3, and G1 cyclin/cdk complexes, and E1A (Johnson, D. G., K. Ohtani, and J. R. Nevins. 1994, *Genes Dev.* 8:1514–25; Neuman, E., E. K. Flemington, W. R. Sellers, and W. G. Kaelin, Jr. 1995. *Mol Cell Biol.* 15:4660; Neuman, E., W. R. Sellers, J. A. McNeil, J. B. Lawrence, and W. G. Kaelin, Jr. 1996, *Gene.* 173:163–9.) Most, if not all, of this regulation has been attributed to the presence of multiple E2F sites present within the E2F-1 promoter. Hence, a virus carrying this (these) modification(s) would be expected to be attenuated in normal cells that contain an intact (wild type) pRb pathway, yet exhibit a normal infection/replication profile in cells that are deficient for pRb's repressive function. In order to maintain the normal infection/replication profile of this mutant virus we have retained the inverted terminal repeat (ITR) at the distal end of the E4 promoter as this contains all of the regulatory elements that are required for viral DNA replication (Hatfield, L. and P. Hearing. 1993, *J. Virol.* 67:3931–9; Rawlins, D. R., P. J. Rosenfeld, R. J. Wides, M. D. Challberg, and T. J. Kelly, Jr. 1984, *Cell.* 37:309–19; Rosenfeld, P. J., E. A. O'Neill, R. J. Wides, and T. J. Kelly. 1987, *Mol Cell Biol.* 7:875–86; Wides, R. J., M. D. Challberg, D. R. Rawlins,

and T. J. Kelly. 1987, *Mol Cell Biol.* 7:864–74). This facilitates attaining wild type levels of virus in pRb pathway deficient tumor cells infected with this virus.

[00198] In some embodiments, the E4 promoter is positioned near the right end of the viral genome and it governs the transcription of multiple open reading frames (ORFs) (Freyer, G. A., Y. Katoh, and R. J. Roberts. 1984, *Nucleic Acids Res.* 12:3503–19; Tigges, M. A., and H. J. Raskas. 1984. Splice junctions in adenovirus 2 early region 4 mRNAs: multiple splice sites produce 18 to 24 RNAs. *J. Virol.* 50:106–17; Virtanen, A. P. Gilardi, A. Naslund, J. M. LeMoullec, U. Pettersson, and M. Perricaudet. 1984, *J. Virol.* 51:822–31.) A number of regulatory elements have been characterized in this promoter that mediate transcriptional activity (Berk, A. J. 1986, *Annu Rev Genet.* 20:45–79; Gilardi, P., and M. Perricaudet. 1986, *Nucleic Acids Res.* 14:9035–49; Gilardi, P., and M. Perricaudet. 1984, *Nucleic Acids Res.* 12:7877–88; Hanaka, S., T. Nishigaki, P. A. Sharp, and H. Handa. 1987, *Mol Cell Biol.* 7:2578–87; Jones, C., and K. A. Lee. 1991, *Mol Cell Biol.* 11:4297–305; Lee, K. A., and M. R. Green. 1987, *Embo J.* 6:1345–53.) In addition to these sequences, the E4 promoter region contains elements that are involved in viral DNA replication (Hatfield, L., and P. Hearing. 1993, *J Virol.* 67:3931–9; Rawlins, D. R., P. J. Rosenfeld, R. J. Wides, M. D. Challberg, and T. J. Kelly, Jr. 1984, *Cell.* 37:309–19; Rosenfeld, P. J., E. A. O'Neill, R. J. Wides, and T. J. Kelly. 1987, *Mol Cell Biol.* 7:875–86; Wides, R. J., M. D. Challberg, D. R. Rawlins, and T. J. Kelly. 1987, *Mol Cell Biol.* 7:864–74.) A depiction of the E4 promoter and the position of these regulatory sequences can be seen in FIGS. 1 and 2. See, also, Jones, C., and K. A. Lee. *Mol Cell Biol.* 11:4297–305 (1991). With these considerations in mind, an E4 promoter shuttle was designed by creating two novel restriction endonuclease sites: a XhoI site at nucleotide 35,576 and a SpeI site at nucleotide 35,815 (see FIG. 3). Digestion with both XhoI and SpeI removes nucleotides from 35,581 to 35,817. This effectively eliminates bases –208 to +29 relative to the E4 transcriptional start site, including all of the sequences that have been shown to have maximal influence on E4 transcription. In particular, this encompasses the two inverted repeats of E4F binding sites that have been demonstrated to have the most significant effect on promoter activation. However, all three Sp1 binding sites, two of the five ATF binding sites, and both of the NF1 and NFIII/Oct-1 binding sites that are critical for viral DNA replication are retained.

[00199] In some embodiments, the E2F responsive promoter is the human E2F-1 promoter. Key regulatory elements in the E2F-1 promoter that mediate the response to the pRb pathway have

been mapped both in vitro and in vivo (Johnson, D. G., K. Ohtani, and J. R. Nevins. 1994, *Genes Dev.* 8:1514–25; Neuman, E., E. K. Flemington, W. R. Sellers, and W. G. Kaelin, Jr. 1995, *Mol Cell Biol.* 15:4660; Parr, M. J., Y. Manome, T. Tanaka, P. Wen, D. W. Kufe, W. G. Kaelin, Jr., and H. A. Fine. 1997, *Nat Med.* 3:1145–9.) Thus, we isolated the human E2F-1 promoter fragment from base pairs –218 to +51, relative to the transcriptional start site, by PCR with primers that incorporated a SpeI and XhoI site into them. This creates the same sites present within the E4 promoter shuttle and allows for direct substitution of the E4 promoter with the E2F-1 promoter.

F. NUCLEIC ACID MOLECULES ENCODING MUTANT IL-2

[00200] In some embodiments the subject IL-2 mutein, either alone or as a part of a chimeric polypeptide, such as those described above, can be obtained by expression of a nucleic acid molecule. Just as IL-2 muteins can be described in terms of their identity with wild-type IL-2 polypeptides, the nucleic acid molecules encoding them will necessarily have a certain identity with those that encode wild-type IL-2. For example, the nucleic acid molecule encoding a subject IL-2 mutein can be at least 50%, at least 65%, preferably at least 75%, more preferably at least 85%, and most preferably at least 95% (e.g., 99%) identical to the nucleic acid encoding wild-type IL-2 (e.g., SEQ ID NO:2).

[00201] The nucleic acid molecules provided can contain naturally occurring sequences, or sequences that differ from those that occur naturally, but, due to the degeneracy of the genetic code, encode the same polypeptide. These nucleic acid molecules can consist of RNA or DNA (for example, genomic DNA, cDNA, or synthetic DNA, such as that produced by phosphoramidite-based synthesis), or combinations or modifications of the nucleotides within these types of nucleic acids. In addition, the nucleic acid molecules can be double-stranded or single-stranded (i.e., either a sense or an antisense strand).

[00202] The nucleic acid molecules are not limited to sequences that encode polypeptides; some or all of the non-coding sequences that lie upstream or downstream from a coding sequence (e.g., the coding sequence of IL-2) can also be included. Those of ordinary skill in the art of molecular biology are familiar with routine procedures for isolating nucleic acid molecules. They can, for example, be generated by treatment of genomic DNA with restriction endonucleases, or by

performance of the polymerase chain reaction (PCR). In the event the nucleic acid molecule is a ribonucleic acid (RNA), molecules can be produced, for example, by *in vitro* transcription.

[00203] Exemplary isolated nucleic acid molecules of the present disclosure can include fragments not found as such in the natural state. Thus, this disclosure encompasses recombinant molecules, such as those in which a nucleic acid sequence (for example, a sequence encoding a mutant IL-2) is incorporated into a vector (e.g., a plasmid or viral vector) or into the genome of a heterologous cell (or the genome of a homologous cell, at a position other than the natural chromosomal location).

[00204] As described above, the subject IL-2 mutein may exist as a part of a chimeric polypeptide. In addition to, or in place of, the heterologous polypeptides described above, a subject nucleic acid molecule can contain sequences encoding a “marker” or “reporter.” Examples of marker or reporter genes include β -lactamase, chloramphenicol acetyltransferase (CAT), adenosine deaminase (ADA), aminoglycoside phosphotransferase (neo^r , G418^r), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding β -galactosidase), and xanthine guanine phosphoribosyltransferase (XGPRT). One of skill in the art will be aware of additional useful reagents, for example, of additional sequences that can serve the function of a marker or reporter.

[00205] The subject nucleic acid molecules can be obtained by introducing a mutation into IL-2-encoding DNA obtained from any biological cell, such as the cell of a mammal. Thus, the subject nucleic acids (and the polypeptides they encode) can be those of a mouse, rat, guinea pig, cow, sheep, horse, pig, rabbit, monkey, baboon, dog, or cat. In one embodiment, the nucleic acid molecules will be those of a human.

G. CHIMERIC ANTIGEN RECEPTORS (CARS)

[00206] Targeted immunotherapy has emerged as promising field of research in the treatment of malignancies and has received a great deal of interest in recent years. Indeed, cures have been reported of lymphoma patients with engineered or genetically modified T cells targeting CD19 malignant cells. This has increased the focus towards antigens present on cancer cells as targets for gene- and immunotherapy. These CARS can be used to target or deliver the IL-2 muteins described herein to the tumor, or even allow for systemic IL-2 mutein expression. In some embodiments, the IL-2 mutein is any IL-2 mutein or variant disclosed herein. In some

embodiments, the IL-2 mutein sequence is 90% identical to any one of SEQ ID NO:2 or SEQ ID NO:6 through SEQ ID NO:10 or SEQ ID NO:16. In some embodiments, the IL-2 mutein includes any one of 5-1 SEQ ID NO:5; 5-2 SEQ ID NO:6; 6-6 SEQ ID NO:7; A2 SEQ ID NO:8; B1 SEQ ID NO:9; B11 SEQ ID NO:10; C5 SEQ ID NO:11; D10 SEQ ID NO:12; E10 SEQ ID NO:13; G8 SEQ ID NO:14; H4 SEQ ID NO:15; and H9 SEQ ID NO:5. In some embodiments, the substitutions in the IL-2 mutein comprise L80F, R81D, L85V, I86V, and I92F, numbered in accordance with wild-type human IL-2 of SEQ ID NO:2.

[00207] Genetic manipulation of autologous or allogeneic T cells or NK cells to specifically target a particular tumor antigen provides a strategy to bypass the failure of cytotoxic immune response induction by most tumor cells. In some embodiments, these genetically manipulated T-cells or NK cells can be used to target the IL-2 muteins described herein to the tumor, for example, so that the IL-2 mutein is expressed at the tumor location. These technologies are based on the genetic modification of human immune cells, where the cells may be extracted from a patient or donor by leukapheresis. Specific cells, usually T-cells, are purified and engineered to express a receptor targeting a cancer antigen of interest. Engineering may utilize transduction by retroviral, lentiviral, transposon, mRNA electroporation, and the like. The immune cells may be expanded to the desired dose, and introduced into a patient. The engineered cells can specifically kill cancer cells through cell-mediated toxicity (cytotoxic T-cells) and/or eliciting an immune response to the cancer cell by immune recognition of tumor, cytokine release and immune cell recruitment.

[00208] For example, the application of chimeric antigen receptors (CAR) for immunogene therapy of malignant tumors is a promising strategy in which an antibody or ligand binding domain is fused with the zeta signaling chain of the T cell receptor. The resulting CAR immune cells are redirected by the neospecificity to attack tumors expressing the surface antigen or receptors recognized by the gene-modified T cell receptors and provide cellular therapy that attacks the tumor through normal host immune response in a highly regulated fashion. These cells are free to circulate throughout the brain and systemic circulation, making the need for colocalization and bioavailability less of a problem.

[00209] A number of generations of CAR immune cells have been developed. CARs are created by the fusion of a tumour-specific scFv antibody or other extracellular ligand binding domain to

either the TCR-associated CD3 ζ signalling domain or another intracellular signalling domains from co-stimulatory protein receptors. This structure allows CARs to have the tumor specificity of the B cell antigen receptor, and to activate T cells through the T cell antigen receptor independently of MHC binding. The first-generation CAR contained one intracellular signalling domain, typically with the CD3 ζ signalling domain to allow for TCR signalling. Second-generation CARs have two intracellular signalling domains: a co-stimulatory domain comprising either a CD28 or a 4-1BB signalling domain, coupled with a CD3 ζ signalling domain. This arrangement enables T-cell activation and proliferation upon antigen recognition by the scFv region of the CAR. The third-generation CARs have two co-stimulatory domains and a CD3 ζ signalling domain. The first co-stimulatory domain is either a CD28 or a 4-1BB domain, with the second co-stimulatory domain consisting of either a CD28, a 4-1BB or a OX40 domain. Fourth-generation “armoured CAR T cells” combine a second-generation CAR with the addition of various genes, including cytokine and co-stimulatory ligands, to enhance the tumoricidal effect of the CAR T cells. See, for example, Batlevi et al. (2016) Nature Reviews Clinical Oncology 13:25-40. See also, US Patent No. 7,741,465 and International Patent Publication No. WO2014127261; all of which are incorporated by reference herein in their entireties.

[00210] Alternative approaches to T cell targeting include T cell antigen couplers, as described in International application WO2015/117229, entitled “Trifunctional T cell antigen Coupler and Methods and Uses thereof”, herein specifically incorporated by reference. The T cell antigen coupler system comprises three linked domains: a target-specific polypeptide ligand; a ligand that binds a protein associated with the TCR complex, for example an scFv binding to CD3 (TCR, T-cell receptor) to stimulate T cell activation; and a T cell receptor signaling domain, for example a CD4 transmembrane and intracellular domain to amplify T cell activation. By stimulating T cell activation through the TCR, TACs were engineered to work with the T cell’s essential molecular machinery.

[00211] Antibody coupled T cell receptors are another approach to T cell targeting. ACTRs are a hybrid approach to CARs and the established monoclonal antibody oncology therapeutics. ACTRs are composed of a typical CAR construct that can bind the heavy chain of an antibody through a high-affinity variant of the Fc receptor CD16. ACTR-T cells can target tumours by binding a ligand targeted to a specific cancer antigen. T cell activation is performed by the CAR module.

[00212] Bispecific T cell exchangers (BiTEs) are bispecific antibodies that can bind the TCR of T cells and target tumour cells through two modules: a cancer targeting ligand; and a CD3-binding scFv domain that bridges T cells to the tumor.

[00213] Targeted therapies have been developed against IL13R α 2, including bacterial toxins conjugated to IL13, nanoparticles, oncolytic virus, as well as immunotherapies using monoclonal antibodies, IL13R α 2-pulsed dendritic cells, and IL13R α 2-targeted chimeric antigen receptors (see Kahlon et al. (2004) *Cancer Research*. 64(24):9160–9166; Kong et al. (2012) *Clinical Cancer Research*. 18(21):5949–5960; Thaci et al. (2014) *Neuro-Oncology*; and clinical trials NCT02208362, NCT00730613 and NCT01082926). In some embodiments, these targeted therapies can be used to deliver the IL-2 muteins to the tumor.

[00214] Biologicals that provide for selective alteration of IL-13 activity are of interest for a number of therapeutic purposes, including the treatment of certain cancers with by engineering of T cell specificities. The present invention addresses this issue.

[00215] Methods and compositions are provided for enhancing anti-tumor immune effector cells, e.g. T cells, NK cells, etc. with targeted compositions, including without limitation chimeric antigen receptors (CARs); T cell antigen couplers (TACs); antibody coupled T cell receptors (ACTRs); and bispecific T cell exchangers (BiTEs), where an IL-13 or IL-4 superkine provides the target-specific ligand. In further embodiments, the immune effector cell expresses an IL-2 mutein.

[00216] Immune cell targeting or expression constructs comprising IL-2 superkine sequences are provided and can include any IL-2 sequence as described herein. Superkines are useful for targeting immune cells to cells, e.g. tumor cells, expressing the at least one receptor. In some embodiments, the IL-2 mutein is any IL-2 mutein or variant disclosed herein. In some embodiments, the IL-2 mutein sequence is 90% identical to any one of SEQ ID NO:2 or SEQ ID NO:6 through SEQ ID NO:10 or SEQ ID NO:16. In some embodiments, the IL-2 mutein includes any one of 5-1 SEQ ID NO:5; 5-2 SEQ ID NO:6; 6-6 SEQ ID NO:7; A2 SEQ ID NO:8; B1 SEQ ID NO:9; B11 SEQ ID NO:10; C5 SEQ ID NO:11; D10 SEQ ID NO:12; E10 SEQ ID NO:13; G8 SEQ ID NO:14; H4 SEQ ID NO:15; and H9 SEQ ID NO:5. In some embodiments, the substitutions in the IL-2 mutein comprise L80F, R81D, L85V, I86V, and I92F, numbered in accordance with wild-type human IL-2 of SEQ ID NO:2.

[00217] The IL-2 superkine or mutein component of the construct may be at least about 50 amino acids in length, at least about 75, at least about 100, at least about 110, at least about 115 amino acids in length, up to the full-length of the wild-type protein at the transmembrane domain, i.e. about 116 amino acids in length. For example, the superkine or mutein may be fused to the hinge, transmembrane or signaling domains of a CAR. Exemplary polypeptide sequences are provided

[00218] Included as superkines or muteins are amino acid and nucleic acid coding sequences that are 90%, 95%, 98% or 99% identical to these sequences, longer sequences that comprise those sequences but also include additional nucleotides at the 3' or 5' end, for example any number of additional nucleotides or codons, such as 3, 6, 9, 12 or more nucleotides, or up to about 12, 20, 50 or 100 additional nucleotides, and any sequence that encodes the same amino acid sequence as these nucleic acids due to the degeneracy of the genetic code. In particular, sequences that are codon optimized (CO) for expression by the desired host are contemplated as part of the invention. In some embodiments, the amino acid sequence is 90% identical. In some embodiments, the amino acid sequence is 95% identical. In some embodiments, the amino acid sequence is 98% identical. In some embodiments, the amino acid sequence is 99% identical. In some embodiments, the polypeptide is linked to an IL-2 superkine immune cell targeting or expression construct. In some embodiments, an IL-2 superkine immune cell targeting or expression construct comprises one or more signaling domains derived from CD3- ζ , CD28, DAP10, OX-40, ICOS and CD137. In some embodiments, an IL-2 superkine immune cell targeting or expression construct or expression comprises one or more signaling domains derived from CD3- ζ . In some embodiments, an IL-2 superkine immune cell targeting or expression construct comprises one or more signaling domains derived from CD28. In some embodiments, an IL-2 superkine immune cell targeting or expression construct comprises one or more signaling domains derived from DAP10. In some embodiments, an IL-2 superkine immune cell targeting or expression construct comprises one or more signaling domains derived from OX-40. In some embodiments, an IL-2 superkine immune cell targeting or expression construct comprises one or more signaling domains derived from CD137. In some embodiments, an IL-2 superkine immune cell targeting or expression construct comprises an IL-2 variant/IL-2 superkine including those provided herein. In some embodiments, an IL-2 superkine immune cell targeting or expression

construct comprises an IL-2 variant/IL-2 superkine including those provided in SEQ ID NO:2 through SEQ ID NO:38.

1. NK cells

[00219] In some embodiments the immune cells are natural killer (NK) cells. NK cells recognize infected or transformed cells through multiple cell surface receptors including NKG2D, CD16, and natural cytotoxicity receptors (NCRs) such as NKp44, NKp46, and NKp30. These receptors activate signaling adapter proteins such as DAP10, DAP12, and CD3 ζ , which contain immuno-tyrosine activation motifs (ITAMs) that initiate the release of cytolytic granules containing perforin and granzymes, as well as mediate production and release of cytokines and chemokines such as IFN- γ and TNF- α . Importantly, NK cell-mediated cytotoxicity does not rely on the presentation of self HLA. Therefore, NK cells hold significant clinical interest as a cell-based therapy for cancer because of their ability to be used in an allogeneic setting and potentially provide an off-the-shelf cellular product.

[00220] Natural killer cells provide an alternative to the use of T cells for adoptive immunotherapy since they do not require HLA matching, so can be used as allogeneic effector cells. Clinical trials of adoptively transferred allogeneic NK cells demonstrate these cells can survive in patients for several weeks to months. Additionally, expression of CARs in NK cells allow these cells to more effectively kill solid tumors that are often resistant to NK cell-mediated activity compared to hematologic malignancies (especially acute myelogenous leukemia) that are typically more NK cell-sensitive. CARs useful in NK cell targeting include, for example, first generation CAR constructs that contain CD3 ζ as the sole signaling domain. Second and third generation CARs are also useful in NK cells. In some embodiments the ectodomain of NKG2D, an NK cell activation receptor, is linked directly to CD3 ζ .

[00221] NK cells for modification include cell lines, or peripheral blood NK cells, which can be isolated from donors through simple blood draws or by apheresis if larger numbers of cells are needed. Activated PB-NK cells express a wider range of activating receptors, such as CD16, NKp44, and NKp46 as well as KIRs, which play an important role in NK cell licensing. In addition, PB-NK cells can be given without irradiating the cells so have the ability to expand *in vivo*. Another source of NK cells suitable for CAR expression are NK cells derived from human pluripotent stem cells – both induced pluripotent stem cells (iPSCs) or human embryonic stem

cells (hESCs). These NK cells display a similar phenotype to PB-NK cells, and hESC/iPSC-NK cells can be grown on a clinical scale.

2. Chimeric Antigen Receptors (CARs)

[00222] In addition to the superkine sequence, CARs contain the signaling domain for CD3 ζ and the signaling domains of one or more costimulatory receptors that further promote the recycling, survival and/or expansion of immune cells expressing the CARs. The signaling domains of the costimulatory receptors are the intracellular portions of each receptor protein that generate the activating signal in the cell. Examples are amino acids 180-220 of the native CD28 molecule and amino acids 214-255 of the native 4-1BB molecule.

[00223] Examples of suitable hinge and transmembrane regions to link the superkine to the signaling region may include without limitation the constant (Fc) regions of immunoglobins, human CD8a, and artificial linkers that serve to move the targeting moiety away from the cell surface for improved access to and binding on target cells. Examples of suitable transmembrane domains include the transmembrane domains of the leukocyte CD markers, preferably that of CD4 or CD28. Examples of intracellular receptor signaling domains include the T cell antigen receptor complex, preferably the zeta chain of CD3, however any transmembrane region sufficient to anchor the CAR in the membrane can be used. Persons of skill are aware of numerous transmembrane regions and the structural elements (such as lipophilic amino acid regions) that produce transmembrane domains in numerous membrane proteins and therefore can substitute any convenient sequence. T cell costimulatory signaling receptors suitable for improving the function and activity of CAR-expressing cells include, but are not limited to, CD28, CD137, and OX-40.

[00224] Signaling via CD28 is required for IL2 production and proliferation, but does not play a primary role in sustaining T cell function and activity. CD137 (a tumor necrosis factor-receptor family member expressed following CD28 activation) and OX-40 are involved in driving long-term survival of T cells, and accumulation of T cells. The ligands for these receptors typically are expressed on professional antigen presenting cells such as dendritic cells and activated macrophages, but not on tumor cells. Expressing a CAR that incorporates CD28 and/or 4-1BB signaling domains in CD4⁺ T cells enhances the activity and anti-tumor potency of those cells

compared to those expressing a CAR that contains only the CD3 ζ signaling domain, which constructs may be referred to as second or third generation CARs.

[00225] Included as CAR constructs of interest are tandem CARs, e.g. see Hegde et al. (2016) *J. Clin. Invest* 126(8):3036-3052, herein specifically incorporated by reference. In such constructs a binding moiety for a tumor specific antigen is combined in tandem with an IL-13 superkine. The binding moiety may be, for example, an scFv specific for a tumor cell antigen, including without limitation HER-2, EGFR, CD20, etc. as known in the art.

[00226] In various embodiments, the antigen binding domain binds to an antigen on a target cell, e.g., a cancer cell. The antigen binding domain can bind an antigen, such as but not limited to a tumor target antigen. In some case, the antigen binding domain binds one or more antigens. Exemplary antigen binding domains can bind to an antigen including, but not limited to, D19; CD123; CD22; CD30; CD171; CS-1 (also referred to as CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); CD33; epidermal growth factor receptor variant III (EGFRvIII); ganglioside G2 (GD2); ganglioside GD3; TNF receptor family member B cell maturation (BCMA); Tn antigen ((Tn Ag) or (GalNAc α Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms-Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2 (IL-13R α 2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha; Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp 100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-

3)bdGalp(1-4)bdGlc(1-1)Cer); transglutaminase 5 (TGS5); high molecular weight-melanoma-associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); Folate receptor beta; tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein-coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostein; surviving; telomerase; prostate carcinoma tumor antigen-1 (PCTA-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MART 1); Rat sarcoma (Ras) mutant; human telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); cytochrome P450 1B1 (CYP1B1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal ubiquitous 1 (RU1); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a;

CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1).

[00227] In some embodiments, the antigen binding domain comprises a monoclonal antibody, a polyclonal antibody, a synthetic antibody, a human antibody, a humanized antibody, a non-human antibody, a nanobody, a single-chain variable fragment (scFv), F(ab')₂, Fab', Fab, Fv, and the like. The antigen binding domain can be linked to the transmembrane domain of the CAR. In some embodiments, a nucleic acid encoding the antigen binding domain is operably linked to a nucleic acid encoding a transmembrane domain of the CAR.

[00228] In some embodiments, the transmembrane domain can be derived from a membrane-bound or transmembrane protein. In certain embodiments, the transmembrane domain comprises one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8 or more amino acid modifications (e.g., substitutions, insertions, and deletions) compared to the wild-type amino acid sequence of the transmembrane domain of the membrane-bound or transmembrane protein. Non-limiting examples of a transmembrane domain of a CAR include at least the transmembrane region(s) of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon (CD3 ξ), CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, or an erythropoietin receptor. In some embodiments, the transmembrane domain includes a human immunoglobulin (Ig) hinge region, e.g., an IgG4Fc hinge. In other embodiments, the transmembrane domain is a recombinant or synthetic domain comprising hydrophobic amino acid residues (e.g., leucine and valine). In some cases, the transmembrane domain includes a phenylalanine, tryptophan and valine at one or both ends of the domain.

[00229] The transmembrane domain links the antigen binding domain to the intracellular signaling domain of the CAR. In some embodiments, the nucleic acid encoding the antigen binding domain is operably linked to the nucleic acid encoding the transmembrane domain that is operably linked to the nucleic acid encoding the intracellular signaling domain.

[00230] In some embodiments, the intracellular signaling domain of a CAR comprises a signal activation or signal transduction domain. As such, an intracellular signaling domain includes any portion of an intracellular signaling domain of a protein sufficient to transduce or transmit a signal, e.g., an activation signal or to mediate a cellular response within a cell. Non-limiting examples include TCR, CD2, CD3 zeta, CD3 gamma, CD3 delta, CD3 epsilon, CD7, CD27, CD86, common FcR gamma, FcR beta, CD79a, CD79b, Fc gamma RIIa, DAP10, DAP12, T cell receptor (TCR), CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, CDS, ICAM-1, GITR, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), CD127, CD160, CD19, CD4, CD8alpha, CD8 beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, any derivative, variant, or fragment thereof. In certain embodiments, the intracellular signaling domain comprises an intracellular domain of a co-stimulatory molecule such as from CD3, CD27, CD28, CD127, ICOS, 4-1BB (CD137), PD-1, T cell receptor (TCR), any derivative thereof, or any variant thereof. In some embodiments, the intracellular signaling domain of the CAR is selected from the group consisting of a MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a signaling lymphocytic activation molecule (SLAM protein), an activating NK cell receptor, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CD11a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229),

CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83.

3. BiTES

[00231] Bi-specific T-cell engagers (BiTES) are fusion proteins comprising an IL-13 superkine fused to an antibody variable region that specifically binds to CD3. In some embodiments the antibody variable region is in a single-chain variable fragment (scFv). The superkine may be fused to the variable region through a linker. An Fc region is optionally provided.

4. TACs

[00232] A TAC construct comprises an IL-2 superkine fused to a ligand that binds a protein associated with the TCR complex; fused to a T cell receptor signaling domain polypeptide. The domains may be separated by linkers. The protein associated with the TCR complex may be CD3. The ligand that binds a protein associated with the TCR complex may be a single chain antibody. The ligand that binds a protein associated with the TCR complex may be UCHT1, or a variant thereof. The T cell receptor signaling domain polypeptide may comprise a cytosolic domain and a transmembrane domain. The cytosolic domain may be a CD4 cytosolic domain and the transmembrane domain is a CD4 transmembrane domain.

5. ACTRs

[00233] ACTRs are a hybrid approach to CARs and the established monoclonal antibody oncology therapeutics. ACTRs are composed of a typical CAR construct that can bind the heavy chain of an antibody through a high-affinity variant of the Fc receptor CD16. A superkine is fused to a moiety recognized by the CAR, which may include, without limitation, an Fc region of an antibody with high affinity for CD16.

[00234] An immune cell targeting or expression construct coding sequence can be produced by any means known in the art, including recombinant DNA techniques. Nucleic acids encoding the several regions of the chimeric receptor can be prepared and assembled into a complete coding sequence by standard techniques of molecular cloning known in the art (genomic library screening, PCR, primer-assisted ligation, site-directed mutagenesis, etc.) as is convenient. The resulting coding region may be inserted into an expression vector and used to transform a

suitable expression host cell line, e.g. a population of allogeneic or autologous T lymphocytes, allogeneic or autologous NK cells, including primary cultures, cell lines, iPSC derived cells, etc. The methods can be used on cells in vitro (e.g., in a cell-free system), in culture, e.g. in vitro or ex vivo. For example, IL-2 superkine CAR-expressing cells can be cultured and expanded in vitro in culture medium.

[00235] An non-IL-2 superkine immune cell targeting or expression construct can also be used specifically direct immune cells to target specific tumor cells. Anti-tumor effector cells, e.g. CD4⁺ or CD8⁺ effector T cells, are generated to be re-directed to recognize such tumor cells by introducing into the T cells an superkine immune cell targeting or expression construct comprising one or more signaling domains derived from CD3- ζ , CD28, DAP10, OX-40, ICOS and CD137. In some embodiments, the cells can further comprise a transgene capable of expressing an IL-2 mutein as described herein. An IL-2 superkine immune cell targeting or expression construct can specifically direct immune cells to target IL-2R expressing cell, including tumor cells. Anti-tumor effector cells, e.g. CD4⁺ or CD8⁺ effector T cells, are generated to be re-directed to recognize such tumor cells by introducing into the T cells an IL-2 superkine immune cell targeting or expression construct comprising one or more signaling domains derived from CD3- ζ , CD28, DAP10, OX-40, ICOS and CD137.

[00236] The IL-2 superkine immune cell targeting or expression construct is infected or transfected into human immune cells, e.g. using a non-viral plasmid vector and electroporation methods; a viral vector and infection methods, etc. as known in the art. A CAR comprising co-stimulatory signaling domains may enhance the duration and/or retention of anti-tumor activity in a manner that can significantly improve the clinical efficacy of adoptive therapy protocols. CD4⁺ and CD8⁺ T cell effector functions, and NK cell functions can be triggered via these receptors, therefore these cell types are contemplated for use with the invention. CD8⁺ T cells expressing the IL13 superkine CARs of this invention may be used to lyse target cells and to produce IL-2 in the presence of target cells, among the other functions of these cells. Expression of the appropriate costimulatory CAR in either or both CD4⁺ and CD8⁺ T cells is used to provide the most effective population of cells for adoptive immunotherapy, consisting therefore of either or both professional helper and killer T cells that exhibit enhanced and/or long term viability and anti-tumor activity. In some embodiments, an IL-2 superkine immune cell targeting or expression construct comprises an IL-2 variant/IL-2 superkine including those provided in

Figure 2. In some embodiments, an IL-2 superkine immune cell targeting or expression construct comprises an IL-2 variant/IL-2 superkine including any of those provided herein.

[00237] Polypeptides of the present invention can be further modified, *e.g.*, joined to a wide variety of other oligopeptides or proteins for a variety of purposes. For example, post-translationally modified, for example by prenylation, acetylation, amidation, carboxylation, glycosylation, pegylation, *etc.* Such modifications can also include modifications of glycosylation, *e.g.* those made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in further processing steps; *e.g.* by exposing the polypeptide to enzymes which affect glycosylation, such as mammalian glycosylating or deglycosylating enzymes.

[00238] Methods which are well known to those skilled in the art can be used to construct T cell targeting construct expression vectors containing coding sequences and appropriate transcriptional/translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. Alternatively, RNA capable of encoding the polypeptides of interest may be chemically synthesized. One of skill in the art can readily utilize well-known codon usage tables and synthetic methods to provide a suitable coding sequence for any of the polypeptides of the invention. The nucleic acids may be isolated and obtained in substantial purity. Usually, the nucleic acids, either as DNA or RNA, will be obtained substantially free of other naturally-occurring nucleic acid sequences, generally being at least about 50%, usually at least about 90% pure and are typically “recombinant,” *e.g.*, flanked by one or more nucleotides with which it is not normally associated on a naturally occurring chromosome. The nucleic acids of the invention can be provided as a linear molecule or within a circular molecule, and can be provided within autonomously replicating molecules (vectors) or within molecules without replication sequences. Expression of the nucleic acids can be regulated by their own or by other regulatory sequences known in the art. The nucleic acids of the invention can be introduced into suitable host cells using a variety of techniques available in the art.

[00239] According to the present invention, immune cell targeting or expression construct vectors and immune cell targeting or expression construct modified cells can be provided in pharmaceutical compositions suitable for therapeutic use, *e.g.* for human treatment. In some

embodiments, pharmaceutical compositions of the present invention include one or more therapeutic entities of the present invention or pharmaceutically acceptable salts, esters or solvates thereof. In some other embodiments, pharmaceutical compositions of the present invention include one or more therapeutic entities of the present invention in combination with another therapeutic agent, *e.g.*, another anti-tumor agent.

[00240] Therapeutic entities of the present invention are often administered as pharmaceutical compositions comprising an active therapeutic agent and another pharmaceutically acceptable excipient. Such formulations can include one or more non-toxic pharmaceutically acceptable carriers, diluents, excipients and/or adjuvants. The preferred form depends on the intended mode of administration and therapeutic application. The compositions can also include, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers or diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological phosphate-buffered saline, Ringer's solutions, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may also include other carriers, adjuvants, or nontoxic, nontherapeutic, nonimmunogenic stabilizers and the like.

[00241] In still some other embodiments, pharmaceutical compositions of the present invention can also include large, slowly metabolized macromolecules such as proteins, polysaccharides such as chitosan, polylactic acids, polyglycolic acids and copolymers (such as latex functionalized SepharoseTM, agarose, cellulose, and the like), polymeric amino acids, amino acid copolymers, and lipid aggregates (such as oil droplets or liposomes).

[00242] The maximum tolerated dose (MTD) of CAR immune cells may be determined during clinical trial development, for example at up to about 10^4 T cells/kg of body weight, up to about 10^5 cells/kg of body weight, up to about 10^6 cells/kg of body weight, up to about 5×10^6 cells/kg of body weight, up to about 10^7 cells/kg of body weight, up to about 5×10^7 cells/kg of body weight, or more, as empirically determined. In some embodiments, the maximum tolerated dose (MTD) of CAR immune cells is up to about 10^4 T cells/kg of body weight. In some embodiments, the maximum tolerated dose (MTD) of CAR immune cells is up to about 10^5 T cells/kg of body weight. In some embodiments, the maximum tolerated dose (MTD) of CAR

immune cells is up to about 10^6 T cells/kg of body weight. In some embodiments, the maximum tolerated dose (MTD) of CAR immune cells is up to about 10^7 T cells/kg of body weight. In some embodiments, the maximum tolerated dose (MTD) of CAR immune cells is up to about 5×10^6 T cells/kg of body weight. In some embodiments, the maximum tolerated dose (MTD) of CAR immune cells is up to about 5×10^7 T cells/kg of body weight.

[00243] Toxicity of the cells described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, by determining the LD₅₀ (the dose lethal to 50% of the population) or the LD₁₀₀ (the dose lethal to 100% of the population). The dose ratio between toxic and therapeutic effect is the therapeutic index. The data obtained from these cell culture assays and animal studies can be used in formulating a dosage range that is not toxic for use in human. The dosage of the described herein lies preferably within a range of circulating concentrations that include the effective dose with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition.

[00244] After a dose escalation phase, patients in the expansion cohort are treated with immune cells at the MTD. An exemplary treatment regime entails administration once every two weeks or once a month or once every 3 to 6 months. Therapeutic entities of the present invention are usually administered on multiple occasions. Intervals between single dosages can be weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of the therapeutic entity in the patient.

[00245] In prophylactic applications, *e.g.* to maintain remission in a patient, a relatively low dosage may be administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In other therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patent can be administered a prophylactic regime.

[00246] Examples of additional therapeutic agents that can be coadministered and/or coformulated with an immune cell targeting or expression construct include: anti-proliferative, or

cytoreductive therapy, which is used therapeutically to eliminate tumor cells and other undesirable cells in a host, and includes the use of therapies such as delivery of ionizing radiation, and administration of chemotherapeutic agents. Chemotherapeutic agents are well-known in the art and are used at conventional doses and regimens, or at reduced dosages or regimens, including for example, topoisomerase inhibitors such as anthracyclines, including the compounds daunorubicin, adriamycin (doxorubicin), epirubicin, idarubicin, anamycin, MEN 10755, and the like. Other topoisomerase inhibitors include the podophyllotoxin analogues etoposide and teniposide, and the anthracenediones, mitoxantrone and amsacrine. Other anti-proliferative agent interferes with microtubule assembly, *e.g.* the family of vinca alkaloids. Examples of vinca alkaloids include vinblastine, vincristine; vinorelbine (NAVELBINE); vindesine; vindoline; vincamine; *etc.* DNA-damaging agent include nucleotide analogs, alkylating agents, *etc.* Alkylating agents include nitrogen mustards, *e.g.* mechlorethamine, cyclophosphamide, melphalan (L-sarcosine), *etc.*; and nitrosoureas, *e.g.* carmustine (BCNU), lomustine (CCNU), semustine (methyl-CCNU), streptozocin, chlorozotocin, *etc.* Nucleotide analogs include pyrimidines, *e.g.* cytarabine (CYTOSAR-U), cytosine arabinoside, fluorouracil (5-FU), floxuridine (FUdR), *etc.*; purines, *e.g.* thioguanine (6-thioguanine), mercaptopurine (6-MP), pentostatin, fluorouracil (5-FU) *etc.*; and folic acid analogs, *e.g.* methotrexate, 10-propargyl-5,8-dideazafolate (PDDF, CB3717), 5,8-dideazatetrahydrofolic acid (DDATHF), leucovorin, *etc.* Other chemotherapeutic agents of interest include metal complexes, *e.g.* cisplatin (cis-DDP), carboplatin, oxaliplatin, *etc.*; ureas, *e.g.* hydroxyurea; and hydrazines, *e.g.* N-methylhydrazine.

[00247] For example, ionizing radiation (IR) is used to treat about 60% of cancer patients, by depositing energy that injures or destroys cells in the area being treated, and for the purposes of the present invention may be delivered at conventional doses and regimens, or at reduced doses. Radiation injury to cells is nonspecific, with complex effects on DNA. The efficacy of therapy depends on cellular injury to cancer cells being greater than to normal cells. Radiotherapy may be used to treat every type of cancer. Some types of radiation therapy involve photons, such as X-rays or gamma rays. Another technique for delivering radiation to cancer cells is internal radiotherapy, which places radioactive implants directly in a tumor or body cavity so that the radiation dose is concentrated in a small area. A suitable dose of ionizing radiation may range from at least about 2 Gy to not more than about 10 Gy, usually about 5 Gy. A suitable dose of

ultraviolet radiation may range from at least about 5 J/m² to not more than about 50 J/m², usually about 10 J/m². The sample may be collected from at least about 4 and not more than about 72 hours following ultraviolet radiation, usually around about 4 hours.

[00248] Treatment may also be combined with immunoregulatory modulating agents, including an agent that agonizes an immune costimulatory molecule, e.g. CD40, OX40, etc.; and/or (iii) an agent that antagonizes an immune inhibitory molecule, e.g. CTLA-4, PD-1, PD-L1, *etc.* The active agents are administered within a period of time to produce an additive or synergistic effect on depletion of cancer cells in the host. Methods of administration include, without limitation, systemic administration, intra-tumoral administration, etc.

[00249] In some embodiments, an individual cancer is selected for treatment with a combination therapy because the cancer is a cancer type that is responsive to a checkpoint inhibitor, e.g. a PD-1 antagonist, a PD-L1 antagonist, a CTLA4 antagonist, a TIM-3 antagonist, a BTLA antagonist, a VISTA antagonist, a LAG3 antagonist; etc. In some embodiments, such an immunoregulatory agent is a CTLA-4, PD1 or PDL1 antagonist, e.g. avelumab, nivolumab, pembrolizumab, ipilimumab, and the like. In some such embodiments the cancer is, without limitation, melanoma or small cell lung cancer. In some such embodiments, the cancer is a type that has a high neoantigen, or mutagenesis, burden (see Vogelstein et al. (2013) Science 339(6127):1546-1558, herein specifically incorporated by reference).

[00250] In some embodiments, an individual cancer is selected for treatment with a combination therapy of the present invention because the cancer is a cancer type that is responsive to an immune response agonist, e.g. a CD28 agonist, an OX40 agonist; a GITR agonist, a CD137 agonist, a CD27 agonist, an HVEM agonist, etc. In some embodiments, such an immunoregulatory agent is an OX40, CD137, or GITR agonist e.g. tremelimumab, and the like. In some such embodiments the cancer is, without limitation, melanoma or small cell lung cancer. In some such embodiments, the cancer is a type that has a high neoantigen, or mutagenesis, burden.

[00251] In some embodiments, the combination therapy includes an antibody known in the art which binds to PD-1 and disrupt the interaction between the PD-1 and its ligand, PD-L1, and stimulate an anti-tumor immune response. In some embodiments, the antibody or antigen-binding portion thereof binds specifically to PD-1. For example, antibodies that target PD-1 and

which can find used in the present invention include, e.g., but are not limited to nivolumab (BMS-936558, Bristol-Myers Squibb), pembrolizumab (lambrolizumab, MK03475 or MK-3475, Merck), humanized anti-PD-1 antibody JS001 (ShangHai JunShi), monoclonal anti-PD-1 antibody TSR-042 (Tesar, Inc.), Pidilizumab (anti-PD-1 mAb CT-011, Medivation), anti-PD-1 monoclonal Antibody BGB-A317 (BeiGene), and/or anti-PD-1 antibody SHR-1210 (ShangHai HengRui), human monoclonal antibody REGN2810 (Regeneron), human monoclonal antibody MDX-1106 (Bristol-Myers Squibb), and/or humanized anti-PD-1 IgG4 antibody PDR001 (Novartis). In some embodiments, the PD-1 antibody is from clone: RMP1-14 (rat IgG) - BioXcell cat# BP0146. Other suitable antibodies include anti-PD-1 antibodies disclosed in U.S. Patent No. 8,008,449, herein incorporated by reference. In some embodiments, the antibody or antigen-binding portion thereof binds specifically to PD-L1 and inhibits its interaction with PD-1, thereby increasing immune activity. Any antibodies known in the art which bind to PD-L1 and disrupt the interaction between the PD-1 and PD-L1, and stimulates an anti-tumor immune response, are suitable for use in the combination treatment methods disclosed herein. For example, antibodies that target PD-L1 and are in clinical trials, include BMS-936559 (Bristol-Myers Squibb) and MPDL3280A (Genetech). Other suitable antibodies that target PD-L1 are disclosed in U.S. Patent No. 7,943,743, herein incorporated by reference. It will be understood by one of ordinary skill that any antibody which binds to PD-1 or PD-L1, disrupts the PD-1/PD-L1 interaction, and stimulates an anti-tumor immune response, is suitable for use in the combination treatment methods.

[00252] In some embodiments, the combination therapy includes an antibody known in the art which binds CTLA-4 and disrupts its interaction with CD80 and CD86. Exemplary antibodies that target CTLA-4 include ipilimumab (MDX-010, MDX-101, Bristol-Myers Squibb), which is FDA approved, and tremelimumab (ticilimumab, CP-675, 206, Pfizer), currently undergoing human trials. Other suitable antibodies that target CTLA-4 are disclosed in WO 2012/120125, U.S. Patents No. 6,984,720, No. 6,682,7368, and U.S. Patent Applications 2002/0039581, 2002/0086014, and 2005/0201994, herein incorporated by reference. It will be understood by one of ordinary skill that any antibody which binds to CTLA-4, disrupts its interaction with CD80 and CD86, and stimulates an anti-tumor immune response, is suitable for use in the combination treatment methods. In some embodiments, the combination therapy includes an antibody known in the art which binds LAG-3 and disrupts its interaction with MHC class II

molecules. An exemplary antibody that targets LAG-3 is IMP321 (Immutep), currently undergoing human trials. Other suitable antibodies that target LAG-3 are disclosed in U.S. Patent Application 2011/0150892, herein incorporated by reference. It will be understood by one of ordinary skill that any antibody which binds to LAG-3, disrupts its interaction with MHC class II molecules, and stimulates an anti-tumor immune response, is suitable for use in the combination treatment methods.

[00253] In some embodiments, the combination therapy includes an antibody known in the art which binds TIM-3 and disrupts its interaction with galectin 9. Suitable antibodies that target TIM-3 are disclosed in U.S. Patent Application 2013/0022623, herein incorporated by reference. It will be understood by one of ordinary skill that any antibody which binds to TIM-3, disrupts its interaction with galectin 9, and stimulates an anti-tumor immune response, is suitable for use in the combination treatment methods.

[00254] In some embodiments, the combination therapy includes an antibody known in the art which binds 4-1BB/CD137 and disrupts its interaction with CD137L. It will be understood by one of ordinary skill that any antibody which binds to 4-1BB/CD137, disrupts its interaction with CD137L or another ligand, and stimulates an anti-tumor immune response or an immune stimulatory response that results in anti-tumor activity overall, is suitable for use in the combination treatment methods.

[00255] In some embodiments, the combination therapy includes an antibody known in the art which binds GITR and disrupts its interaction with its ligand. It will be understood by one of ordinary skill that any antibody which binds to GITR, disrupts its interaction with GITRL or another ligand, and stimulates an anti-tumor immune response or an immune stimulatory response that results in anti-tumor activity overall, is suitable for use in the combination treatment methods.

[00256] In some embodiments, the combination therapy includes an antibody known in the art which binds OX40 and disrupts its interaction with its ligand. It will be understood by one of ordinary skill that any antibody which binds to OX40, disrupts its interaction with OX40L or another ligand, and stimulates an anti-tumor immune response or an immune stimulatory response that results in anti-tumor activity overall, is suitable for use in the combination treatment methods.

[00257] In some embodiments, the combination therapy includes an antibody known in the art which binds CD40 and disrupts its interaction with its ligand. It will be understood by one of ordinary skill that any antibody which binds to CD40, disrupts its interaction with its ligand, and stimulates an anti-tumor immune response or an immune stimulatory response that results in anti-tumor activity overall, is suitable for use in the combination treatment methods.

[00258] In some embodiments, the combination therapy includes an antibody known in the art which binds ICOS and disrupts its interaction with its ligand. It will be understood by one of ordinary skill that any antibody which binds to ICOS, disrupts its interaction with its ligand, and stimulates an anti-tumor immune response or an immune stimulatory response that results in anti-tumor activity overall, is suitable for use in the combination treatment methods.

[00259] In some embodiments, the combination therapy includes an antibody known in the art which binds CD28 and disrupts its interaction with its ligand. It will be understood by one of ordinary skill that any antibody which binds to CD28, disrupts its interaction with its ligand, and stimulates an anti-tumor immune response or an immune stimulatory response that results in anti-tumor activity overall, is suitable for use in the combination treatment methods.

[00260] In some embodiments, the combination therapy includes an antibody known in the art which binds IFN α and disrupts its interaction with its ligand. It will be understood by one of ordinary skill that any antibody which binds to IFN α , disrupts its interaction with its ligand, and stimulates an anti-tumor immune response or an immune stimulatory response that results in anti-tumor activity overall, is suitable for use in the combination treatment methods.

[00261] An “anti-cancer therapeutic” is a compound, composition, or treatment (e.g., surgery) that prevents or delays the growth and/or metastasis of cancer cells. Such anti-cancer therapeutics include, but are not limited to, surgery (e.g., removal of all or part of a tumor), chemotherapeutic drug treatment, radiation, gene therapy, hormonal manipulation, immunotherapy (e.g., therapeutic antibodies and cancer vaccines) and antisense or RNAi oligonucleotide therapy. Examples of useful chemotherapeutic drugs include, but are not limited to, hydroxyurea, busulphan, cisplatin, carboplatin, chlorambucil, melphalan, cyclophosphamide, Ifosphamide, danorubicin, doxorubicin, epirubicin, mitoxantrone, vincristine, vinblastine, Navelbine.RTM. (vinorelbine), etoposide, teniposide, paclitaxel, docetaxel, gemcitabine, cytosine, arabinoside, bleomycin, neocarzinostatin, suramin, taxol, mitomycin C, Avastin,

Herceptin.RTM., flurouracil, and temozolamide and the like. The compounds are also suitable for use with standard combination therapies employing two or more chemotherapeutic agents. It is to be understood that anti-cancer therapeutics includes novel compounds or treatments developed in the future.

[00262] The pharmaceutical compositions and/or formulations described above include one or more therapeutic entities in an amount effective to achieve the intended purpose. Thus the term “therapeutically effective dose” refers to the amount of the therapeutic entities that ameliorates the symptoms of cancer. Determination of a therapeutically effective dose of a compound is well within the capability of those skilled in the art. For example, the therapeutically effective dose can be estimated initially either in cell culture assays, or in animal models, such as those described herein. Animal models can also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in other animals, including humans, using standard methods known in those of ordinary skill in the art.

[00263] Also within the scope of the invention are kits comprising the compositions of the invention and instructions for use. The kit may further contain a least one additional reagent, e.g. a chemotherapeutic drug, anti-tumor antibody, *etc.* Kits typically include a label indicating the intended use of the contents of the kit. The term label includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit.

[00264] The invention now being fully described, it will be apparent to one of ordinary skill in the art that various changes and modifications can be made without departing from the spirit or scope of the invention. In some embodiments, the kit comprises an IL-2 superkine immune cell targeting or expression construct comprising an IL-2 variant/IL-2 superkine as described herein. In some embodiments, the kit comprises an IL-2 superkine immune cell targeting or expression construct comprising an IL-2 variant/IL-2 superkine including those provided herein. In some embodiments, an IL-2 superkine immune cell targeting or expression construct comprises an IL-2 variant/IL-2 superkine including those provided herein.

6. Exemplary Immune Cell Targeting or Expression Construct Embodiments

[00265] An immune cell targeting or expression construct comprising: an interleukin-2 receptor β (IL-2R β) binding protein, wherein the equilibrium dissociation constant for the IL-2R β of said binding protein is less than that of wild-type human IL-2 (hIL-2); linked to an immune cell targeting or expression construct.

[00266] In some embodiments, the immune cell targeting or expression construct exhibits a cytotoxic effect on a T-cell, for example a CD8⁺ T-cell or a CD4⁺ T-cell.

[00267] In some embodiments, the construct is a chimeric antigen receptor (CAR) and wherein the IL-2 superkine is fused to a transmembrane domain; linked to an intracellular signaling region.

[00268] In some embodiments, the intracellular signaling region comprises a CD3 signaling domain.

[00269] In some embodiments, the intracellular signaling region comprises one or more of a CD28 signaling domain, a CD137 signaling domain, an OX-40 signaling domain, an ICOS signaling domain, a DAP10 signaling domain.

[00270] In some embodiments, the construct is a T cell antigen coupler (TAC), wherein the IL-2 superkine is fused to a ligand that binds a protein associated with the TCR complex; fused to a T cell receptor signaling domain polypeptide.

[00271] In some embodiments, the protein associated with the TCR complex is CD3.

[00272] In some embodiments, the T cell receptor signaling domain polypeptide comprises CD4 cytosolic domain and CD4 transmembrane domain.

[00273] In some embodiments, the construct is an antibody coupled T cell receptors (ACTR), comprising a chimeric antigen receptor component that binds to the IL-2 superkine at a high affinity.

[00274] In some embodiments, the CAR component comprises CD16, and the IL-2 superkine is fused to an Fc sequence.

[00275] In some embodiments, the construct is a bispecific T cell exchanger (BiTE) comprising an IL-2 superkine fused to a variable region of an antibody that binds to a component of a T cell receptor.

[00276] In some embodiments, the BiTE component of a T cell receptor is CD3.

[00277] In some embodiments, the IL-2R β binding protein comprises the following amino acid substitutions: L80F, R81D, L85V, I86V, and I92F, numbered in accordance with wild-type hIL-2.

[00278] In some embodiments, the a nucleic acid encoding and IL-2 described herein is provided. In some embodiments, the vector comprising the nucleic acid is provided.

[00279] In some embodiments, a T cell comprising a construct according to any of the above is provided. In some embodiments, an NK cell comprising a construct according to any of the above is provided. In some embodiments, the T cell is a CD4⁺ T cell. In some embodiments, the T cell is a CD8⁺ T cell.

[00280] Also provided are an isolated population of immune cells described above. Also provided are pharmaceutical formulations comprising the immune cell population described above.

H. EXPRESSION OF MUTANT IL-2 GENE PRODUCTS

[00281] The nucleic acid molecules described above can be contained within a vector that is capable of directing their expression in, for example, a cell that has been transduced with the vector. Accordingly, in addition to the subject IL-2 muteins, expression vectors containing a nucleic acid molecule encoding a subject IL-2 mutein and cells transfected with these vectors are among the preferred embodiments.

[00282] It should of course be understood that not all vectors and expression control sequences will function equally well to express the DNA sequences described herein. Neither will all hosts function equally well with the same expression system. However, one of skill in the art may make a selection among these vectors, expression control sequences and hosts without undue experimentation. For example, in selecting a vector, the host must be considered because the vector must replicate in it. The vector's copy number, the ability to control that copy number, and the expression of any other proteins encoded by the vector, such as antibiotic markers,

should also be considered. For example, vectors that can be used include those that allow the DNA encoding the IL-2 muteins to be amplified in copy number. Such amplifiable vectors are well known in the art. They include, for example, vectors able to be amplified by DHFR amplification (*see, e.g.*, Kaufman, U.S. Pat. No. 4,470,461, Kaufman and Sharp, "Construction of a Modular Dihydrofolate Reductase cDNA Gene: Analysis of Signals Utilized for Efficient Expression", *Mol. Cell. Biol.*, 2, pp. 1304-19 (1982)) or glutamine synthetase ("GS") amplification (*see, e.g.*, U.S. Pat. No. 5,122,464 and European published application 338,841).

[00283] In some embodiments, the human IL-2 muteins of the present disclosure will be expressed from vectors, preferably expression vectors. The vectors are useful for autonomous replication in a host cell or may be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome (e.g., nonepisomal mammalian vectors). Expression vectors are capable of directing the expression of coding sequences to which they are operably linked. In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids (vectors). However, other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses, and adeno-associated viruses) are included also.

[00284] Exemplary recombinant expression vectors can include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, operably linked to the nucleic acid sequence to be expressed.

[00285] The expression constructs or vectors can be designed for expression of an IL-2 mutein or variant thereof in prokaryotic or eukaryotic host cells.

[00286] Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. Suitable methods for transforming or transfecting host cells can be found in Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, N.Y.) and other standard molecular biology laboratory manuals.

[00287] Expression of proteins in prokaryotes is most often carried out in *Escherichia coli* with vectors containing constitutive or inducible promoters. Strategies to maximize recombinant protein expression in *E. coli* can be found, for example, in Gottesman (1990) in *Gene Expression Technology: Methods in Enzymology* 185 (Academic Press, San Diego, Calif.), pp. 119-128 and

Wada et al. (1992) *Nucleic Acids Res.* 20:2111-2118. Processes for growing, harvesting, disrupting, or extracting the IL-2 mutein or variant thereof from cells are substantially described in, for example, U.S. Pat. Nos. 4,604,377; 4,738,927; 4,656,132; 4,569,790; 4,748,234; 4,530,787; 4,572,798; 4,748,234; and 4,931,543, herein incorporated by reference in their entireties.

[00288] In some embodiments the recombinant IL-2 muteins or biologically active variants thereof can also be made in eukaryotes, such as yeast or human cells. Suitable eukaryotic host cells include insect cells (examples of Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf9 cells) include the pAc series (Smith et al. (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39)); yeast cells (examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari et al. (1987) *EMBO J.* 6:229-234), pMFa (Kurjan and Herskowitz (1982) *Cell* 30:933-943), pJRY88 (Schultz et al. (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and pPicZ (Invitrogen Corporation, San Diego, Calif.)); or mammalian cells (mammalian expression vectors include pCDM8 (Seed (1987) *Nature* 329:840) and pMT2PC (Kaufman et al. (1987) *EMBO J.* 6:187:195)). Suitable mammalian cells include Chinese hamster ovary cells (CHO) or COS cells. In mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus, and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells, see Chapters 16 and 17 of Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual* (2nd ed., Cold Spring Harbor Laboratory Press, Plainview, N.Y.). *See*, Goeddel (1990) in *Gene Expression Technology: Methods in Enzymology* 185 (Academic Press, San Diego, Calif.).

[00289] The sequences encoding the human IL-2 muteins of the present disclosure can be optimized for expression in the host cell of interest. The G-C content of the sequence can be adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. Methods for codon optimization are well known in the art. Codons within the IL-2 mutein coding sequence can be optimized to enhance expression in the host cell, such that about 1%, about 5%, about 10%, about 25%, about 50%, about 75%, or up to 100% of the codons within the coding sequence have been optimized for expression in a particular host cell.

[00290] Vectors suitable for use include T7-based vectors for use in bacteria (*see*, for example, Rosenberg et al., *Gene* 56:125, 1987), the pMSXND expression vector for use in mammalian cells (Lee and Nathans, *J. Biol. Chem.* 263:3521, 1988), and baculovirus-derived vectors (for example, the expression vector pBacPAK9 from Clontech, Palo Alto, Calif.) for use in insect cells.

[00291] In some embodiments nucleic acid inserts, which encode the subject IL-2 muteins in such vectors, can be operably linked to a promoter, which is selected based on, for example, the cell type in which expression is sought.

[00292] In selecting an expression control sequence, a variety of factors should also be considered. These include, for example, the relative strength of the sequence, its controllability, and its compatibility with the actual DNA sequence encoding the subject IL-2 mutein, particularly as regards potential secondary structures. Hosts should be selected by consideration of their compatibility with the chosen vector, the toxicity of the product coded for by the DNA sequences of this invention, their secretion characteristics, their ability to fold the polypeptides correctly, their fermentation or culture requirements, and the ease of purification of the products coded for by the DNA sequences.

[00293] Within these parameters one of skill in the art may select various vector/expression control sequence/host combinations that will express the desired DNA sequences on fermentation or in large scale animal culture, for example, using CHO cells or COS 7 cells.

[00294] The choice of expression control sequence and expression vector, in some embodiments, will depend upon the choice of host. A wide variety of expression host/vector combinations can be employed. Useful expression vectors for eukaryotic hosts, include, for example, vectors with expression control sequences from SV40, bovine papilloma virus, adenovirus and cytomegalovirus. Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from *E. coli*, including col E1, pCRI, pER32z, pMB9 and their derivatives, wider host range plasmids, such as RP4, phage DNAs, e.g., the numerous derivatives of phage lambda, e.g., NM989, and other DNA phages, such as M13 and filamentous single stranded DNA phages. Useful expression vectors for yeast cells include the 2 μ plasmid and derivatives thereof. Useful vectors for insect cells include pVL 941 and pFastBac™ 1 (GibcoBRL, Gaithersburg, Md.). Cate et al., "Isolation Of The Bovine And Human Genes For

Mullerian Inhibiting Substance And Expression Of The Human Gene In Animal Cells”, *Cell*, 45, pp. 685-98 (1986).

[00295] In addition, any of a wide variety of expression control sequences can be used in these vectors. Such useful expression control sequences include the expression control sequences associated with structural genes of the foregoing expression vectors. Examples of useful expression control sequences include, for example, the early and late promoters of SV40 or adenovirus, the lac system, the trp system, the TAC or TRC system, the major operator and promoter regions of phage lambda, for example PL, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, *e.g.*, PhoA, the promoters of the yeast a-mating system, the polyhedron promoter of Baculovirus, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof.

[00296] A T7 promoter can be used in bacteria, a polyhedrin promoter can be used in insect cells, and a cytomegalovirus or metallothionein promoter can be used in mammalian cells. Also, in the case of higher eukaryotes, tissue-specific and cell type-specific promoters are widely available. These promoters are so named for their ability to direct expression of a nucleic acid molecule in a given tissue or cell type within the body. Skilled artisans are well aware of numerous promoters and other regulatory elements which can be used to direct expression of nucleic acids.

[00297] In addition to sequences that facilitate transcription of the inserted nucleic acid molecule, vectors can contain origins of replication, and other genes that encode a selectable marker. For example, the neomycin-resistance (neo^r) gene imparts G418 resistance to cells in which it is expressed, and thus permits phenotypic selection of the transfected cells. Those of skill in the art can readily determine whether a given regulatory element or selectable marker is suitable for use in a particular experimental context.

[00298] Viral vectors that can be used in the invention include, for example, retroviral, adenoviral, and adeno-associated vectors, herpes virus, simian virus 40 (SV40), and bovine papilloma virus vectors (*see*, for example, Gluzman (Ed.), *Eukaryotic Viral Vectors*, CSH Laboratory Press, Cold Spring Harbor, N.Y.).

[00299] Prokaryotic or eukaryotic cells that contain and express a nucleic acid molecule that encodes a subject IL-2 mutein disclosed herein are also features of the invention. A cell of the invention is a transfected cell, i.e., a cell into which a nucleic acid molecule, for example a nucleic acid molecule encoding a mutant IL-2 polypeptide, has been introduced by means of recombinant DNA techniques. The progeny of such a cell are also considered within the scope of the invention.

[00300] The precise components of the expression system are not critical. For example, an IL-2 mutein can be produced in a prokaryotic host, such as the bacterium *E. coli*, or in a eukaryotic host, such as an insect cell (*e.g.*, an Sf21 cell), or mammalian cells (*e.g.*, CHO, HEK293, COS cells, NIH 3T3 cells, or HeLa cells). These cells are available from many sources, including the American Type Culture Collection (Manassas, Va.). In selecting an expression system, it matters only that the components are compatible with one another. Artisans of ordinary skill are able to make such a determination. Furthermore, if guidance is required in selecting an expression system, skilled artisans may consult Ausubel et al. (*Current Protocols in Molecular Biology*, John Wiley and Sons, New York, N.Y., 1993) and Pouwels et al. (*Cloning Vectors: A Laboratory Manual*, 1985 Suppl. 1987).

[00301] The expressed polypeptides can be purified from the expression system using routine biochemical procedures, and can be used, *e.g.*, as therapeutic agents, as described herein.

[00302] In some embodiments, IL-2 muteins obtained will be glycosylated or unglycosylated depending on the host organism used to produce the mutein. If bacteria are chosen as the host then the IL-2 mutein produced will be unglycosylated. Eukaryotic cells, on the other hand, will glycosylate the IL-2 muteins, although perhaps not in the same way as native-IL-2 is glycosylated. The IL-2 mutein produced by the transformed host can be purified according to any suitable method. Various methods are known for purifying IL-2. See, *e.g.* *Current Protocols in Protein Science*, Vol 2. Eds: John E. Coligan, Ben M. Dunn, Hidde L. Ploehg, David W. Speicher, Paul T. Wingfield, Unit 6.5 (Copyright 1997, John Wiley and Sons, Inc. IL-2 muteins can be isolated from inclusion bodies generated in *E. coli*, or from conditioned medium from either mammalian or yeast cultures producing a given mutein using cation exchange, gel filtration, and/or reverse phase liquid chromatography.

[00303] Another exemplary method of constructing a DNA sequence encoding the IL-2 muteins is by chemical synthesis. This includes direct synthesis of a peptide by chemical means of the protein sequence encoding for an IL-2 mutein exhibiting the properties described. This method can incorporate both natural and unnatural amino acids at positions that affect the interactions of IL-2 with the IL-2R α , the IL-2R β and/or the IL-2R γ . Alternatively a gene which encodes the desired IL-2 mutein can be synthesized by chemical means using an oligonucleotide synthesizer. Such oligonucleotides are designed based on the amino acid sequence of the desired IL-2 mutein, and preferably selecting those codons that are favored in the host cell in which the recombinant mutein will be produced. In this regard, it is well recognized that the genetic code is degenerate—that an amino acid may be coded for by more than one codon. For example, Phe (F) is coded for by two codons, TIC or TTT, Tyr (Y) is coded for by TAC or TAT and his (H) is coded for by CAC or CAT. Trp (W) is coded for by a single codon, TGG. Accordingly, it will be appreciated that for a given DNA sequence encoding a particular IL-2 mutein, there will be many DNA degenerate sequences that will code for that IL-2 mutein. For example, it will be appreciated that in addition to the preferred DNA sequence for mutein H9, there will be many degenerate DNA sequences that code for the IL-2 mutein shown. These degenerate DNA sequences are considered within the scope of this disclosure. Therefore, “degenerate variants thereof” in the context of this invention means all DNA sequences that code for and thereby enable expression of a particular mutein.

[00304] The biological activity of the IL-2 muteins can be assayed by any suitable method known in the art. Such assays include PHA-blast proliferation and NK cell proliferation.

I. ANTI-PD-1 ANTIBODIES AND COMBINATIONS

[00305] Anti-PD-1 antibodies for use according to the invention and methods described herein include but are not limited to nivolumab, BMS-936558, MDX-1106, ONO-4538, AMP224, CT-011, and MK-3475 (pembrolizumab), cemiplimab (REGN2810), SHR-1210 (CTR20160175 and CTR20170090), SHR-1210 (CTR20170299 and CTR20170322), JS-001 (CTR20160274), IBI308 (CTR20160735), BGB-A317 (CTR20160872) and/or a PD-1 antibody as recited in U.S. Patent Publication No. 2017/0081409. There are two approved anti-PD-1 antibodies, pembrolizumab (Keytruda®; MK-3475-033) and nivolumab (Opdivo®; CheckMate078) and many more in development which can be used in combination described herein. Exemplary anti-

PD-1 antibody sequences are shown in Figure 10 and any of these can be used with the combination methods with the IL-2 muteins as described herein.

[00306] In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination with an anti-PD-1 antibody or inhibitor. In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination with nivolumab. In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination with pembrolizumab. In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination with cemiplimab. In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination BMS-936558. In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination MDX-1106. In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination ONO-4538. In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination AMP224. In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination CT-011. In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination MK-3475. In some embodiments, the IL-2 mutein further comprises F42A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises Y45A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises E62A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2.

[00307] In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination with any of the referenced antibodies. In some embodiments, the IL-2 mutein further comprises F42A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises Y45A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises E62A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. . In some embodiments, the IL-2 mutein further comprises E62A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein is any IL-2 mutein or variant disclosed herein. In some embodiments, the IL-2 mutein sequence is 90% identical to any one of SEQ ID NO:2 or SEQ ID NO:6 through SEQ ID NO:10 or SEQ ID NO:16. In some embodiments, the IL-2 mutein includes any one of 5-1 SEQ ID NO:5; 5-2 SEQ ID NO:6; 6-6 SEQ ID NO:7; A2 SEQ ID NO:8; B1 SEQ ID NO:9; B11 SEQ ID NO:10; C5 SEQ ID NO:11; D10 SEQ ID NO:12; E10 SEQ ID NO:13; G8 SEQ ID NO:14; H4 SEQ ID NO:15; and H9 SEQ ID NO:5. In some embodiments, the IL-2 mutein used in combination with an anti-PD-1 antibody is a fusion mutein as described herein. In some embodiments, the IL-2 mutein used in combination with an anti-PD-1 antibody is a fusion mutein as described herein.

J. ANTI-PD-L1 ANTIBODIES AND COMBINATIONS

[00308] In some embodiments, any of the IL-2 muteins described herein can be used in combination with an anti-PD-1 antibody. There are three approved anti-PD-L1 antibodies, atezolizumab (TECENTRIQ®; MPDL3280A), avelumab (BAVENCIO®; MSB001071 8C), and Durvalumab (MEDI4736), as well as other anti-PD-L1 antibodies in development. Numerous anti-PD-L1 antibodies are available and many more in development which can be used in combination with the IL-2 muteins as described herein. In some embodiments, the PD-L1 antibody is one described in U.S. Patent Publication No. 2017/0281764 as well as International Patent Publication No. WO 2013/079174 (avelumab) and WO 2010/077634 (or U.S. Patent Application No. 20160222117 or U.S. Patent No. 8,217,149; atezolizumab). In some embodiments, the PD-L1 antibody comprises a heavy chain sequence of SEQ ID NO:34 and a light chain sequence of SEQ ID NO:36 (from US 2017/281764). In some embodiments, the PD-

L1 antibody is atezolizumab (TECENTRIQ®; MPDL3280A; IMpower110). In some embodiments, the PD-L1 antibody is avelumab (BAVENCIO®; MSB001071 8C). In some embodiments, the PD-L1 antibody is durvalumab (MEDI4736). In some embodiments, the PD-L1 antibody includes, for example, Atezolizumab (IMpower133), BMS-936559/MDX-1105, and/or RG-7446/MPDL3280A, and/or YW243.55.S70, as well as any of the exemplary anti-PD-L1 antibodies provided herein in Figure 11. In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination with any of the referenced antibodies. In some embodiments, the IL-2 mutein further comprises F42A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises Y45A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises E62A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. . In some embodiments, the IL-2 mutein further comprises E62A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein is any IL-2 mutein or variant disclosed herein. In some embodiments, the IL-2 mutein sequence is 90% identical to any one of SEQ ID NO:2 or SEQ ID NO:6 through SEQ ID NO:10 or SEQ ID NO:16. In some embodiments, the IL-2 mutein includes any one of 5-1 SEQ ID NO:5; 5-2 SEQ ID NO:6; 6-6 SEQ ID NO:7; A2 SEQ ID NO:8; B1 SEQ ID NO:9; B11 SEQ ID NO:10; C5 SEQ ID NO:11; D10 SEQ ID NO:12; E10 SEQ ID NO:13; G8 SEQ ID NO:14; H4 SEQ ID NO:15; and H9 SEQ ID NO:5. In some embodiments, the IL-2 mutein used in combination with an anti-PD-L1 antibody is a fusion mutein as described herein. In some embodiments, the IL-2 mutein used in combination with an anti-PD-L1 antibody is a fusion mutein as described herein.

K. OTHER IMMUNOTHERAPY COMBINATIONS

[00309] Other antibodies and/or immunotherapies for use according to the methods of the present invention include but are not limited to, anti-CTLA4 mAbs, such as ipilimumab, tremelimumab; anti-PD-L1 antagonistic antibodies such as BMS-936559/MDX-1105, MEDI4736, RG-7446/MPDL3280A; anti-LAG-3 such as IMP-321; agonistic antibodies targeting immunostimulatory proteins, including anti-CD40 mAbs such as CP-870,893, lucatumumab, dacetuzumab; anti-CD137 mAbs (anti-4-1-BB antibodies) such as BMS-663513

urelumab (anti-4-1BB antibody; see, for example, US Patent Nos. 7,288,638 and 8,962,804, incorporated by reference herein in their entireties); lirilumab (anti-KIR mAb; IPH2102/BMS-986015; blocks NK cell inhibitory receptors) and PF-05082566 (utomilumab; see, for example, US Patent Nos. 8,821,867; 8,337,850; and 9,468,678, as well as International Patent Application Publication No. WO 2012/032433, incorporated by reference herein in their entireties); anti-OX40 mAbs (see, for example, WO 2006/029879 or WO 2010/096418, incorporated by reference herein in their entireties); anti-GITR mAbs such as TRX518 (see, for example, US Patent No. 7,812,135, incorporated by reference herein in its entirety); anti-CD27 mAbs, such as varlilumab CDX-1127 (see, for example, WO 2016/145085 and U.S. Patent Publication Nos. US 2011/0274685 and US 2012/0213771, incorporated by reference herein in their entireties) anti-ICOS mAbs (for example, MEDI-570, JTX-2011, and anti-TIM-3 antibodies (see, for example, WO 2013/006490 or U.S. Patent Publication No US 2016/0257758, incorporated by reference herein in their entireties). In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination with any of the referenced antibodies. In some embodiments, the IL-2 mutein further comprises F42A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises Y45A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises E62A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. . In some embodiments, the IL-2 mutein further comprises E62A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein is any IL-2 mutein or variant disclosed herein. In some embodiments, the IL-2 mutein sequence is 90% identical to any one of SEQ ID NO:2 or SEQ ID NO:6 through SEQ ID NO:10 or SEQ ID NO:16. In some embodiments, the IL-2 mutein includes any one of 5-1 SEQ ID NO:5; 5-2 SEQ ID NO:6; 6-6 SEQ ID NO:7; A2 SEQ ID NO:8; B1 SEQ ID NO:9; B11 SEQ ID NO:10; C5 SEQ ID NO:11; D10 SEQ ID NO:12; E10 SEQ ID NO:13; G8 SEQ ID NO:14; H4 SEQ ID NO:15; and H9 SEQ ID NO:5.

[00310] Other antibodies can also include monoclonal antibodies to prostate cancer, ovarian cancer, breast cancer, endometrial cancer, multiple myeloma, melanoma, lymphomas, lung cancers including small cell lung cancer, kidney cancer, colorectal cancer, pancreatic cancer,

gastric cancer, brain cancer (*see*, generally www.clinicaltrials.gov). In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination with any of the referenced antibodies. In some embodiments, the IL-2 mutein further comprises F42A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises Y45A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises E62A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein is any IL-2 mutein or variant disclosed herein. In some embodiments, the IL-2 mutein sequence is 90% identical to any one of SEQ ID NO:2 or SEQ ID NO:6 through SEQ ID NO:10 or SEQ ID NO:16. In some embodiments, the IL-2 mutein includes any one of 5-1 SEQ ID NO:5; 5-2 SEQ ID NO:6; 6-6 SEQ ID NO:7; A2 SEQ ID NO:8; B1 SEQ ID NO:9; B11 SEQ ID NO:10; C5 SEQ ID NO:11; D10 SEQ ID NO:12; E10 SEQ ID NO:13; G8 SEQ ID NO:14; H4 SEQ ID NO:15; and H9 SEQ ID NO:5.

[00311] Antibodies can also include antibodies for antibody-dependent cell-mediated cytotoxicity (ADCC). In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination with an antibody for antibody-dependent cell-mediated cytotoxicity (ADCC). In some embodiments, the IL-2 mutein further comprises F42A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises Y45A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises E62A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2.

L. METHODS OF TREATMENT

[00312] In some embodiments, subject IL-2 muteins, and/or nucleic acids expressing them, can be administered to a subject to treat a disorder associated with abnormal apoptosis or a differentiative process (*e.g.*, cellular proliferative disorders or cellular differentiative disorders, such as cancer, by, for example, producing an active or passive immunity). In the treatment of

such diseases, the disclosed IL-2 muteins may possess advantageous properties, such as reduced vascular leak syndrome. In some embodiments, the IL-2 mutein is any IL-2 mutein or variant disclosed herein. In some embodiments, the IL-2 mutein sequence is 90% identical to any one of SEQ ID NO:2 or SEQ ID NO:6 through SEQ ID NO:10 or SEQ ID NO:16. In some embodiments, the IL-2 mutein includes any one of 5-1 SEQ ID NO:5; 5-2 SEQ ID NO:6; 6-6 SEQ ID NO:7; A2 SEQ ID NO:8; B1 SEQ ID NO:9; B11 SEQ ID NO:10; C5 SEQ ID NO:11; D10 SEQ ID NO:12; E10 SEQ ID NO:13; G8 SEQ ID NO:14; H4 SEQ ID NO:15; and H9 SEQ ID NO:5. In some embodiments, the substitutions in the IL-2 mutein comprise L80F, R81D, L85V, I86V, and I92F, numbered in accordance with wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein is a fusion protein. In some embodiments, the IL-2 mutein is associated with and/or expressed by a CAR-T construct. In some embodiments, the IL-2 mutein is expressed by and/or associated with an oncolytic virus.

[00313] Examples of cellular proliferative and/or differentiative disorders include cancer (e.g., carcinoma, sarcoma, metastatic disorders or hematopoietic neoplastic disorders, e.g., leukemias). A metastatic tumor can arise from a multitude of primary tumor types, including but not limited to those of prostate cancer, ovarian cancer, breast cancer, endometrial cancer, multiple myeloma, melanoma, lymphomas, lung cancers including small cell lung cancer, kidney cancer, liver cancer, colon cancer, colorectal cancer, pancreatic cancer, gastric cancer, and brain cancer.

[00314] The mutant IL-2 polypeptides can be used to treat patients who have, who are suspected of having, or who may be at high risk for developing any type of cancer, including renal carcinoma or melanoma, or any viral disease. Exemplary carcinomas include those forming from tissue of the cervix, lung, prostate, breast, head and neck, colon and ovary. The term also includes carcinosarcomas, which include malignant tumors composed of carcinomatous and sarcomatous tissues.

[00315] Additional examples of proliferative disorders include hematopoietic neoplastic disorders.

[00316] Alternatively, or in addition to methods of direct administration to patients, in some embodiments, mutant IL-2 polypeptides can be used in *ex vivo* methods. For example, cells (e.g., peripheral blood lymphocytes or purified populations of lymphocytes isolated from a patient and placed or maintained in culture) can be cultured *in vitro* in culture medium and the contacting

step can be affected by adding the IL-2 mutant to the culture medium. The culture step can include further steps in which the cells are stimulated or treated with other agents, e.g., to stimulate proliferation, or to expand a population of cells that is reactive to an antigen of interest (e.g., a cancer antigen or a viral antigen). The cells are then administered to the patient after they have been treated.

[00317] Anti-PD-1 antibodies for use in combination with the IL-2 muteins disclosed herein for the treatment methods include but are not limited to nivolumab, BMS-936558, MDX-1106, ONO-4538, AMP224, CT-011, and MK-3475.

[00318] In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination with an anti-PD-1 antibody or inhibitor for the treatment of cancer. In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination with nivolumab for the treatment of cancer. In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination BMS-936558 for the treatment of cancer. In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination MDX-1106 for the treatment of cancer. In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination ONO-4538 for the treatment of cancer. In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination AMP224 for the treatment of cancer. In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination CT-011 for the treatment of cancer. In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination MK-3475 for the treatment of cancer. In some embodiments, the IL-2 mutein further comprises F42A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises K43N

substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises F42A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises Y45A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises E62A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2.

[00319] In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination with an antibody and/or immunotherapy including but not limited to, anti-CTLA4 mAbs, such as ipilimumab, tremelimumab; anti-PD-L1 antagonistic antibodies such as BMS-936559/MDX-1105, MEDI4736, RG-7446/MPDL3280A; anti-LAG-3 such as IMP-321; agonistic antibodies targeting immunostimulatory proteins, including anti-CD40 mAbs such as CP-870,893, lucatumumab, dacetuzumab; anti-CD137 mAbs (anti-4-1-BB antibodies) such as BMS-663513 urelumab (anti-4-1BB antibody; see, for example, US Patent Nos. 7,288,638 and 8,962,804, incorporated by reference herein in their entireties); lirilumab (anti-KIR mAb; IPH2102/BMS-986015; blocks NK cell inhibitory receptors) and PF-05082566 (utomilumab; see, for example, US Patent Nos. 8,821,867; 8,337,850; and 9,468,678, as well as International Patent Application Publication No. WO 2012/032433, incorporated by reference herein in their entireties); anti-OX40 mAbs (see, for example, WO 2006/029879 or WO 2010/096418, incorporated by reference herein in their entireties); anti-GITR mAbs such as TRX518 (see, for example, US Patent No. 7,812,135, incorporated by reference herein in its entirety); anti-CD27 mAbs, such as varlilumab CDX-1127 (see, for example, WO 2016/145085 and U.S. Patent Publication Nos. US 2011/0274685 and US 2012/0213771, incorporated by reference herein in their entireties) anti-ICOS mAbs (for example, MEDI-570, JTX-2011, and anti-TIM-3 antibodies (see, for example, WO 2013/006490 or U.S. Patent Publication No US 2016/0257758, incorporated by reference herein in their entireties) for the treatment of cancer.

[00320] In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination with another antibody which can include monoclonal antibodies to prostate cancer, ovarian cancer, breast cancer, endometrial cancer, multiple myeloma, melanoma, lymphomas,

lung cancers including small cell lung cancer, kidney cancer, colorectal cancer, pancreatic cancer, gastric cancer, brain cancer (*see*, generally www.clinicaltrials.gov), for the treatment of cancer.

[00321] In some embodiments, the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2) is used in combination with antibodies for antibody-dependent cell-mediated cytotoxicity (ADCC) for the treatment of cancer.

M. PHARMACEUTICAL COMPOSITIONS AND METHODS OF ADMINISTRATION

[00322] In some embodiments, subject IL-2 muteins and nucleic acids can be incorporated into compositions, including pharmaceutical compositions. Such compositions typically include the polypeptide or nucleic acid molecule and a pharmaceutically acceptable carrier. Such compositions can also comprise anti-PD-1 antibodies. In some embodiments, the composition comprises an IL-2 mutein that is a fusion protein and/or is associated with a CAR-T construct and/or expressed by or associated with an oncolytic virus.

[00323] The anti-PD-1 antibodies and IL-2 muteins can be administered as a co-composition, simultaneously as two separate compositions, and/or sequentially as two separate compositions. In some embodiments, the anti-PD-1 antibody or inhibitor and IL-2 mutein are administered together as a single co-composition (*i.e.*, co-formulated). In some embodiments, the anti-PD-1 antibody or inhibitor and IL-2 mutein are administered simultaneously as two separate compositions (*i.e.*, separate formulations). In some embodiments, the anti-PD-1 antibody or inhibitor and IL-2 mutein are administered sequentially as separate compositions (*i.e.*, separate formulations). In some embodiments, when the anti-PD-1 antibody or inhibitor and IL-2 mutein are administered sequentially as separate compositions, the anti-PD-1 antibody or inhibitor is administered before the IL-2 mutein. In some embodiments, when the anti-PD-1 antibody or inhibitor and IL-2 mutein are administered sequentially as separate compositions, the IL-2 mutein is administered before the anti-PD-1 antibody or inhibitor. In some embodiments, the anti-PD-1 antibodies include but are not limited to nivolumab, BMS-936558, MDX-1106, ONO-4538, AMP224, CT-011, and MK-3475. In some embodiments, the IL-2 mutein is the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance

with human wild-type IL-2 (SEQ ID NO:2). In some embodiments, the IL-2 mutein further comprises F42A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises K43N substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises F42A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises Y45A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises E62A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2.

[00324] The other immunotherapy agents as described and IL-2 muteins can be administered as a co-composition, simultaneously as two separate compositions, and/or sequentially as two separate compositions. In some embodiments, the other immunotherapy agents and IL-2 mutein are administered together as a single co-composition (*i.e.*, co-formulated). In some embodiments, the other immunotherapy agents and IL-2 mutein are administered simultaneously as two separate compositions (*i.e.*, separate formulations). In some embodiments, the other immunotherapy agents and IL-2 mutein are administered sequentially as separate compositions (*i.e.*, separate formulations). In some embodiments, when the other immunotherapy agents and IL-2 mutein are administered sequentially as separate compositions, the anti-PD-1 antibody or inhibitor is administered before the IL-2 mutein. In some embodiments, when other immunotherapy agents and IL-2 mutein are administered sequentially as separate compositions, the IL-2 mutein is administered before other immunotherapy agents. In some embodiments, the IL-2 mutein is the IL-2 mutein comprising substitutions L80F, R81D, L85V, I86V, and I92F, numbered in accordance with human wild-type IL-2 (SEQ ID NO:2). In some embodiments, the IL-2 mutein further comprises F42A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises K43N substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises F42A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2 mutein further comprises Y45A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2. In some embodiments, the IL-2

mutein further comprises E62A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2.

[00325] A pharmaceutical composition is formulated to be compatible with its intended route of administration. The anti-PD-1 antibodies and/or mutant IL-2 polypeptides of the invention may be given orally, but it is more likely that they will be administered through a parenteral route, including for example intravenous administration. Examples of parenteral routes of administration include, for example, intravenous, intradermal, subcutaneous, transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as mono- and/or di-basic sodium phosphate, hydrochloric acid or sodium hydroxide (*e.g.*, to a pH of about 7.2-7.8, *e.g.*, 7.5). The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00326] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™. (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition should be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants, *e.g.*, sodium dodecyl sulfate. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and

the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[00327] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00328] Oral compositions, if used, generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, e.g., gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel™, or corn starch; a lubricant such as magnesium stearate or Sterotes™; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[00329] In the event of administration by inhalation, anti-PD-1 antibodies and/or IL-2 muteins, or the nucleic acids encoding them, are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer. Such methods include those described in U.S. Pat. No. 6,468,798.

[00330] Systemic administration of the anti-PD-1 antibodies and/or IL-2 muteins or nucleic acids can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation.

Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[00331] In some embodiments, compounds (anti-PD-1 antibodies and/or mutant IL-2 polypeptides or nucleic acids) can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[00332] In some embodiments, compounds (subject IL-2 muteins or nucleic acids) can also be administered by transfection or infection using methods known in the art, including but not limited to the methods described in McCaffrey et al. (Nature 418:6893, 2002), Xia et al. (Nature Biotechnol. 20: 1006-1010, 2002), or Putnam (Am. J. Health Syst. Pharm. 53: 151-160, 1996, erratum at Am. J. Health Syst. Pharm. 53:325, 1996).

[00333] In one embodiment, the anti-PD-1 antibodies and/or IL-2 muteins or nucleic acids are prepared with carriers that will protect the anti-PD-1 antibodies and/or mutant IL-2 polypeptides against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Such formulations can be prepared using standard techniques. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

[00334] Dosage, toxicity and therapeutic efficacy of such anti-PD-1 antibodies, IL-2 muteins, or nucleic acids compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the

ratio LD₅₀/ED₅₀. Compounds that exhibit high therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[00335] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[00336] As defined herein, a therapeutically effective amount of a subject IL-2 mutein (i.e., an effective dosage) and/or the anti-PD-1 antibody or inhibitor depends on the polypeptide or antibody selected. In some embodiments, single dose amounts of the IL-2 mutein can be in the range of approximately 0.001 mg/kg to 0.1 mg/kg of patient body weight can be administered. In some embodiments, single dose amounts of the anti-PD-1 antibody or inhibitor can be in the range of approximately 1 mg/kg to 20 mg/kg, or about 5 mg/kg to about 15 mg/kg, or about 10 mg/kg of patient body weight can be administered. In some embodiments, doses of the anti-PD-1 antibody or inhibitor and/or the IL-2 mutein of about 0.005 mg/kg, 0.01 mg/kg, 0.025 mg/kg, 0.05 mg/kg, 0.1 mg/kg, 0.25 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 5.0 mg/kg, 10.0 mg/kg may be administered. In some embodiments, 600,000 IU/kg is administered (IU can be determined by a lymphocyte proliferation bioassay and is expressed in International Units (IU) as established by the World Health Organization 1st International Standard for Interleukin-2 (human)). The dosage may be similar to, but is expected to be less than, that prescribed for PROLEUKIN®. The compositions can be administered one from one or more times per day to one or more times per week; including once every other day. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited

to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of the subject IL-2 muteins can include a single treatment or, can include a series of treatments. In one embodiment, the compositions are administered every 8 hours for five days, followed by a rest period of 2 to 14 days, e.g., 9 days, followed by an additional five days of administration every 8 hours. In some embodiments, administration is 3 doses administered every 4 days.

[00337] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[00338] The following examples are provided to describe certain embodiments of the invention provided herein and are not to be construed to as limiting.

EXEMPLARY EMBODIMENTS

1. A method of treating cancer comprising administering a combination treatment comprising:
 - (i) an anti-PD-1 antibody or inhibitor or an anti-PD-L1 antibody or inhibitor and
 - (ii) an IL-2 mutein comprising the following amino acid substitutions L80F, R81D, L85V, I86V, and I92F, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2.
2. The method of claim 1, wherein said anti-PD-1 antibody or inhibitor is selected from the group consisting of nivolumab, BMS-936558, MDX-1106, ONO-4538, AMP224, CT-011, and MK-3475(pembrolizumab), cemiplimab (REGN2810), SHR-1210 (CTR20160175 and CTR20170090), SHR-1210 (CTR20170299 and CTR20170322), JS-001 (CTR20160274), IBI308 (CTR20160735), BGB-A317 (CTR20160872) and a PD-1 antibody as recited in U.S. Patent Publication No. 2017/0081409.
3. The method of claim 1, wherein said anti-PD-L1 antibody or inhibitor is selected from the group consisting of atezolizumab, avelumab, and Durvalumab.

4. The method of any one of claims 1 to 3, wherein said IL-2 mutein further comprises F42A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2.
5. The method of any one of claims 1 to 3, wherein said IL-2 mutein further comprises K43N substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2.
6. The method of any one of claims 1 to 3, wherein said IL-2 mutein further comprises F42A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2.
7. The method of any one of claims 1 to 3, wherein said IL-2 mutein further comprises Y45A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2.
8. The method of any one of claims 1 to 3, wherein said IL-2 mutein further comprises E62A substitution, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2.
9. The method of any one of claims 1 to 8, wherein said IL-2 mutein is a fusion protein.
10. The method of claim 9, wherein said fusion protein comprises said IL-2 linked to an Fc antibody fragment.
11. The method of claim 10, wherein said Fc antibody fragment is a human Fc antibody fragment.
12. The method of claim 10, wherein said Fc antibody fragment comprises a N297A substitution.

13. The method of claim 9, wherein said fusion protein comprises said IL-2 linked to an albumin.
14. The method of any one of claims 1 to 13, wherein said cancer is selected from the group consisting of prostate cancer, ovarian cancer, breast cancer, endometrial cancer, multiple myeloma, melanoma, lymphomas, lung cancers including small cell lung cancer, kidney cancer, liver cancer, colon cancer, colorectal cancer, pancreatic cancer, gastric cancer, and brain cancer.
15. The method of claim 14, wherein said cancer is colon cancer.
16. The method of any one of claims 1 to 15, wherein said IL-2 mutein exhibits increased binding capacity for IL-2R β as compared to wild-type human IL-2.
17. The method of any one of claims 1 to 15, wherein said IL-2 mutein exhibits a greater binding affinity for IL-2R β as compared to wild-type human IL-2.
18. The method of any one of claims 4 to 8, wherein said IL-2 mutein exhibits decreased binding affinity for CD25 as compared to wild-type human IL-2.
19. A pharmaceutical composition comprising an anti-PD-1 antibody or inhibitor, the IL-2 mutein of any of claims 1 to 13 or 16 to 18, and a pharmaceutically acceptable carrier.
20. An immune cell targeting or expression construct comprising: an interleukin-2 receptor β (IL-2R β) binding protein, wherein the equilibrium dissociation constant for the IL-2R β of said binding protein is less than that of wild-type human IL-2 (hIL-2); linked to an immune cell targeting or expression construct comprising at least one other targeting moiety.

21. The construct of claim 20, wherein the immune cell targeting or expression construct exhibits a cytotoxic effect on a T-cell, for example a CD8+ T-cell or a CD4+ T-cell.
22. The construct of any one of claims 20 to 21, wherein the construct is a chimeric antigen receptor (CAR) and wherein the IL-2 mutein or other targeting moiety is fused to a transmembrane domain; linked to an intracellular signaling region.
23. The construct of claim 22, wherein the intracellular signaling region comprises a CD3 signaling domain.
24. The construct of claim 23, wherein the intracellular signaling region comprises one or more of a CD28 signaling domain, a CD137 signaling domain, an OX-40 signaling domain, an ICOS signaling domain, a DAP10 signaling domain.
25. The construct of claim 21, wherein the construct is a T cell antigen coupler (TAC), wherein the IL-2 mutein or other targeting moiety is fused to a ligand that binds a protein associated with the TCR complex; fused to a T cell receptor signaling domain polypeptide.
26. The construct of claim 25, wherein the protein associated with the TCR complex is CD3.
27. The construct of claim 26, wherein the T cell receptor signaling domain polypeptide comprises CD4 cytosolic domain and CD4 transmembrane domain.
28. The construct of claim 20, wherein the construct is an antibody coupled T cell receptors (ACTR), comprising a chimeric antigen receptor component that binds to the IL-2 mutein or other targeting moiety at a high affinity.

29. The construct of claim 20, wherein the CAR component comprises CD16, and the IL-2 mutein is fused to an Fc sequence.
30. The construct of claim 20, wherein the construct is a bispecific T cell exchanger (BiTE) comprising an IL-2 mutein or other targeting moiety fused to a variable region of an antibody that binds to a component of a T cell receptor.
31. The construct of claim 30, wherein the BiTE component of a T cell receptor is CD3.
32. The construct of claim 31, wherein IL-2R β binding protein comprises the following amino acid substitutions: L80F, R81D, L85V, I86V, and I92F, numbered in accordance with wild-type hIL-2.
33. A nucleic acid encoding a construct according to any one of claims 20 to 32.
34. A vector comprising the nucleic acid construct of claim 33.
35. A T cell comprising a construct according to claim 33 or a vector according to claim 34.
36. An NK cell comprising a construct according to claim 33 or a vector according to claim 34.
37. The T-cell of claim 35, wherein the T cell is a CD4⁺ T cell.
38. The T-cell of claim 35, wherein the T cell is a CD8⁺ T cell.

39. An isolated population of immune cells of claim 35 or claim 36.
40. A pharmaceutical formulation comprising the immune cell population of claim 39.
41. A method of targeting a cancer cell, including a cell expressing an IL-2 receptor, the method comprising contacting a cell with a formulation of claim 40.
42. The method of claim 41, wherein the contacting is *in vitro*.
43. The method of claim 41, wherein the contacting is *in vivo*.
44. A method of treating cancer, the method comprising contacting an individual having cancer with an effective dose of a formulation of claim 40.
45. The method of claim 44, wherein the cancer is a leukemia, lymphoma, glioblastoma, medulloblastoma, breast cancer, head and neck cancer, kidney cancer, ovarian cancer, Kaposi's sarcoma, acute myelogenous leukemia, B-lineage malignancies, colorectal, pancreatic, kidney, or mesothelioma.
46. A method of targeting an IL-2 mutein protein to a cancer cell comprising contacting said cancer cell with an IL-2 mutein oncolytic virus combination, wherein said combination comprises an IL-2 mutein conjugated to or expressed by an oncolytic virus, and wherein said oncolytic virus is capable of targeting a cancer cell.
47. The method of claim 46, wherein said contacting occurs *in vitro*.

48. The method of claim 46, wherein said contacting occurs *in vivo*.
49. The method of any one of claims 46 to 48, wherein the oncolytic virus is selected from the group consisting of an adenovirus, a self-replicating alphavirus, a vaccinia virus, a Seneca Valley Virus, a Newcastle disease Virus, a Maraba virus, vesicular stomatitis virus (VSV), a Herpes virus (including HSV-1 and HSV-2), a measles virus, a poliovirus, a reovirus, a coxsackie virus, a lentivirus, a morbillivirus, an influenza virus, Sinbis virus, myxoma virus, and a retrovirus.
50. The method of claim 47, wherein the vaccinia virus genome comprises thymidine kinase gene is inactivated by a substitution in the thymidine kinase (TK) gene and/or an open reading frame ablating deletion of at least one nucleotide providing a partially deleted thymidine kinase gene, the vaccinia growth factor gene is deleted, and the modified vaccinia virus vector comprises at least one nucleic acid sequence encoding an IL-2 mutein as described herein.
51. The method of claim 48, wherein said *in vivo* contacting results in an increased concentration of the IL-2 mutein protein in the tumor microenvironment as compared to the concentration of an IL-2 mutein protein not conjugated to an oncolytic virus.
52. The method of any one of claims 46 or 48 to 51, wherein the modified oncolytic virus targets the IL-2 mutein to the immunosuppressive cells of the tumor microenvironment (TME), such as tumor associated macrophages and MDSCs (myeloid-derived suppressor cells) in order to have an improved therapeutic benefit.

53. The method of any one of claims 46 or 48 to 52, wherein the modified oncolytic virus targets the IL-2 mutein to one or more immunosuppressive cells expressing one or more tumor antigens.
54. The method of any one of claims 46 or 48 to 53, wherein the modified oncolytic virus targets the IL-2 mutein to the TME.
55. The method of any one of claims 46 or 48 to 54, wherein the IL-2 mutein protein enhances effector T cells and/or NK cells.
56. The method of any one of claims 46 or 48 to 55, wherein the IL-2 mutein suppresses Treg activity.
57. The method of any one of claims 46 or 48 to 56, wherein the IL-2 comprises the following amino acid substitutions L80F, R81D, L85V, I86V, and I92F, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2.
58. A modified vaccinia virus vector, characterized in that the vector comprises a vaccinia virus genome wherein the thymidine kinase gene is inactivated by a substitution in the thymidine kinase (TK) gene and/or an open reading frame ablating deletion of at least one nucleotide providing a partially deleted thymidine kinase gene, the vaccinia growth factor gene is deleted, and the modified vaccinia virus vector comprises at least one nucleic acid sequence encoding an IL-2 mutein as described herein.
59. A modified oncolytic adenovirus comprising (i) a modified nucleic acid, wherein optionally the nucleotides encoding amino acids 122-129 of the encoded E1A polypeptide are deleted, and (ii) an expression cassette comprising a polynucleotide encoding an IL-2 mutein as described herein.

60. The modified virus of claim 58 or claim 59, where the IL-2 mutein directs the modified oncolytic virus to the immunosuppressive cells of the tumor microenvironment (TME), such as tumor associated macrophages and MDSCs (myeloid-derived suppressor cells) in order to have an improved therapeutic benefit.
61. The modified virus of claim 58 or claim 59, where the IL-2 mutein protein directs the modified oncolytic virus to one or more tumor antigens.
62. The modified virus of claim 58 or claim 59, where the IL-2 mutein protein directs modified oncolytic virus to the TME.
63. The modified virus of claim 58 or claim 59, where the IL-2 mutein protein enhances effector T cells and NK cells.
64. The modified virus of claim 58 or claim 59, where the IL-2 mutein suppresses Treg activity.
65. A method of treating cancer comprising administering and oncolytic virus capable of expressing an IL-2 mutein to a subject in need thereof.
66. The method of claim 65, wherein the IL-2 mutein comprises the following amino acid substitutions L80F, R81D, L85V, I86V, and I92F, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2.
67. The method of any one of claims 65-66, wherein the oncolytic virus is selected from the group consisting of an adenovirus, a self-replicating alphavirus, a vaccinia virus, a

Seneca Valley Virus, a Newcastle disease Virus, a Maraba virus, vesicular stomatitis virus (VSV), a Herpes virus (including HSV-1 and HSV-2), a measles virus, a poliovirus, a reovirus, a coxsackie virus, a lentivirus, a morbillivirus, an influenza virus, Sinbis virus, myxoma virus, and a retrovirus.

EXAMPLES

Example 1: H9 Synergizes with Anti-PD-1 Immunotherapy in mouse MC38 colon cancer model.

[00339] This example provides data showing that combination therapy produces robust responses in a dose-dependent fashion.

[00340] Table 11 below shows the substitution matrix for the H9 IL-2 mutein used in this example.

Table 11: H9 substitution matrix

residue #	74	80	81	85	86	89	92	93	K_d (nM)
<i>wt IL-2</i>	<i>Q</i>	<i>L</i>	<i>R</i>	<i>L</i>	<i>I</i>	<i>I</i>	<i>I</i>	<i>V</i>	280
H9		F	D	V	V		F		1.3

[00341] An anti-PD-1 antibody was administered at 10 mg/kg intravenously with 3 doses administered every 4 days (10 mg/kg IV q4dx3). H9 (IL-2 mutein having the amino acid substitutions L80F, R81D, L85V, I86V, and I92F, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2) was administered at the indicated dosage of 5 µg q.d. or 25 µg q.d. (µg/mouse), according to the same dosing regimen. MC38 colon cancer model mice were then monitored for up to 40 days post-tumor implant. The combination of anti-PD-1 antibody plus H9 resulted in an increase in the number of cured mice at both the low and high dose, with a substantial increase at the 25 ug q.d, dose of H9.

[00342] As provided in the data in Figure 1, H9 and anti-PD-1 produce limited efficacy alone. However, the combination treatment is sufficient to cure most mice at a well-tolerated H9 dose. Increased efficacy of the combination did not result in new or increased toxicities.

[00343] The examples set forth above are provided to give those of ordinary skill in the art a complete disclosure and description of how to make and use the embodiments of the compositions, systems and methods of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Modifications of the above-described modes for carrying out the invention that are obvious to persons of skill in the art are intended to be within the scope of the following claims. All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

[00344] All headings and section designations are used for clarity and reference purposes only and are not to be considered limiting in any way. For example, those of skill in the art will appreciate the usefulness of combining various aspects from different headings and sections as appropriate according to the spirit and scope of the invention described herein.

[00345] All references cited herein are hereby incorporated by reference herein in their entireties and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

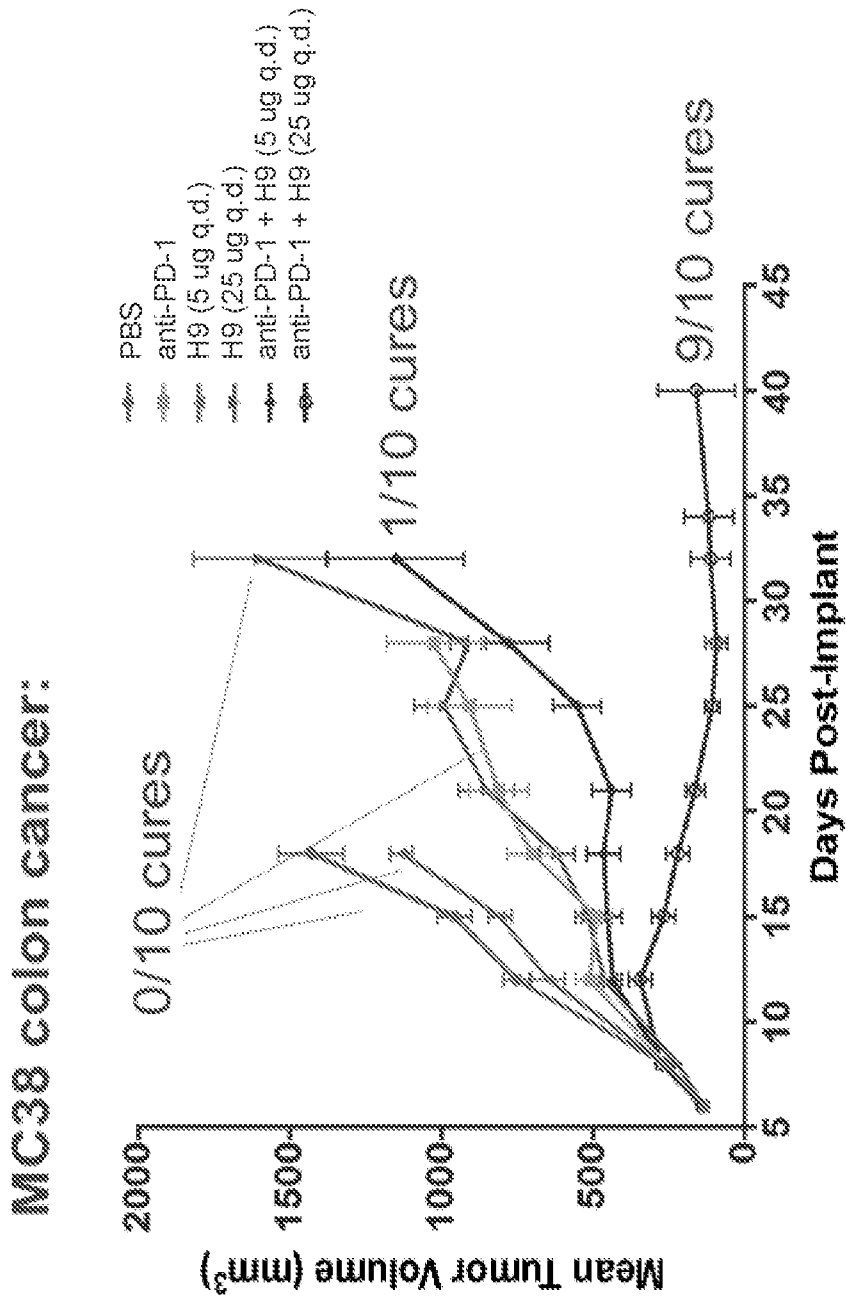
[00346] Many modifications and variations of this application can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments and examples described herein are offered by way of example only, and the application is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which the claims are entitled.

WHAT IS CLAIMED IS:

1. A method of treating cancer comprising administering a combination treatment comprising:
 - (i) an anti-PD-1 antibody or inhibitor or an anti-PD-L1 antibody or inhibitor and
 - (ii) an IL-2 mutein comprising the following amino acid substitutions L80F, R81D, L85V, I86V, and I92F, wherein numbering is in accordance with the wild-type human IL-2 of SEQ ID NO:2, and wherein the IL-2 mutein comprises SEQ ID NO: 9.
2. The method of claim 1, wherein said anti-PD-1 antibody or inhibitor is selected from the group consisting of nivolumab, BMS-936558, MDX-1106, ONO-4538, AMP224, CT-011, and MK-3475(pembrolizumab), cemiplimab (REGN2810), SHR-1210 (CTR20160175 and CTR20170090), SHR-1210 (CTR20170299 and CTR20170322), JS-001 (CTR20160274), IBI308 (CTR20160735), and BGB-A317 (CTR20160872).
3. The method of claim 1, wherein said anti-PD-L1 antibody or inhibitor is selected from the group consisting of atezolizumab, avelumab, and Durvalumab.
4. The method of any one of claims 1 to 3, wherein said IL-2 mutein is a fusion protein.
5. The method of claim 4, wherein said fusion protein comprises said IL-2 linked to an Fc antibody fragment.
6. The method of claim 5, wherein said Fc antibody fragment is a human Fc antibody fragment.
7. The method of claim 5, wherein said Fc antibody fragment comprises a N297A substitution.
8. The method of claim 4, wherein said fusion protein comprises said IL-2 linked to an albumin.

9. The method of any one of claims 1 to 8, wherein said cancer is selected from the group consisting of prostate cancer, ovarian cancer, breast cancer, endometrial cancer, multiple myeloma, melanoma, lymphomas, lung cancers including small cell lung cancer, kidney cancer, liver cancer, colon cancer, colorectal cancer, pancreatic cancer, gastric cancer, and brain cancer.
10. The method of claim 9, wherein said cancer is colon cancer.
11. The method of any one of claims 1 to 10, wherein said IL-2 mutein exhibits increased binding capacity for IL-2R β as compared to wild-type human IL-2.
12. The method of any one of claims 1 to 10, wherein said IL-2 mutein exhibits a greater binding affinity for IL-2R β as compared to wild-type human IL-2.
13. The method of any one of claims 1 to 3, wherein said IL-2 mutein exhibits decreased binding affinity for CD25 as compared to wild-type human IL-2.
14. A pharmaceutical composition comprising an anti-PD-1 antibody or inhibitor, the IL-2 mutein of any of claims 1 to 8 or 11 to 13, and a pharmaceutically acceptable carrier.

Figure 1



* 10 mg/kg IV q4dx3

Figure 2A

CH1																						
EU Index	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	
IgG1	A	S	T	K	G	P	S	V	F	P	L	A	P	S	S	K	S	T	S	G	G	
IgG2	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	E	S	
IgG3	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	G	G	
IgG4	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	E	S	
EU Index	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	
IgG1	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	
IgG2	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	
IgG3	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	
IgG4	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	
EU Index	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	
IgG1	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y	
IgG2	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y	
IgG3	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y	
IgG4	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y	
EU Index	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	
IgG1	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q	T	Y	I	C	N	
IgG2	S	L	S	S	V	V	T	V	P	S	S	N	F	G	T	Q	T	Y	T	C	N	
IgG3	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q	T	Y	T	C	N	
IgG4	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	K	T	Y	T	C	N	
EU Index	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220			
IgG1	V	N	H	K	P	S	N	T	K	V	D	K	K	V	E	P	K	S	C			
IgG2	V	D	H	K	P	S	N	T	K	V	D	K	T	V	E	R	K	C	C			
IgG3	V	N	H	K	P	S	N	T	K	V	D	K	R	V	E	L	K	T	P			
IgG4	V	D	H	K	P	S	N	T	K	V	D	K	R	V	E	S	K	Y	G			
Hinge										Fc >												
EU Index	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236						
IgG1	D			K	T	H	T	C	P	P												
IgG2				V		E		C	P	P												
IgG3	L	G	D	T	T	H	T	C	P	R	C	P	E	P	K	S	C	D	T	P	P	
IgG4						P	P	C	P	S												
EU Index											Fc >											
IgG1											C	P	A	P	E	L	L	G				
IgG2											C	P	A	P	P	V	A					
IgG3											E	P	K	S	C	D	T	P	P	C	P	R
IgG4											C	P	A	P	E	F	L	G				

Figure 2B

CH2												
EU Index	237	238	239	240	241	242	243	244	245	246	247	248
1gG1	G	P	S	V	F	L	F	P	P	K	P	K
1gG2	G	P	S	V	F	L	F	P	P	K	P	K
1gG3	G	P	S	V	F	L	F	P	P	K	P	K
1gG4	G	P	S	V	F	L	F	P	P	K	P	K
EU Index	258	259	260	261	262	263	264	265	266	267	268	269
1gG1	E	V	T	C	V	V	V	D	V	S	H	E
1gG2	E	V	T	C	V	V	V	D	V	S	H	E
1gG3	E	V	T	C	V	V	V	D	V	S	H	E
1gG4	E	V	T	C	V	V	V	D	V	S	H	E
EU Index	270	271	272	273	274	275	276	277	278			
1gG1	V	O	G	V	E	V	H	N	A			
1gG2	V	O	G	V	E	V	H	N	A			
1gG3	V	O	G	V	E	V	H	N	A			
1gG4	V	O	G	V	E	V	H	N	A			
EU Index	300	301	302	303	304	305	306	307	308			
1gG1	Y	R	V	V	S	V	L	T	V			
1gG2	Y	R	V	V	S	V	L	T	V			
1gG3	Y	R	V	V	S	V	L	T	V			
1gG4	Y	R	V	V	S	V	L	T	V			
EU Index	321	322	323	324	325	326	327	328	329			
1gG1	C	K	V	S	N	K	A	L	P			
1gG2	C	K	V	S	N	K	A	L	P			
1gG3	C	K	V	S	N	K	A	L	P			
1gG4	C	K	V	S	N	K	A	L	P			
CH3												
EU Index	341	342	343	344	345	346	347	348	349			
1gG1	G	G	P	R	E	P	Q	V	Y			
1gG2	G	G	P	R	E	P	Q	V	Y			
1gG3	G	G	P	R	E	P	Q	V	Y			
1gG4	G	G	P	R	E	P	Q	V	Y			
EU Index	362	363	364	365	366	367	368	369	370			
1gG1	D	V	S	L	T	C	L	V	K			
1gG2	D	V	S	L	T	C	L	V	K			
1gG3	D	V	S	L	T	C	L	V	K			
1gG4	D	V	S	L	T	C	L	V	K			
EU Index	383	384	385	386	387	388	389	390	391			
1gG1	S	N	C	G	P	E	N	N	Y			
1gG2	S	N	C	G	P	E	N	N	Y			
1gG3	S	N	C	G	P	E	N	N	Y			
1gG4	S	N	C	G	P	E	N	N	Y			
EU Index	404	405	406	407	408	409	410	411	412			
1gG1	F	F	L	Y	S	K	L	T	V			
1gG2	F	F	L	Y	S	K	L	T	V			
1gG3	F	F	L	Y	S	K	L	T	V			
1gG4	F	F	L	Y	S	K	L	T	V			
EU Index	426	427	428	429	430	431	432	433	434			
1gG1	C	S	V	M	H	E	A	L	H			
1gG2	C	S	V	M	H	E	A	L	H			
1gG3	C	S	V	M	H	E	A	L	H			
1gG4	C	S	V	M	H	E	A	L	H			
EU Index	446	447										
1gG1	G	N										
1gG2	G	N										
1gG3	G	N										
1gG4	G	N										

Figure 3

H9-Fc Fusions

Annotation Key:

- Signal Peptide (MYRMQLLSICIALSLALVTNS)** (SEQ ID NO:20)
- Gene of Interest**
- Linker (GGGGSGGGSGGGGS)** (SEQ ID NO:17)
- Tag (Fc)**
- H9 Mutations (L80F, R81D, L85V, I86V, and I92F)**
- Other IL-2 Mutations (F42A, Y45A, E62A)**
- Fc variant (N297A)**

Protein: wtIL2-Fc (Native IL-2 ss, hIgG1 N297A) 3x GGGGS linker
 Isotype: Human IgG1 (N297A)

MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMI~~L~~NGINNYKNPKLTRMLT~~F~~KFYMPKKATELKH~~L~~QCLEE
 ELKPLEEVLNLAQSKNFH~~L~~RPDLI~~S~~NI~~N~~VIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGGSGGGG
 SGGGSDKTHTCPPCPAPELLGGPSVFLFPPPKD~~T~~LMI~~S~~RTP~~E~~VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
 YASTYRVVSVLTVLHQD~~W~~LN~~G~~KEYKCKVSNKALPAPIEKTI~~S~~KA~~K~~GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD
 IAVEWESNGQPENNYK~~T~~TPPVLDSDGSFFLYSKLTV~~D~~KSRWQQGNV~~F~~SCSV~~M~~HEALHNHYTQKSLSLSPGK* (SEQ ID
 NO:11)

Protein: H9-Fc (Native IL-2 ss, hIgG1 N297A) 3x GGGGS linker
 Isotype: Human IgG1 (N297A)

MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMI~~L~~NGINNYKNPKLTRMLT~~F~~KFYMPKKATELKH~~L~~QCLEE
 ELKPLEEVLNLAQSKNFH~~L~~RPDLI~~S~~NI~~N~~VIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGGSGGGG
 SGGGSDKTHTCPPCPAPELLGGPSVFLFPPPKD~~T~~LMI~~S~~RTP~~E~~VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
 YASTYRVVSVLTVLHQD~~W~~LN~~G~~KEYKCKVSNKALPAPIEKTI~~S~~KA~~K~~GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD
 IAVEWESNGQPENNYK~~T~~TPPVLDSDGSFFLYSKLTV~~D~~KSRWQQGNV~~F~~SCSV~~M~~HEALHNHYTQKSLSLSPGK* (SEQ ID
 NO:12)

Protein: H9-FYAA-Fc (Native IL-2 ss, hIgG1 N297A) 3x GGGGS linker
 Isotype: Human IgG1 (N297A)

MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMI~~L~~NGINNYKNPKLTRMLT~~A~~KFA~~M~~PKKATELKH~~L~~QCLEE
 ELKPLEEVLNLAQSKNFH~~L~~RPDLI~~S~~NI~~N~~VIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGGSGGGG
 SGGGSDKTHTCPPCPAPELLGGPSVFLFPPPKD~~T~~LMI~~S~~RTP~~E~~VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
 YASTYRVVSVLTVLHQD~~W~~LN~~G~~KEYKCKVSNKALPAPIEKTI~~S~~KA~~K~~GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD
 IAVEWESNGQPENNYK~~T~~TPPVLDSDGSFFLYSKLTV~~D~~KSRWQQGNV~~F~~SCSV~~M~~HEALHNHYTQKSLSLSPGK* (SEQ ID
 NO:13)

Protein: H9-FEAA-Fc (Native IL-2 ss, hIgG1 N297A) 3x GGGGS linker
 Isotype: Human IgG1 (N297A)

MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMI~~L~~NGINNYKNPKLTRMLT~~A~~KFYMPKKATELKH~~L~~QCLEE
 ALKPLEEVLNLAQSKNFH~~L~~RPDLI~~S~~NI~~N~~VIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGGSGGGG
 SGGGSDKTHTCPPCPAPELLGGPSVFLFPPPKD~~T~~LMI~~S~~RTP~~E~~VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
 YASTYRVVSVLTVLHQD~~W~~LN~~G~~KEYKCKVSNKALPAPIEKTI~~S~~KA~~K~~GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD
 IAVEWESNGQPENNYK~~T~~TPPVLDSDGSFFLYSKLTV~~D~~KSRWQQGNV~~F~~SCSV~~M~~HEALHNHYTQKSLSLSPGK* (SEQ ID
 NO:14)

Protein: H9-FYEAAA-Fc (Native IL-2 ss, hIgG1 N297A) 3x GGGGS linker
 Isotype: Human IgG1 (N297A)

MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMI~~L~~NGINNYKNPKLTRMLT~~A~~KFA~~M~~PKKATELKH~~L~~QCLEE
 ALKPLEEVLNLAQSKNFH~~L~~RPDLI~~S~~NI~~N~~VIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGGSGGGG
 SGGGSDKTHTCPPCPAPELLGGPSVFLFPPPKD~~T~~LMI~~S~~RTP~~E~~VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
 YASTYRVVSVLTVLHQD~~W~~LN~~G~~KEYKCKVSNKALPAPIEKTI~~S~~KA~~K~~GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD
 IAVEWESNGQPENNYK~~T~~TPPVLDSDGSFFLYSKLTV~~D~~KSRWQQGNV~~F~~SCSV~~M~~HEALHNHYTQKSLSLSPGK* (SEQ ID
 NO:15)

Figure 4

ID	II-130a2 binding constants										II-130a2 binding constants								
	10	11	14	18	86	87	88	89	101	104	105	107	108	K1 (M ⁻¹ s ⁻¹)	K-1 (s ⁻¹)	KD (nM)	K1 (M ⁻¹ s ⁻¹)	K-1 (s ⁻¹)	KD (nM)
A5	I	R	I	V	I	K	G	S	R	R	K	F	R	5.21E+06	2.20E-02	4.28 nM	5.00E+07	9.40E-05	0.001 nM
A6	I	I	L	M	K	S			R	R	A	M		2.80E+06	9.20E-03	2.94 nM	-	-	50 nM
A7	I	I	I	I	G	S			R	R	A			1.77E+06	3.30E-03	1.9 nM	1.07E+07	2.80E-03	0.267 nM
A8	I	I	I	I	S	S			R	R	A			2.80E+06	3.40E-03	1.12 nM	1.80E+07	4.10E-03	0.218 nM
A11	V	I	I	I	S	S			R	R	Y			1.64E+06	3.30E-03	2.061 nM	1.01E+07	2.60E-03	0.263 nM
B1	I	I	I	I	S	S			F	R	Y			1.64E+07	1.30E-03	0.004 nM	1.62E+07	6.00E-03	0.391 nM
B2	I	I	I	I	S	S			Y	R	A			1.70E+06	1.70E-03	0.273 nM	7.60E+06	1.00E-02	1.5 nM
B4	S	I	I	I	K	G	S	M	Y	R	Y			1.36E+06	2.40E-02	1.8 nM	-	-	108 nM
B5	I	I	I	I	G	S			Y	R	Y			1.40E+06	9.20E-03	9.9 nM	-	-	53 nM
C2	D	I	I	I	K	K	R	R		R				1.50E+06	1.50E-04	0.025 nM	5.60E+06	1.50E-04	0.025 nM
C3	A	I	I	I	T	G	R	R	R	R	A			6.00E+06	2.00E-03	0.379 nM	6.00E+06	2.00E-03	0.379 nM
C4	V	I	I	I	I	S			R	R	E			1.94E+06	7.60E-04	0.393 nM	1.94E+06	7.60E-04	0.393 nM
C7	S	I	I	I	I	S			R	R	A			2.10E+06	5.70E-04	0.272 nM	2.10E+06	5.70E-04	0.272 nM
C9	M	I	I	I	R	E			R	R	Y			2.03E+06	5.20E-04	0.289 nM	2.03E+06	5.20E-04	0.289 nM
C10	M	L	I	I	I	M			R	R				1.19E+06	6.10E-03	5 nM	1.19E+06	6.10E-03	5 nM
C11	M	I	I	I	T	G	R		R	R				9.41E+06	9.18E-07	0.0001 nM	9.41E+06	9.18E-07	0.0001 nM
C12	M	I	I	I	M	S			R	R	A			7.61E+06	4.04E-04	0.053 nM	7.61E+06	4.04E-04	0.053 nM
D7	A	I	I	I	F	K	K	R	I	R				1.77E+07	5.37E-05	0.003 nM	1.77E+07	5.37E-05	0.003 nM

Figure 5

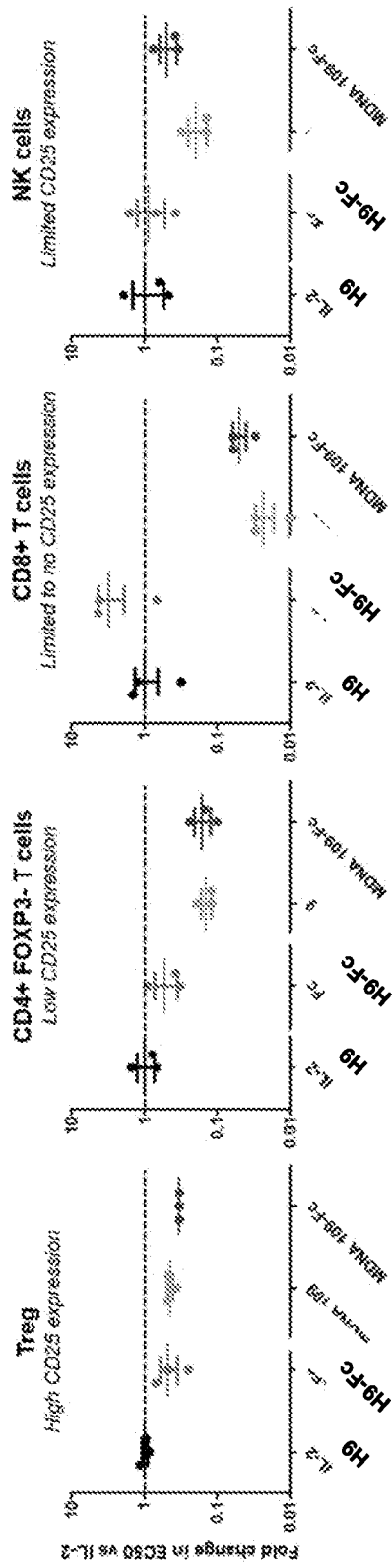


Figure 6

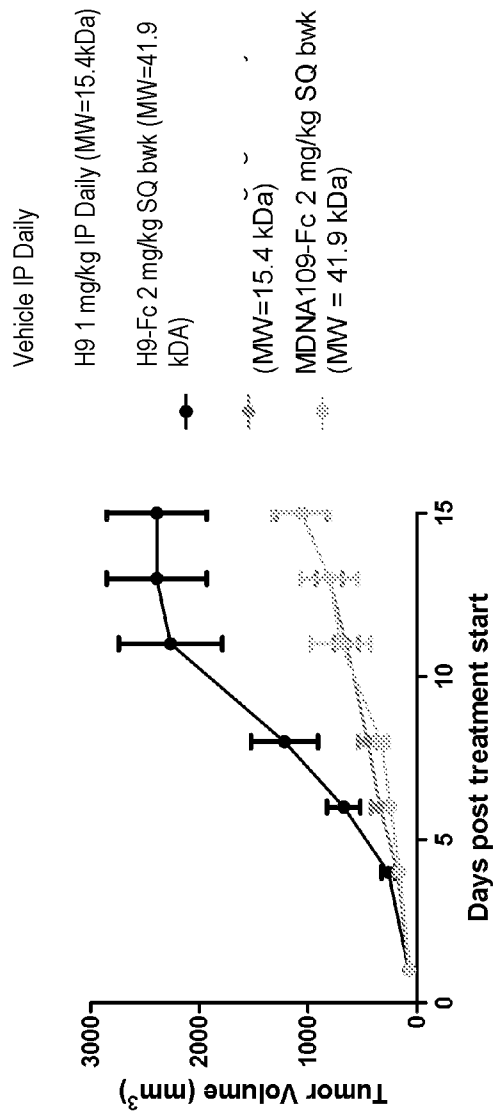


Figure 7A

Pembrolizumab (Anti-PD1 hgG4)	SEQ ID NO:
What sequence	
Variable heavy (vh) domain	EVQLVLSGGGFVQPGGSLKLSCAASGFTFSSYAMSWWRQNPERRLVVWVATITGGGRNTYYPDSVKGRFTIS RDNAKNTLYLQMSSLRSEDTAMYCTRQGDYTWFAWGGQGLTVVSS EVQLVLSGGGFVQPGGSLKLSCAASGFTFSSYAMSWWRQNPERRLVVWVATITGGGRNTYYPDSVKGRFTIS RDNAKNTLYLQMSSLRSEDTAMYCTRQGDYTWFAWGGQGLTVVSSASTKGPSVFLAPCSRSTSEST AALGCLVKDYFPEPTVSWNSGALTSVHTFPAVLQSSGLYSLSSVTPVSSSLGKTKYTCNVDHKPSNTKV DKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSDQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSV HEALHNHYTQKSLSLSPGK
Full length HC	
Variable light (vl) domain	DIVLTQSPTSLAVSLGQRATISCRASESDNSGISFMNWFQQKPGQPPKLLIYAASNPGSGVPAARFSGSGSGT DFSLNIHPMEEDDTAMYFCQQSQKEVPWTFGGGTELEIKR
Full length light chain	DIVLTQSPTSLAVSLGQRATISCRASESDNSGISFMNWFQQKPGQPPKLLIYAASNPGSGVPAARFSGSGSGT DFSLNIHPMEEDDTAMYFCQQSQKEVPWTFGGGTELEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNINFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C
Pembrolizumab (Anti-PD1 from WO2016028656)	SEQ ID NO:
What sequence	
Full length HC (SEQ ID NO:33 from WO2016028656)	QVQLVQSGVEVKKPGASVKVCKASGYTFTNYYMYWWRQAPGQGLEWMGINPSNGGTFNFKNRVTL TTDSSITTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGQGTITVSSASTKGPSVFLAPCSRSTSES TAALGCLVKDYFPEPTVSWNSGALTSVHTFPAVLQSSGLYSLSSVTPVSSSLGKTKYTCNVDHKPSNTK VDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSDQEDPEVQFNWYVDGVEV HNAKTKPREEQFNSTYRVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVYTLPPSQE E MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSV HEALHNHYTQKSLSLSPGK
Full length light chain (SEQ ID NO:34 from WO2016028656)	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESGVPAARFSGSGSGT DFTLTISLLEPEDFAVYYCQHSRDPLITFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNINFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 7B

Nivololumab (Anti-PD1 hlgG4)	SEQ ID NO:
What sequence	
Variable heavy (vh) domain	QVQLVESGGGVWQPGRSLRLDCKASGITFSNSGMHWRQAPGKGLEWAVIWDGSKRYADSVKGRFTI SRDNSKNTLFLQMNSLRAEDTAVYCATNDDYWGQGLVTVSS QVQLVESGGGVWQPGRSLRLDCKASGITFSNSGMHWRQAPGKGLEWAVIWDGSKRYADSVKGRFTI SRDNSKNTLFLQMNSLRAEDTAVYCATNDDYWGQGLTVVSSASTKGPSVFLAPCSRSTSESTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSWTPSSSLGKTYTCNVDHKPSNTKVDKRVESK YGPCCPCPAPEFLGGPSVFLPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPRE EQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVYTLPPSQEEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHREALHNY TQKLSLSLGLK
Full length HC	
Variable light (vl) domain	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLYDASNRATGIPARFSGSGGTDFTL TISLEPEDFAVYCCQQSSNWRPRTFGQGTKEIKR
Full length light chain	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLYDASNRATGIPARFSGSGGTDFTL TISLEPEDFAVYCCQQSSNWRPRTFGQGTKEIKRRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
Nivololumab (Anti-PD1 from WO2016028656)	SEQ ID NO:
What sequence	
Full length heavy chain (SEQ ID NO:35 from WO2016028656)	QVQLVESGGGVWQPGRSLRLDCKASGITFSNSGMHWRQAPGKGLEWAVIWDGSKRYADSVKGRFTI SRDNSKNTLFLQMNSLRAEDTAVYCATNDDYWGQGLTVVSSASTKGPSVFLAPCSRSTSESTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSWTPSSSLGKTYTCNVDHKPSNTKVDKRVESK YGPCCPCPAPEFLGGPSVFLPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPRE EQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVYTLPPSQEEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHREALHNY TQKLSLSLGLK
Full length light chain (SEQ ID NO:36 from WO2016028656)	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLYDASNRATGIPARFSGSGGTDFTL TISLEPEDFAVYCCQQSSNWRPRTFGQGTKEIKRRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 7C

Cemiplimab (REGN2810; anti-PD1)	SEQ ID NO:
What sequence	
Variable heavy (vh) domain	EVQLLESGGVLVQPGGSLRLSCAASGFTFSNFGMTWVRQAPGKGLEWWSGISGGGRDITYF ADSVKGRFTISRDNKNTLYLQMNSLKGEDTAVYYCVKWGNIYFDYWGGQLVTYSS
(SEQ ID NO:1 from US20170174779)	
Variable light (vl) domain	DIQMTQSPSSLSASVGDSTITCRASLINTFLNWWYQQKPKGKAPNLLIYAASSLHGGVPS
(SEQ ID NO:2 from US20170174779)	RFSGSGSGTDFTLTIRTLQPEDFATYYCQQSSNTPFTFGPGTVVDFR
Cemiplimab (REGN2810; anti-PD1)	SEQ ID NO:
What sequence	
Full length heavy chain	EVQLLESGGVLVQPGGSLRLSCAASGFTFSNFGMTWVRQAPGKGLEWWSGISGGGRDITYFADSVKGRFTIS RDNKNTLYLQMNSLKGEDTAVYYCVKWGNIYFDYWGGQLVTYSSASTKGPSVFLAPCSRSTSESTAAL GCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVWTPSSSLGKTYTCNVDHKPSNTKVKDKR VESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDITLISRTEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKT KPREEQFNSTYRWVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVYTLPPSQEEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNIVFSCSVMHREAL HNHYTQKSLSLGLGK
(SEQ ID NO:9 from US20170174779)	
Full length light chain	DIQMTQSPSSLSASVGDSTITCRASLINTFLNWWYQQKPKGKAPNLLIYAASSLHGGVPSRFSGGSGTDFTLTI RTLQPEDFATYYCQQSSNTPFTFGPGTVVDFRRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
(SEQ ID NO:10 from US20170174779)	

Figure 8A

From W02017100541 (Medimmune, LLC; also US20170306025; Durvalumab)	SEQ ID NO:
What Heavy chain (SEQ ID NO:2 from W02017100541)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMSWWRQAPGKGLEWAWISPYGGSTYYADSVKGR FTISRDNAKNSLYLQMNSLRAEDTAVYCARREGGWFGELAFDYWGQGLTVTVSS
Light chain (SEQ ID NO:1 from W02017100541)	EIVLTQSPGTLSPGERATLSCRASQRVSSSYLAWYQQKPGQAPRLLIYDASSRATGIPDRFSGSGSGT DFTLTISRLEPEDFAVYYCQQYGSLPWTFGGQGTKVEIK
From US20170281764 (JN Biosciences)	SEQ ID NO:
What Heavy chain (SEQ ID NO:34 from US2017281764)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWRQAPGKGLEWAWISPYGGSTYYADSVKGR FTISADTSKNTAYLQMNSLRAEDTAVYCARHWPGGFDYWGQGLTVVSAASTKGPSVFLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH KPSNTKVDKKEPKKSCDKHTHTCPPCPAPELGGPSVFLFPPKPKDITLMISRTEPEVTCWVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYASTYRVSVLTVLHQDWLNGKEYCKVSNKALPAIEKTIISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSGFFLYSKLTV DKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG
Light chain (SEQ ID NO:36 from US2017281764)	DIQMTQSPSSLSASVGRVTITCRASQDVSTAVAWYQQKPKAPKLLIYASFLYSGVPSRFSGSGSGT DFTLTISLQPEDFATYCCQYLFTPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKS FNRGEC

Figure 8B

From WO2015009856 (GENENTECH, INC. and F. HOFFMANN-LA ROCHE AG)			
What	sequence		SEQ ID NO:
Heavy chain (SEQ ID NO:23 from WO2015009856)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWWRQAPGKGLEWAWISPYGGSTYYADSVKGR FTISADTSKNTAYLQMNSLRAEDTAVYCARRHWPGGFDYWGQGLTVTSSA		
Light chain (SEQ ID NO:24 from WO2015009856)	DIQMTQSPSSLSASVGDRTITCRASQDVSTAVAWYQQKPKAPKLLIYSASFLYSGVPSRFSFGSGSGT DFTLTISSLQPEDFATYYCQQYLHPATFGGQTKVEIKR		
From WO2015009856 (GENENTECH, INC. and F. HOFFMANN-LA ROCHE AG)			
What	sequence		SEQ ID NO:
Heavy chain (SEQ ID NO:40 from WO2015009856)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWWRQAPGKGLEW A WISPYGG STYY ADSVKGRFTISADTSKNTA YLQMNSLRAEDTA VYCARRHWPGGFDYWGQG TLTVTSSASTK		
Light chain (SEQ ID NO:24 from WO2015009856)	DIQMTQSPSSLSASVGDRTITCRASQDVSTA VA WYQQKPKGKAPKLLIYSASF L YSG VPSRFSFGSGGTDFTLTISSLQPEDF ATYYCQQYL YHPATFGGQTKVEIKR		
From WO2015009856 (GENENTECH, INC. and F. HOFFMANN-LA ROCHE AG)			
What	sequence		SEQ ID NO:
Heavy chain (SEQ ID NO:41 from WO2015009856)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWWRQAPGKGLEWAWISPYGG STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTA VYCARRHWPGGFDYWGQG TLTVTSS		
Light chain (SEQ ID NO:24 from WO2015009856)	DIQMTQSPSSLSASVGDRTITCRASQDVSTAVAWYQQKPKAPKLLIYSASF L YSGVPSRFSFGSGSGT DFTLTISSLQPEDFATYYCQQYLHPATFGGQTKVEIKR		

Figure 8C

From US20160222117 (GENENTECH, INC.)			
What	sequence		SEQ ID NO:
Heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWWRQAPGKGLEWAWISPYGGSTYYADSVKGR		
(SEQ ID NO:20 from US20160222117)	FTISRDTSKNTAYLQMNSLRAEDTAVYFCARRHWPGGFDYWGQGLTVTUSA		
Heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWWRQAPGKGLEWAWISPYGGSTYYADSVKGR		
(SEQ ID NO:23 from US20160222117)	FTISRDTSKNTAYLQMNSLRAEDTAVYFCARRHWPGGFDYWGQGLTVTUSA		
Heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWWRQAPGKGLEWAWILPYGGSSYYADSVKGR		
(SEQ ID NO:24 from US20160222117)	FTISRDTSKNTAYLQMNSLRAEDTAVYFCARRHWPGGFDYWGQGLTVTUSA		
From US20160222117 (GENENTECH, INC.)			
What	sequence		SEQ ID NO:
Light chain	DIQMTQSPSSLSASVGDRTITCRASQDVSTAVAWYQQKPKAPKLLIYSASFLYSGVPSRFSGSGSGT		
(SEQ ID NO:21 from US20160222117)	DFTLTISLQPEDFATYCCQQYLHPATFGQGTKVEIKR		
Light chain	DIQMTQSPSSLSASVGDRTITCRASQDVSTAVAWYQQKPKAPKLLIYSASFLYSGVPSRFSGSGSGT		
(SEQ ID NO:26 from US20160222117)	DFTLTISLQPEDFATYCCQQYINVPWTFGQGTKVEIKR		
Light chain	DIQMTQSPSSLSASVGDRTITCRASQDVSTAVAWYQQKPKAPKLLIYSASFLYSGVPSRFSGSGSGT		
(SEQ ID NO:27 from US20160222117)	DFTLTISLQPEDFATYCCQQYAPPWTFGQGTKVEIKR		

Figure 8D

From US20160222117 (GENENTECH, INC.)		
What	sequence	SEQ ID NO:
Light chain (SEQ ID NO:28 from US20160222117)	DIQMTQSPSSLSASVGDRTVITCRASQDVSTAVAWYQQKPKAPKLLIYSASFLYSGVPSRFRFSGSGGT DFTLTISLQPEDFATYCCQQYYTVPTWTFGGGKTKVEIKR	
Light chain (SEQ ID NO:29 from US20160222117)	DIQMTQSPSSLSASVGDRTVITCRASQVINTFLAWYQQKPKAPKLLIYSASTLASGVPSRFRFSGSGGTD FTLTISLQPEDFATYCCQQYYTVPTWTFGGGKTKVEIKR	
Light chain (SEQ ID NO:30 from US20160222117)	DIQMTQSPSSLSASVGDRTVITCRASQDVSTAVAWYQQKPKAPKLLIYSASFLYSGVPSRFRFSGSGGT DFTLTISLQPEDFATYCCQQYGVPRTFGGGKTKVEIKR	
Light chain (SEQ ID NO:31 from US20160222117)	DIQMTQSPSSLSASVGDRTVITCRASQDVSTAVAWYQQKPKAPKLLIYSASFLYSGVPSRFRFSGSGGT DFTLTISLQPEDFATYCCQYLFTPTTFGGGKTKVEIKR	
Light chain (SEQ ID NO:32 from US20160222117)	DIQMTQSPSSLSASVGDRTVITCRASQDVSTAVAWYQQKPKAPKLLIYSASFLYSGVPSRFRFSGSGGT DFTLTISLQPEDFATYCCQYFPTTFGGGKTKVEIKR	
Light chain (SEQ ID NO:33 from US20160222117)	DIQMTQSPSSLSASVGDRTVITCRASQDVSTAVAWYQQKPKAPKLLIYSASFLYSGVPSRFRFSGSGGT DFTLTISLQPEDFATYCCQQYYTPTTFGGGKTKVEIKR	
Light chain (SEQ ID NO:34 from US20160222117)	DIQMTQSPSSLSASVGDRTVITCRASQDVSTAVAWYQQKPKAPKLLIYSASFLYSGVPSRFRFSGSGGT DFTLTISLQPEDFATYCCQFFYPTPTTFGGGKTKVEIKR	
From WO2013079174 (Merck; Avelumab or A09-246- 2)	sequence	SEQ ID NO:
What	sequence	
Heavy chain (SEQ ID NO:32 from WO2013079174)	EVQLLESGGGLVQPGGSLRSLSCAASGFTFSYIMMWVRRQAPGKGLWVSSIYPSGGITFYADTVKGRFT ISRDNSKNTLYLQMNSLRAEDTAVYCARIKLGTVTVDYWGQGLTVTVSSASTKGPSVFPFLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHK PSNTKVDKVKVEPKSCDKTHITCPAPELLEGPSVFLFPPKPKDTLMISRTPEVTCVAVDVSHEDEPKF NWAYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTIKAKGQPR EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	
Light chain (SEQ ID NO:33 from WO2013079174)	QSALTQPASVSGSPGQSITISCTGTSSDVGNGYNYVSWYQQHPGKAPKLMYDVSNRPSGVSNRFGSGK SGNTASLTISGLQAEADYYCSSYTSSTRVFGTGKTVLGGPKANPTVTLFPPSSEELQANKATLVK LISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNINKYAASSYLSLTPEQWKSHRYSQCQVTHEGSTV EKTVAPTESCS	

Figure 8F

From US8217149 (Hoffman La Roche: Atezolizumab)		
What	sequence	SEQ ID NO:
Heavy chain (VH) (SEQ ID NO:20 from US8217149)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWWRQAPGKGLEWAWISPYGGSTYYADSVKGR FTISADTSKNTAYLQMNSLRAEDTAVYCARHWPGGFDYWGQGLTVTSA	
Light chain (VL) (SEQ ID NO:21 from US8217149)	DIQMTQSPSSLSASVGDRTTTCRASQDVSTAVAWYQQKPKGKAPKWWYASFLYSGVPSRFRSGSGGTD FTLTISLQPEDFATYYC QQYLYH PATFGGQTKVEIKR	
Atezolizumab (alternate sequence)	sequence	SEQ ID NO:
What		
Heavy chain (from IMGt database or DrugBank database)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWWRQAPGKGLEWAWISPYGGSTYYADSVKGR FTISADTSKNTAYLQMNSLRAEDTAVYCARHWPGGFDYWGQGLTVTSSASTKGPSVFLAPSSKST SGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH KPSNTKVDKKEPKSCDKTHITCPAPELGGPSVFLFPPKPKDTLMISRTPEVTCWVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYASTYRWVSLTVLHQDWLNGKEYCKVSNKALPAIEKTIKAKGQP REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDSDGFFFLYSKLT DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	
Light chain (from IMGt database or DrugBank database)	DIQMTQSPSSLSASVGDRTTTCRASQDVSTAVAWYQQKPKGKAPKLLYSASFLYSGVPSRFRSGSGGT DFTLTISLQPEDFATYYCQQYLYHPATFGGQTKVEIKRTVAAPSVFIFPPSDEQLKSGTASWCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSSLSPVTKS FNRGEC	

Figure 9A

<u>Adenoviruses used as oncolytic agents</u>		
Name (serotype)	Basis of tumor-selective propagation	Therapeutic traits
Ad wild type (various serotypes)	None	Oncolysis
Ad5/IFN (Ad5)	None	Oncolysis & immuno-stimulatory gene therapy
A1520 or Onyx015 (Ad2/5)	E1b55kDa-deletion abrogates p53 binding	Oncolysis
AdTK ^{R/C}	E1b55kDa-deletion abrogates p53 binding	Oncolysis & suicide gene therapy (TK)
Ad-5-CD-TKrep or FGR (ad5)	E1b55kDa-deletion abrogates p53 binding	Oncolysis & suicide gene therapy (CD + TK)
AdvE1AΔB-F/K20 (Ad5)	E1b55kDa-deletion abrogates p53 binding	Oncolysis with enhanced infectivity
AxElAdB (Ad5) & AdCAhIL-2 (Ad5)	E1b55kDa-deletion abrogates p53 binding	Oncolysis & immuno-stimulatory gene therapy
AdD24 (Ad5)	E1a deletion abrogates Rb binding	Oncolysis
CN706 (Ad5)	Regulation of E1a under the PSA promoter	Oncolysis
CN763 (Ad5)	Regulation of E1a under the kallikrein 2 promoter	Oncolysis
CN764 (Ad5)	Regulation of E1a under the PSA promoter and E1b under the kallikrein 2 promoter	Oncolysis
CV739	Regulation of E1a under rat probasin promoter and E1b under human PSA promoter	Oncolysis
<u>Adenoviruses used as oncolytic agents</u>		
Name (serotype)	Basis of tumor-selective propagation	Therapeutic traits
CV787	Regulation of E1a under rat probasin promoter and E1b under human PSA promoter	Oncolysis (enhanced compared with CV739 due to the presence of E)
AvE1a941 GT5610 (Ad5) + AdH1B (Ad5)	Regulation of E1a under the AFP promoter Regulation of E1a under the AFP promoter	Oncolysis Oncolysis
DE337 (Ad5)	None	Oncolysis (enhanced due to E1b-19 kDa deletion)
D1316 (Ad5)	The complete deletion of E1a makes this mutant dependent on Nrmic or ML-6-induced E1a-like activity	Oncolysis
D1118 (Ad5)	The complete deletion of E1b abrogates p53 binding; however E1a-induced apoptosis is not inhibited by E1b-19 kDa	Oncolysis

Figure 9B

<u>Replication-Selective Viruses in Clinical Trials</u>					
Parental Strain	Agent	Clinical phase	Tumor targets in clinical trials	Genetic alterations	Cell phenotype allowing selective replication
<u>Engineered</u>					
Adenovirus (2/5 chimera)	②1520②	I-III	SCCHN Colorectal Ovarian Pancreatic	E1B-55-kD gene deletion	Controversial cells lacking p53 function (for example, deletion, mutation), other?
Adenovirus (serotype 5)	CN706 CN787	I I	Prostate	E1A expression driven by PSE element E1A driven by rat probasin promoter/ E1B by PSE/promoter/enhancer	Prostate cells (malignant, normal)
Adenovirus (2/5 chimera)	Ad5-CD9k-rep	I	Prostate	E1B-55-kD gene deletion	Controversial cells lacking p53 function (for example, deletion, mutation), other?
Herpes simplex virus-1	G207	I-II	GBM	Insertion of HSV-tk/CD fusion gene ribonucleotide reductase disruption (locZ insertion into ICP6 gene) neuropathogenesis gene mutation (γ-34.5 gene)--both copies	Proliferating cells
Herpes simplex virus-1	NV1020	I	Colorectal	neuropathogenesis gene mutation (γ-34.5 gene)--single copy	Proliferating cells
Vaccinia virus	Wild-type = GM-CSF	I	Melanoma	For selectivity: none or ② deletion immunostimulatory gene (GM-CSF) insertion	Unknown
<u>Non-engineered</u>					
Newcastle Disease virus	73-T	I	Bladder SCCHN Ovarian	Unknown (serial passage on tumor cells)	Loss of IFN response in tumor cells
Autonomous parvoviruses	R-I	I		None	Transformed cells ↑ proliferation ↓ differentiation ras, p53 mutation
Reovirus	Reofysin	I	SCCHN	None	Ras-pathway activation (for example, ras mutation, EGFR signalling)