

control sequences active in the target cell. In some embodiments, the pharmaceutically acceptable formulation may comprise one or more supplementary agents.

[00030] The present disclosure provides a method for the prevention and/or treatment of neoplastic disease in a mammalian subject the method comprising the step of administering to the subject a pharmaceutically acceptable dosage form comprising a therapeutically or prophylactically effective amount of an hIL2 mutein or vector encoding a hIL2 mutein of the present disclosure to a subject in need of treatment.

[00031] The present disclosure further provides a kit, the kit comprising a pharmaceutically acceptable dosage form of an hIL2 mutein of the present disclosure and instructions for use. The pharmaceutical dosage form may be provided in a pre-filled syringe. The kit may optionally further provide a quantity of a solution for admixture with the pharmaceutically acceptable dosage form of an hIL2 mutein, wherein the solution includes but is not limited to one or more of diluents, reconstitution buffers, activating agents, formulators, tonicity agents, or components of a controlled release formulation including but not limited to biodegradable or bioerodible biocompatible polymers. The kit may optionally provide a pharmaceutically acceptable formulation of comprising one or more supplementary agents. The kit may optionally include medical devices to facilitate administration (e.g. a syringe, or autoinjector device). The kit may also provide one or more components for maintaining the kit and its components at a refrigerated temperature for extended periods of time such as one or more gel ice packs and/or insulated packaging.

[00032] Garcia, *et al.* (International Application Number PCT/US2018/062122, PCT International Publication No. WO 2019/104092 A1 published May 31, 2019, hereinafter “Garcia ‘092”) describes certain IL2 muteins having modifications including positions 18, 22 and 126 that, among other things, exhibit diminished binding for CD132 while retaining partial IL2 activity that are useful in the practice of the presently described methods.

[00033] In some embodiments, the disclosure provides a method of treating a subject suffering from a neoplastic disease, disorder or condition by the administration to said subject of polypeptide that is at least 95% homologous to a polypeptide of the formula: 1 ([SEQ ID NO: 10](#)):

[00034] (AA1)a-(AA2)b-(AA3)c-(AA4)d-(AA5)e-(AA6)f-(AA7)g-(AA8)h-(AA9)i-T10-Q11-L12-Q13-L14-E15-H16-L17-(AA18)-L19-D20-L21-(AA22)-M23-I24-L25-N26-G27-I28-N29-N30-Y31-K32-N33-P34-(AA35)-L36-T37-(AA38)-(AA39)-L40-T41-F42-K43-F44-Y45-M46-P47-K48-K49-A50-T51-

E52-L53-K54-(AA55)-L56-Q57-C58-L59-E60-E61-E62-L63-K64-P65-L66-E67-E68-(AA69)-L70-N71-L72-A73-(AA74)-S75-K76-N77-F78-H79-(AA80-(AA81)-P82-R83-D84-(AA85)-(AA86)-S87-N88-(AA89)-N90-(AA91)-(AA92)-V93-L94-E95-L96-(AA97)-G98-S99-E100-T101-T102-F103-(AA104)-C105-E106-Y107-A108-(AA109)-E110-T111-A112-(AA113)-I114-V115-E116-F117-L118-N119-R120-W121-I122-T123-F124-(AA125)-(AA126)-S127-I128-I129-(AA130)-T131-L132-T133

wherein:

each of a, b, c, d, e, f, g, h, and i is individually selected from 0 or 1;

AA1 is A (wild type, a=1) or deleted (a=0);

AA2 is P (wild type, b=1) or deleted (b=0);

AA3 is T (wild type, c=1), C, A, G, Q, E, N, D, R, K, P, or deleted (c=0);

AA4 is S (wild type, d=1) or deleted (d=0);

AA5 is S (wild type, e=1) or deleted (e=0);

AA6 is S (wild type, f=1) or deleted (f=0);

AA7 is T (wild type, g=1) or deleted (g=0);

AA8 is K (wild type, h=1) or deleted (h=0);

AA9 is K (wild type, i=1) or deleted (i=0);

AA18 is L (wild type) or R, L, G, M, F, E, H, W, K, Q, S, V, I, Y, H, D or T;

AA22 is Q (wild type) or F, E, G, A, L, M, F, W, K, S, V, I, Y, H, R, N, D, T, or F;

AA35 is K (wildtype) or E;

AA38 is R (wild type), W or G;

AA39 is M (wildtype), L or V;

AA55 is H (wildtype) or Y;

AA69 is V (wildtype) or A;

AA74 is Q (wild type), P, N, H, S;

AA80 is L (wild type), F or V;

AA81 is R (wild type), I, D or T;

AA85 is L (wild type) or V;

AA86 is I (wild type) or V;

AA89 is I (wild type) or V;

[AA91 is V \(wild type\), R or K;](#)

AA92 is I (wild type) or F;

AA97 is K (wild type) or Q;

[00046] In some embodiments, the neoplastic disease disorder or condition is selected from the group consisting of: adenomas, fibromas, hemangiomas, hyperplasia, atypia, metaplasia, dysplasia, carcinomas, leukemias, breast cancers, sarcomas, leukemias, lymphomas, genitourinary cancers, ovarian cancers, urethral cancers, bladder cancers, prostate cancers, gastrointestinal cancers, colon cancers, esophageal cancers, stomach cancers, lung cancers; myelomas; pancreatic cancers; liver cancers; kidney cancers; endocrine cancers; skin cancers; gliomas, neuroblastomas, astrocytomas, myelodysplastic disorders; cervical carcinoma-in-situ; intestinal polyposes; oral leukoplakias; histiocytoses, hyperproliferative scars including keloid scars, respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, breast carcinomas, prostatic carcinomas, endocrine system carcinomas, melanomas, adenocarcinomas, myeloproliferative neoplasms, myeloid and lymphoid disorders with eosinophilia, myeloproliferative/myelodysplastic neoplasms, myelodysplastic syndromes, acute myeloid leukemia and related precursor neoplasms, and acute leukemia of ambiguous lineage, promyeloid leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML), precursor lymphoid neoplasms, mature B-cell neoplasms, mature T-cell neoplasms, Hodgkin's Lymphoma, and immunodeficiency-associated lymphoproliferative disorders, 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). erythroblastic leukemia and acute megakaryoblastic leukemia, malignant lymphomas including, but are not limited to, non-Hodgkins lymphoma and variants thereof, peripheral T cell lymphomas, adult T-cell leukemia/lymphoma (ATL), cutaneous T cell lymphoma (CTCL), large granular lymphocytic leukemia (LGL), Hodgkin's disease and Reed-Stemberg disease.

[00047] In some embodiments, the method comprises administering to said subject a therapeutically effective amount of an hIL2 mutein of sufficient to maintain a serum concentration greater than about 50% of a period of time of at least 24 hours at or above the effective concentration of the IL2 mutein sufficient to promote proliferation of CD3-activated primary human T-cells at or below a serum concentration of such IL2 mutein sufficient to induce activation of T-cells with respect to such IL2 mutein.

[00047a] [The present disclosure further provides a use of a polypeptide comprising a sequence of formula 1:](#)

(AA1)-(AA2)-(AA3)-(AA4)-(AA5)-(AA6)-(AA7)-(AA8)-
 (AA9)-T10-Q11-L12-Q13-L14-E15- H16-L17-(AA18)-L19-D20-L21-
 (AA22)-M23-I24-L25- N26-G27-I28-N29-N30-Y31-K32-N33-P34-
 (AA35)-L36- T37-(AA38)-(AA39)-L40-T41-F42-K43-F44-Y45-M46-
 P47-K48-K49-A50-T51-E52-L53-K54-(AA55)-L56-Q57-C58-L59-
 E60-E61-E62-L63-K64-P65-L66-E67-E68-(AA69)-L70-N71-L72-
 A73-(AA74)-S75-K76-N77-F78- H79-(AA80)-(AA81)-P82-R83-D84-
 (AA85)-(AA86)-S87- N88-(AA89)-N90-(AA91)-(AA92)-V93-L94-
 E95-L96- (AA97)-G98-S99-E100-T101-T102-F103-(AA104)-C105-
 E106-Y107-A108-(AA109)-E110-T111-A112-(AA113)-I114-V115-
 E116-F117-L118-N119-R120-W121-I122- T123-F124-(AA125)-
 (AA126)-S127-I128-I129- (AA130)-T131-L132-T133

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in the manufacture of a medicament for treating a human subject suffering from a neoplastic disease,
wherein the polypeptide is to be administered to said subject,

wherein:

AA18 is selected from the group consisting of L, R, G, M, F, E, W, K, Q, S, V, I, Y, H, D and T;

AA22 is selected from the group consisting of Q, E, G, A, L, M, W, K, S, V, I, Y, H, R, N, D, T and
F; and

AA126 is selected from the group consisting of H, M, K, C, D, E, G, I, R and S,

wherein AA18 is not L when AA22 is Q,

and wherein:

AA1 is A or deleted;

AA2 is P or deleted;

AA3 is T, C, A, G, Q, E, N, D, R, K, P or deleted;

AA4 is S or deleted;

AA5 is S or deleted;

AA6 is S or deleted;

AA7 is T or deleted;

AA8 is K or deleted;

AA9 is K or deleted;

AA35 is K or E;

AA38 is R, W or G;

AA39 is M, L or V;

AA55 is H or Y;

AA69 is V or A;

AA74 is Q, P, N, H or S;

AA80 is L, F or V;

AA81 is R, I, D or T;

AA85 is L or V;

AA86 is I or V;

AA89 is I or V;

AA91 is V, R or K

AA92 is I or F;

AA97 is K or Q;

AA104 is M or A;

AA109 is D or C;

AA113 is T or N;

AA125 is C, A or S;

AA130 is S, T, G or R; and

wherein (a) said polypeptide does not comprise the group of amino acid substitutions 80F, 81D, 85V, 86V and 92F; (b) the polypeptide exhibits diminished binding to CD132 relative to wild-type hIL2 of SEQ ID NO:1 and (c) (i) the ratio of pSTAT5 induction in CD25^{pos} T cells relative to pSTAT5 induction in CD25^{neg} T cells upon contacting the CD25^{pos} and CD25^{neg} T cells with the polypeptide, is greater than (ii) the ratio of pSTAT5 induction in CD25^{pos} T cells relative to pSTAT5 induction in CD25^{neg} T cells upon contacting with the CD25^{pos} and CD25^{neg} T cells with wild-type hIL2 of SEQ ID NO:1.

[00047b] The present disclosure further provides Use of a polypeptide comprising a sequence of formula 1:

(AA1)-(AA2)-(AA3)-(AA4)-(AA5)-(AA6)-(AA7)-(AA8)-
 (AA9)-T10-Q11-L12-Q13-L14-E15- H16-L17-(AA18)-L19-D20-L21-
 (AA22)-M23-I24-L25- N26-G27-I28-N29-N30-Y31-K32-N33-P34-
 (AA35)-L36- T37-(AA38)-(AA39)-L40-T41-F42-K43-F44-Y45-M46-
 P47-K48-K49-A50-T51-E52-L53-K54-(AA55)-L56-Q57-C58-L59-
 E60-E61-E62-L63-K64-P65-L66-E67-E68-(AA69)-L70-N71-L72-
 A73-(AA74)-S75-K76-N77-F78- H79-(AA80)-(AA81)-P82-R83-D84-
 (AA85)-(AA86)-S87- N88-(AA89)-N90-(AA91)-(AA92)-V93-L94-
 E95-L96- (AA97)-G98-S99-E100-T101-T102-F103-(AA104)-C105-
 E106-Y107-A108-(AA109)-E110-T111-A112-(AA113)-I114-V115-
 E116-F117-L118-N119-R120-W121-I122- T123-F124-(AA125)-
 (AA126)-S127-I128-I129- (AA130)-T131-L132-T133

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in the manufacture of a medicament for treating a human subject suffering from a neoplastic disease,

wherein

AA18 is selected from the group consisting of L, R, G, M, F, E, W, K, Q, S, V, I, Y, H, D and T;

AA22 is selected from the group consisting of Q, E, G, A, L, M, W, K, S, V, I, Y, H, R, N, D, T and F; and

AA126 is selected from the group consisting of H, M, K, C, D, E, G, I, R and S,

wherein AA18 is not L when AA22 is Q,

and wherein:

AA1 is A or deleted;

AA2 is P or deleted;

AA3 is T, C, A, G, Q, E, N, D, R, K, P or deleted;

AA4 is S or deleted;

AA5 is S or deleted;

AA6 is S or deleted;

AA7 is T or deleted;

AA8 is K or deleted;

AA9 is K or deleted;

AA35 is K;

AA38 is R, W or G;

AA39 is M, L or V;

AA55 is H or Y;

AA69 is V or A;

AA74 is Q, N, H or S;

AA80 is L, F or V;

AA81 is R, I, D or T;

AA85 is L or V;

AA86 is I or V;

AA89 is I or V;

AA91 is V, R or K

AA92 is I or F;

AA97 is K or Q;

AA104 is M or A;

AA109 is D or C;

AA113 is T or N;

AA125 is C, A or S;

AA130 is S, T or R; and

wherein (a) said polypeptide does not comprise the group of amino acid substitutions 80F, 81D, 85V, 86V and 92F; (b) the polypeptide exhibits diminished binding to CD132 relative to wild-type hIL2 of SEQ ID NO:1 and (c) (i) the ratio of pSTAT5 induction in CD25^{pos} T cells relative to pSTAT5 induction in CD25^{neg} T cells upon contacting the CD25^{pos} and CD25^{neg} T cells with the polypeptide, is greater than (ii) the ratio of pSTAT5 induction in CD25^{pos} T cells relative to pSTAT5 induction in CD25^{neg} T cells upon contacting with the CD25^{pos} and CD25^{neg} T cells with wild-type IL2 of SEQ ID NO:1.

BRIEF DESCRIPTION OF THE FIGURES

Nomenclature Conventions:

[000154] In some embodiments, the hIL2 muteins of the present disclosure comprise substitutions, deletions, or insertions relative to the wt hIL2 (SEQ ID NO:1) amino acid sequence. Residues may be designated herein by the one-letter or three-letter amino acid code followed by the wt hIL2 amino acid position, e.g., “Cys125” or “C125” refers to the cysteine residue at position 125 of wt hIL2 (SEQ ID NO:1). The following nomenclature is used herein to refer to substitutions, deletions or insertions. Substitutions are designated herein by the one letter amino acid code for the wt hIL2 residue followed by the IL2 amino acid position followed by the single letter amino acid code for the new substituted amino acid. For example, “K35A” refers to a substitution of the lysine (K) residue at position 35 of Sequence ID No. 1 with an alanine (A) residue. A deletion is referred to as “des” followed by the amino acid residue and its position in wt hIL2 (SEQ ID NO:1). For example the term “des-Ala1” or “desA1” refers to the deletion of the alanine at position 1 of the polypeptide of wt hIL2 (SEQ ID NO:1).

hIL2 Muteins

[000155] In some embodiments, the hIL2 muteins useful in the practice of the methods of the present disclosure that are partial agonists have one or more reduced functions as compared to wt hIL2. In some embodiments, the hIL2 mutein consists of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acid substitutions, modifications or deletions as compared to wt hIL2 (SEQ ID NO: 1).

[000156] The present disclosure provides compositions comprising and methods employing a human IL2 (“hIL2”) mutein useful in the treatment and/or prevention of neoplastic disease, wherein the human IL2 mutein exhibits decreased binding affinity for CD132 relative to wild-type hIL2 (“wt hIL2”, SEQ ID NO:1) polypeptides, comprising the amino acid sequence according to the following Formula 1 ([SEQ ID NO: 10](#)):

- AA81 is R (wild type), I, D or T;
- AA85 is L (wild type) or V;
- AA86 is I (wild type) or V;
- AA89 is I (wild type) or V;
- [AA91 is V \(wild type\), R or K;](#)
- AA92 is I (wild type) or F;
- AA97 is K (wild type) or Q;
- AA104 is M (wild type) or A;
- AA109 is D (wildtype), C or a non-natural amino acid with an activated side chain;
- AA113 is T (wild type) or N;
- AA125 is C (wild type), A or S;
- AA126 is Q (wild type) or H, M, K, C, D, E, G, I, R, S, or T; and
- AA130 is S (wild type), T, G or R.

[000157] The hIL2 muteins of the present disclosure possesses decreased binding affinity to hCD132 (SEQ ID NO:5) or the extracellular domain of hCD132 if the hIL2 mutein binds with <70%, alternatively <65%, alternatively <60%, alternatively <55%, alternatively <50%, alternatively <45%, alternatively <40%, alternatively <35%, alternatively <25%, alternatively <20%, alternatively <15%, alternatively <10%, or alternatively <5% of the affinity of wt hIL2 (SEQ ID NO:1) for hCD132 (or the extracellular domain thereof).

[000158] In certain embodiments, the hIL2 mutein disrupts the association of the CD122 with the CD132 such that this CD122/CD132 interaction is reduced by about 2%, about 5%, about 10%, about 15%, about 20%, about 50%, about 75%, about 90%, about 95% or more relative to wild type hIL2.

[000159] In some embodiments, the hIL2 mutein exhibits decreased binding affinity for CD132 relative wt hIL2 and retains significant binding affinity for CD122 and/or CD25.

[000160] In some embodiments, the hIL2 mutein exhibits decreased binding affinity for CD132 relative to wt hIL2 and exhibits binding affinity for hCD122 (SEQ ID NO:3), or the ECD thereof (SEQ ID NO:4), comparable to or greater than wt hIL2. An IL2 mutein retains binding affinity for hCD122 (or the ECD thereof) comparable to or greater than wt hIL2 if the hIL2 mutein binds to hCD122 (or the ECD thereof) with greater than about 50%, alternatively >60%, alternatively >65%, alternatively >70%, alternatively >75%, alternatively

(Figure 6). As indicated by the data presented, expression of the trimeric, high affinity IL-2R renders the NKL cells responsive to STK-012. The biological activity of both wild-type human IL-2 and STK-012 was evaluated in a proliferation assay using the NKL cell line, a human lymphoblastic NK cell line. Specificity of STK-012 for CD25 expressing cells was established in a pSTAT5 activity assay in YT cells and YT CD25. As shown in the data presented in Figure 7 of the attached drawings, human IL-2 and STK-012 induced proliferation of NKL cells in a similar dose range demonstrating that STK-012 retains the ability to induce pSTAT5 in a human immune cell comparable to wt hIL2.

[000201] STK-014: An STK-012 Murine Surrogate For Efficacy Studies in Mice: The interface between IL2 and its receptor components is slightly different between rodent (e.g mouse) IL2 and primate (e.g., human) IL2 molecules. Consequently, to demonstrate the activity of the human IL2 muteins in murine models, a representative human IL2 mutein of the present disclosure was selected (STK-012) and a murine IL2 mutein surrogate (STK-014) was developed for *in vivo* studies in mice to correlate activity between the rodent (mouse) and primate (human) environments. The amino acid sequence of the murine IL2 (mIL2) polypeptide component of STK-014 is:

A P T S S S T S S S T A E A Q Q Q Q Q H L E Q L R M D L E E L L S R M E
 N Y R N L K L P R M L T F K F Y L P K Q A T E L K D L Q C L E D E L G
 P L R H V L D L T Q S K S F Q L E D A E N F I S N I R V T V V K L K G S
 D N T F E C Q F D D E S A T V V D F L R R W I A F C H S I I S T S P Q
[\(SEQ ID NO: 11\)](#)

The foregoing IL2 polypeptide is N-terminally PEGylated with a PEG linker of the structure as used in the preparation of STK-010 and STK-012 above to complete the STK-014 molecule.

[000202] To demonstrate that STK-014 represents is a valid surrogate for STK-012, a study was conducted to evaluate target specificity on mouse and human cells. A study was performed to evaluate the relative potencies of human wild type IL-2 (huIL-2) and STK-012 by comparing the EC50 of each molecule in inducing phospho-STAT5 (pSTAT5) in primary human CD8+ T cells, activated by anti-CD3/anti-CD28 stimulation, and in primary human NK cells. Both cell types were isolated from fresh donor peripheral blood monocyctic cells (PBMCs). As the STK-014 is a murine IL2 mutein, it was tested on equivalent cell populations freshly isolated from mouse spleen. The results of this study are provided in Table 8 below:

practice of the present invention are peptides comprising 3-6 contiguous histidine residues ([SEQ ID NO: 12](#)) such as a six-histidine peptide (His)₆ ([SEQ ID NO: 13](#)) and are frequently referred to in the art as “His-tags.”

[000256] Targeted hIL2 Muteins: In some embodiments, the IL2 mutein is provided as a fusion protein with a polypeptide sequence (“targeting domain”) to facilitate selective binding to particular cell type or tissue expressing a cell surface molecule that specifically binds to such targeting domain, optionally incorporating a linker molecule of from 1-40 (alternatively 2-20, alternatively 5-20, alternatively 10-20) amino acids between the IL2 mutein sequence and the sequence of the targeting domain of the fusion protein. In other embodiments, a chimeric polypeptide including a hIL2 mutein 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. Nucleic Acid Molecules Encoding Mutant IL2.

[000257] In some embodiments, the targeting domain of the hIL2 mutein fusion protein specifically binds to a cell surface molecule of a tumor cell. In one embodiment wherein the ECD of the CAR of a CAR-T cell specifically binds to CD-19, the IL2 mutein may be provided as a fusion protein with a CD-19 targeting moiety. For example, in one embodiment wherein the ECD of the CAR of an CAR-T cell is an scFv molecule that provides specific binding to CD-19, the IL2 mutein is provided as a fusion protein with a CD-19 targeting moiety such as a single chain antibody (e.g., an scFv or VHH) that specifically binds to CD-19. In some embodiments, the fusion protein comprises an hIL2 mutein and the anti-CD19 scFv FMC63 (Nicholson, et al. (1997) Mol Immunol 34: 1157–1165).

[000258] Similarly, in some embodiments wherein the ECD of the CAR of the CAR-T cell specifically binds to BCMA, the hIL2 mutein may be provided as a fusion protein with a BCMA targeting moiety, such as antibody comprising the CDRs of anti-BCMA antibodies as described in Kalled, *et al.* (United States Patent 9,034,324 issued May 9, 2015) or antibodies comprising the CDRs as described in Brogdon, et al., (United States Patent No. 10,174,095 issued January 8, 2019). In some embodiments the hIL2 mutein may be provided as a fusion protein with a GD2 targeting moiety, such as an antibody comprising the CDRs of described in Cheung, et al., (United States Patent No. 9,315,585 issued April 19, 2016) or the

the herpes simplex gD signal. When the recombinant host cell is a yeast cell such as *Saccharomyces cerevisiae*, the alpha mating factor secretion signal sequence may be employed to achieve extracellular secretion of the IL2 mutein into the culture medium as described in Singh, United States Patent No. 7,198,919 B1 issued April 3, 2007.

[000274] In the event the IL2 mutein to be expressed is to be expressed as a chimera (e.g., a fusion protein comprising an IL2 mutein and a heterologous polypeptide sequence), the chimeric protein can be encoded by a hybrid nucleic acid molecule comprising a first sequence that encodes all or part of the hIL2 mutein and a second sequence that encodes all or part of the heterologous polypeptide. For example, subject hIL2 muteins described herein may be fused to a hexa-histidine tag ([SEQ ID NO: 13](#)) to facilitate purification of bacterially expressed protein, or to a hemagglutinin tag to facilitate purification of protein expressed in eukaryotic cells. By first and second, it should not be understood as limiting to the orientation of the elements of the fusion protein and a heterologous polypeptide can be linked at either the N-terminus and/or C-terminus of the IL2 mutein. For example, the N-terminus may be linked to a targeting domain and the C-terminus linked to a hexa-histidine tag ([SEQ ID NO: 13](#)) purification handle.

[000275] The complete amino acid sequence of the polypeptide (or fusion/chimera) to be expressed can be used to construct a back-translated gene. A DNA oligomer containing a nucleotide sequence coding for hIL2 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.

[000276] Codon Optimization: In some embodiments, the nucleic acid sequence encoding the IL2 mutein may be “codon optimized” to facilitate expression in a particular host cell type. Techniques for codon optimization in a wide variety of expression systems, including mammalian, yeast and bacterial host cells, are well known in the art and there are online tools to provide for a codon optimized sequences for expression in a variety of host cell types. See e.g. Hawash, et al., (2017) 9:46-53 and Mauro and Chappell in Recombinant Protein Expression in Mammalian Cells: Methods and Protocols, edited by David Hacker (Human Press New York). Additionally, there are a variety of web based on-line software packages that are freely available to assist in the preparation of codon optimized nucleic acid sequences.

Expression Vectors:

the art upon review of this disclosure and are to be included within the spirit and purview of this application.

Examples

[000408] The following examples are provided to describe certain embodiments of the invention provided herein and are not to be construed to as limiting.

Example 1: Generation of the human IL2 expression vector pcDNA3.1/hygro(+)-huIL2

[000409] The human IL2 DNA open reading frame (“ORF”) (Genbank NM_000586.3) was synthesized (Life Technologies GeneArt Service, Carlsbad, CA), and amplified via PCR using Platinum SuperFi II DNA polymerase kit (commercially available as catalog #12361050, ThermoFisher) in substantial accordance with the manufacturer’s protocol, and using primers 5’ TATAGTCAGCGCCACcCATGTACAGGATGCAACTCCTGTC 3’ ([SEQ ID NO: 14](#)), which incorporates an NheI restriction site, and 5’ TATAGGGCCCTATCAAGTCAGTGTTGAGATG 3’ ([SEQ ID NO: 15](#)), which incorporates an ApaI restriction site. The PCR fragment was visualized on a 1% agarose gel (item #54803, Lonza, Rockland, ME), excised from the gel and purified using a QIAquick PCR Purification kit (commercially available as catalog #28106, Qiagen, Germany) according to the manufacturer’s protocol.

[000410] The purified PCR fragment and mammalian expression vector pcDNA 3.1/Hygro(+) (commercially available as catalog #V87020, ThermoFisher, Carlsbad CA) were digested with NheI and ApaI (commercially available as catalog #R0111S and #R0114L, New England Biolabs, Ipswich, MA) restriction enzymes. The expression vector was further treated with a Quick Dephosphorylation kit (commercially available as catalog #M0508L, New England Biolabs) in substantial accordance with the manufacturer’s protocol. The PCR fragment was ligated into pcDNA 3.1/Hygro(+) using the Rapid DNA Ligation Kit (commercially available as catalog #11635379001, Sigma Aldrich, St. Louis, MO) in substantial accordance with the manufacturer’s protocol, transformed into One Shot TOP10 Chemically Competent E. coli (commercially available as catalog #C404006, Life Technologies, Carlsbad, CA), plated onto LB Agar plates containing 100ug/ml carbenicillin (commercially available as catalog #L1010, Teknova, Hollister, CA), and grown overnight at 37C.

into E. coli, as well as the isolation and sequence analysis of the plasmid DNA, was performed using the same protocol as in the generation of the pcDNA3.1/Hygro-huIL2 expression vector.

Table 15. Quik Change Mutagenesis					
Full ORF #	38	42	146	*Templates	
Mature Peptide #	18	22	126	IL2: pcDNA3.1/hygro(+)-huIL2 IL2 REH: pcDNA3.1/Hygro(+)-huIL2 REH IL2 REK: pcDNA3.1/Hygro(+)-huIL2 REK IL2 AEH: pcDNA3.1/Hygro(+)-huIL2 AEH IL2 EEH: pcDNA3.1/Hygro(+)-huIL2 EEH IL2 VEH: pcDNA3.1/Hygro(+)-huIL2 VEH IL2 HEH: pcDNA3.1/Hygro(+)-huIL2 HEH IL2 IEH: pcDNA3.1/Hygro(+)-huIL2 IEH IL2 RTH: pcDNA3.1/Hygro(+)-huIL2 RTH	
hIL2 Wild Type human IL2 Residue	L	Q	Q	Primer Set (5'→3')	Template *
REE	R	E	E	GATGGATTACCTTTTGTGAGAGCATCATCTCAACA (SEQ ID NO: 16) TGTGAGATGATGCTCTCACAAAAGGTAATCCATC (SEQ ID NO: 17)	IL2 REK
REM	R	E	M	GGATTACCTTTTGTATGAGCATCATCTCAAC (SEQ ID NO: 18) GTTGAGATGATGCTCATACAAAAGGTAATCC (SEQ ID NO: 19)	IL2 REK
REV	R	E	V	GGATTACCTTTTGTGTGAGCATCATCTCAACAC (SEQ ID NO: 20) GTGTTGAGATGATGCTCACACAAAAGGTAATCC (SEQ ID NO: 21)	IL2 REK
REL	R	E	L	GGATTACCTTTTGTCTGAGCATCATCTCAACAC (SEQ ID NO: 22) GTGTTGAGATGATGCTCAGACAAAAGGTAATCC (SEQ ID NO: 23)	IL2 REK
REF	R	E	F	GGATTACCTTTTGTTCAGCATCATCTCAACAC (SEQ ID NO: 24) GTGTTGAGATGATGCTGAAACAAAAGGTAATCC (SEQ ID NO: 25)	IL2 REK

REN	R	E	N	GGATTACCTTTTGTAAACAGCATCATCTCAACAC (SEQ ID NO: 26) GTGTTGAGATGATGCTGTTACAAAAGGTAATCC (SEQ ID NO: 27)	IL2 REK
RER	R	E	R	GGATTACCTTTTGTAGGAGCATCATCTCAACAC (SEQ ID NO: 28) GTGTTGAGATGATGCTCCTACAAAAGGTAATCC (SEQ ID NO: 29)	IL2 REK
REY	R	E	Y	GGATTACCTTTTGTACAGCATCATCTCAACAC (SEQ ID NO: 30) GTGTTGAGATGATGCTGTAACAAAAGGTAATCC (SEQ ID NO: 31)	IL2 REK
AEK	A	E	K	GGATTACCTTTTGTAAAGAGCATCATCTC (SEQ ID NO: 32)	IL2 AEH

				GAGATGATGCTCTTACAAAAGGTAATCC (SEQ ID NO: 33)	
EEK	E	E	K	GGATTACCTTTTGTAAAGAGCATCATCTC (SEQ ID NO: 32) GAGATGATGCTCTTACAAAAGGTAATCC (SEQ ID NO: 33)	IL2 EEH
VEK	V	E	K	GGATTACCTTTTGTAAAGAGCATCATCTC (SEQ ID NO: 32) GAGATGATGCTCTTACAAAAGGTAATCC (SEQ ID NO: 33)	IL2 VEH
HEK	H	E	K	GGATTACCTTTTGTAAAGAGCATCATCTC (SEQ ID NO: 32) GAGATGATGCTCTTACAAAAGGTAATCC (SEQ ID NO: 33)	IL2 HEH
IEK	I	E	K	GGATTACCTTTTGTAAAGAGCATCATCTC (SEQ ID NO: 32) GAGATGATGCTCTTACAAAAGGTAATCC (SEQ ID NO: 33)	IL2 IEH
RTK	R	T	K	GGATTACCTTTTGTAAAGAGCATCATCTC (SEQ ID NO: 32) GAGATGATGCTCTTACAAAAGGTAATCC (SEQ ID NO: 33)	IL2 RTH

Table 16. hIL2 Ortholog Constructs		
Name	Primer Set (5' → 3') +	Template
REE	GATGGATTACCTTTTGTGAGAGCATCATCTCAA CA (SEQ ID NO: 16) TGTTGAGATGATGCTCTCACAAAAGGTAATCC ATC (SEQ ID NO: 17)	pExSyn2.0 - hIL2 REK
REM	GGATTACCTTTTGTATGAGCATCATCTCAAC (SEQ ID NO: 18) GTTGAGATGATGCTCATACAAAAGGTAATCC (SEQ ID NO: 19)	pExSyn2.0 - hIL2 REK
REV	GGATTACCTTTTGTGTGAGCATCATCTCAACAC (SEQ ID NO: 20) GTGTTGAGATGATGCTCACACAAAAGGTAATC C (SEQ ID NO: 21)	pExSyn2.0 - hIL2 REK
REL	GGATTACCTTTTGTCTGAGCATCATCTCAACAC (SEQ ID NO: 22) GTGTTGAGATGATGCTCAGACAAAAGGTAATC C (SEQ ID NO: 23)	pExSyn2.0 - hIL2 REK
REF	GGATTACCTTTTGTTCAGCATCATCTCAACAC (SEQ ID NO: 24) GTGTTGAGATGATGCTGAAACAAAAGGTAATC C (SEQ ID NO: 25)	pExSyn2.0 - hIL2 REK

REN	GGATTACCTTTTGTAAACAGCATCATCTCAACAC (SEQ ID NO: 26) GTGTTGAGATGATGCTGTTACAAAAGGTAATC C (SEQ ID NO: 27)	pExSyn2.0 - hIL2 REK
RER	GGATTACCTTTTGTAGGAGCATCATCTCAACAC (SEQ ID NO: 28) GTGTTGAGATGATGCTCCTACAAAAGGTAATC C (SEQ ID NO: 29)	pExSyn2.0 - hIL2 REK
REY	GGATTACCTTTTGTACAGCATCATCTCAACAC (SEQ ID NO: 30) GTGTTGAGATGATGCTGTAACAAAAGGTAATC C (SEQ ID NO: 31)	pExSyn2.0 - hIL2 REK
REK + N88R	GACTTAATCAGCCGTATCAACGTAATA (SEQ ID NO: 34) TATTACGTTGATACGGCTGATTAAGTC (SEQ ID NO: 35)	pExSyn2.0 - hIL2 REK
REK + N88D	GGACTTAATCAGCGATATCAACGTAAT (SEQ ID NO: 36) ATTACGTTGATATCGCTGATTAAGTCC (SEQ ID NO: 37)	pExSyn2.0 - hIL2 REK
REK + N88G	GGGACTTAATCAGCGGTATCAACGTAAT (SEQ ID NO: 38) ATTACGTTGATACCGCTGATTAAGTCCC (SEQ ID NO: 39)	pExSyn2.0 - hIL2 REK
REK + N88I	GGACTTAATCAGCATTATCAACGTAAT (SEQ ID NO: 40) ATTACGTTGATAATGCTGATTAAGTCC (SEQ ID NO: 41)	pExSyn2.0 - hIL2 REK
REK + D20I	GCATTTAAGGCTGATTTTAGAGATGATTTTG (SEQ ID NO: 42) CAAAATCATCTCTAAAATCAGCCTTAAATGC (SEQ ID NO: 43)	pExSyn2.0 - hIL2 REK
REK + D20H	GAGCATTTAAGGCTGCATTTAGAGATG (SEQ ID NO: 44) CATCTCTAAATGCAGCCTTAAATGCTC (SEQ ID NO: 45)	pExSyn2.0 - hIL2 REK
REK + D20T	GCATTTAAGGCTGACTTTAGAGATGATTTTG (SEQ ID NO: 46) CAAAATCATCTCTAAAGTCAGCCTTAAATGC (SEQ ID NO: 47)	pExSyn2.0 - hIL2 REK
REK + D20G	GCATTTAAGGCTGGGTTTAGAGATGA (SEQ ID NO: 48) TCATCTCTAAACCCAGCCTTAAATGC (SEQ ID NO: 49)	pExSyn2.0 - hIL2 REK

Table 16. hIL2 Ortholog Constructs		
Name	Primer Set (5'→3') +	Template
REK + D20A	GCATTTAAGGCTGGCTTTAGAGATGATTTTG (SEQ ID NO: 50) CAAAATCATCTCTAAAGCCAGCCTTAAATGC (SEQ ID NO: 51)	pExSyn2.0 - hIL2 REK
AEH + V91K	CAGCAATATCAACAAGATAGTTCTGGAAC (SEQ ID NO: 52) GTTCCAGAACTATCTTGTTGATATTGCTG (SEQ ID NO: 53)	pExSyn2.0 - hIL2 AEH
EEH + V91K	CAGCAATATCAACAAGATAGTTCTGGAAC (SEQ ID NO: 52) GTTCCAGAACTATCTTGTTGATATTGCTG (SEQ ID NO: 53)	pExSyn2.0 - hIL2 EEH
VEH + V91K	CAGCAATATCAACAAGATAGTTCTGGAAC (SEQ ID NO: 52) GTTCCAGAACTATCTTGTTGATATTGCTG (SEQ ID NO: 53)	pExSyn2.0 - hIL2 VEH
HEH + V91K	CAGCAATATCAACAAGATAGTTCTGGAAC (SEQ ID NO: 52) GTTCCAGAACTATCTTGTTGATATTGCTG (SEQ ID NO: 53)	pExSyn2.0 - hIL2 HEH
IEH + V91K	CAGCAATATCAACAAGATAGTTCTGGAAC (SEQ ID NO: 52) GTTCCAGAACTATCTTGTTGATATTGCTG (SEQ ID NO: 53)	pExSyn2.0 - hIL2 IEH
RTH + V91K	CAGCAATATCAACAAGATAGTTCTGGAAC (SEQ ID NO: 52) GTTCCAGAACTATCTTGTTGATATTGCTG (SEQ ID NO: 53)	pExSyn2.0 - hIL2 RTH
REE + V91K	CAGCAATATCAACAAGATAGTTCTGGAAC (SEQ ID NO: 52) GTTCCAGAACTATCTTGTTGATATTGCTG (SEQ ID NO: 53)	pExSyn2.0 - hIL2 REE
AEK	GGATTACCTTTTGTAAGAGCATCATCTC (SEQ ID NO: 32) GAGATGATGCTCTTACAAAAGGTAATCC (SEQ ID NO: 33)	pExSyn2.0 - hIL2 AEH
EEK	GGATTACCTTTTGTAAGAGCATCATCTC (SEQ ID NO: 32) GAGATGATGCTCTTACAAAAGGTAATCC (SEQ ID NO: 33)	pExSyn2.0 - hIL2 EEH
VEK	GGATTACCTTTTGTAAGAGCATCATCTC (SEQ ID NO: 32) GAGATGATGCTCTTACAAAAGGTAATCC (SEQ ID NO: 33)	pExSyn2.0 - hIL2 VEH

HEK	GGATTACCTTTTGTAAGAGCATCATCTC (SEQ ID NO: 32) GAGATGATGCTCTTACAAAAGGTAATCC (SEQ ID NO: 33)	pExSyn2.0 - hIL2 HEH
IEK	GGATTACCTTTTGTAAGAGCATCATCTC (SEQ ID NO: 32) GAGATGATGCTCTTACAAAAGGTAATCC (SEQ ID NO: 33)	pExSyn2.0 - hIL2 IEH
RTK	GGATTACCTTTTGTAAGAGCATCATCTC (SEQ ID NO: 32) GAGATGATGCTCTTACAAAAGGTAATCC (SEQ ID NO: 33)	pExSyn2.0 - hIL2 RTH
N88R	GACTTAATCAGCCGTATCAACGTAATA (SEQ ID NO: 34) TATTACGTTGATACGGCTGATTAAGTC (SEQ ID NO: 35)	pExSyn2.0 - hIL2

Example 5. Transient Transfections in HEK293 cells

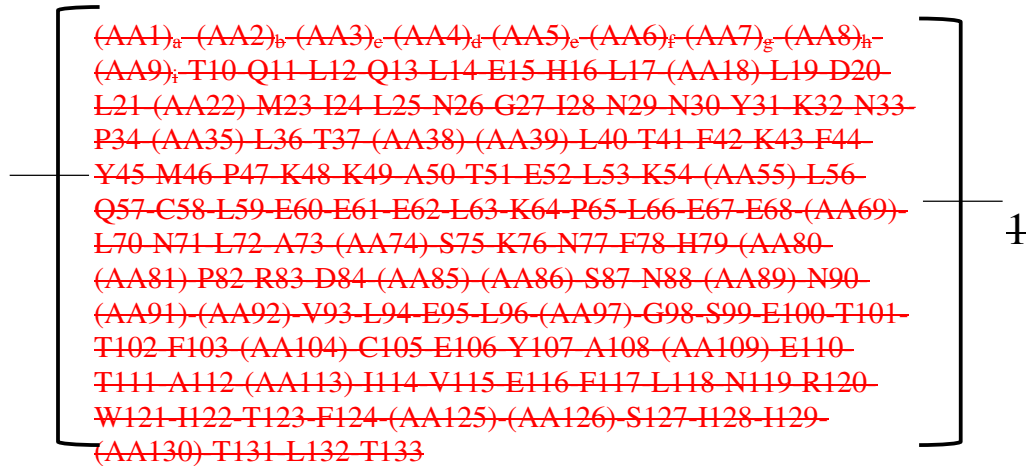
[000415] All expression vectors were transiently transfected into HEK293 cells (#CRL-1573, ATCC, Manassas, VA). ~1E6 HEK293 cells were plated into each well of a 6 well tissue culture plate in 2ml of DMEM (#10569044, Life Technologies) supplemented with 10% Fetal Bovine serum (#SH30071.03, Fisher Scientific, Chicago, IL), and grown overnight at 37C and 5% CO₂. The next day the cells were transfected using Lipofectamine 3000 Reagent (#L3000150, Life Technologies) following the manufacturer's protocol, using 2.5ug DNA, 5ul P3000 reagent, and 7.5ul Lipofectamine 3000 per transfection. The transfected cells were grown at 37C, 5% CO₂ for 48 – 72 hours and then the conditioned media was harvested.

Example 6. Analysis of protein expression

Claims:

We claim:

1. ~~A method of treating a subject suffering from a neoplastic disease, disorder or condition by the administration to said subject of polypeptide that is at least 95% homologous to a polypeptide of the formula: 1:~~



wherein:

- ~~• each of a, b, c, d, e, f, g, h, and i is individually selected from 0 or 1;~~
- ~~• AA1 is A (wild type, a=1) or deleted (a=0);~~
- ~~• AA2 is P (wild type, b=1) or deleted (b=0);~~
- ~~• AA3 is T (wild type, c=1), C, A, G, Q, E, N, D, R, K, P, or deleted (c=0);~~
- ~~• AA4 is S (wild type, d=1) or deleted (d=0);~~
- ~~• AA5 is S (wild type, e=1) or deleted (e=0);~~
- ~~• AA6 is S (wild type, f=1) or deleted (f=0);~~
- ~~• AA7 is T (wild type, g=1) or deleted (g=0);~~
- ~~• AA8 is K (wild type, h=1) or deleted (h=0);~~
- ~~• AA9 is K (wild type, i=1) or deleted (i=0);~~
- ~~• AA18 is L (wild type) or R, L, G, M, F, E, H, W, K, Q, S, V, I, Y, H, D or T;~~
- ~~• AA22 is Q (wild type) or F, E, G, A, L, M, F, W, K, S, V, I, Y, H, R, N, D, T, or F;~~
- ~~• AA35 is K (wildtype) or E;~~
- ~~• AA38 is R (wild type), W or G;~~
- ~~• AA39 is M (wildtype), L or V;~~

- ~~AA55 is H (wildtype) or Y;~~
- ~~AA69 is V (wildtype) or A;~~
- ~~AA74 is Q (wild type), P, N, H, S;~~
- ~~AA80 is L (wild type), F or V;~~
- ~~AA81 is R (wild type), I, D or T;~~
- ~~AA85 is L (wild type) or V;~~
- ~~AA86 is I (wild type) or V;~~
- ~~AA89 is I (wild type) or V;~~
- ~~AA92 is I (wild type) or F;~~
- ~~AA97 is K (wild type) or Q;~~
- ~~AA104 is M (wild type) or A;~~
- ~~AA109 is D (wildtype), C or a non-natural amino acid with an activated side chain;~~
- ~~AA113 is T (wild type) or N;~~
- ~~AA125 is C (wild type), A or S;~~
- ~~AA126 is Q (wild type) or H, M, K, C, D, E, G, I, R, S, or T; and~~
- ~~AA130 is S (wild type), T, G or R.~~

2. ~~The method of Claim 1 wherein the polypeptide comprises the following mutations:~~

- ~~AA18 is selected from the group consisting of L (wild type) or R, L, G, M, F, E, H, W, K, Q, S, V, I, Y, H, D or T;~~
- ~~AA22 is selected from the group consisting of Q (wild type) or F, E, G, A, L, M, F, W, K, S, V, I, Y, H, R, N, D, T, or F; and~~
- ~~AA126 is selected from the group consisting of Q (wild type) or H, M, K, C, D, E, G, I, R, S, or T.~~

3. ~~The method of Claim 1 wherein the polypeptide comprises the following mutations:~~

~~a=0;~~

- ~~AA18 is selected from the group consisting of L (wild type) or R, L, G, M, F, E, H, W, K, Q, S, V, I, Y, H, D or T;~~
- ~~AA22 is selected from the group consisting of Q (wild type) or F, E, G, A, L, M, F, W, K, S, V, I, Y, H, R, N, D, T, or F; and~~

- AA126 is selected from the group consisting of Q (wild type) or H, M, K, C, D, E, G, I, R, S, or T.

4. The method of claim 1 wherein the polypeptide comprises a set of mutations selected from the following sets of mutations: L18R, Q22E, and Q126H; L18R, Q22E, and Q126K; L18R, Q22E and Q126M; L18R, Q22E Q126T; L18R; Q22E; V91K; V91R; Q126H; L18R, and Q126H; Q22E, and Q126H; L18G, Q22E and Q126H; L18A, Q22E and Q126H; L18M, Q22E and Q126H; L18F, Q22E and Q126H; L18W, Q22E and Q126H; L18K, Q22E and Q126H; L18Q, Q22E and Q126H; L18E, Q22E and Q126H; L18S, Q22E and Q126H; L18V, Q22E and Q126H; L18I, Q22E and Q126H; L18Y, Q22E and Q126H; L18H, Q22E and Q126H; L18N, Q22E and Q126H; L18D, Q22E and Q126H; L18T, Q22E and Q126H; L18R, Q22G and Q126H; L18R, Q22A and Q126H; L18R, Q22L and Q126H; L18R, Q22M and Q126H; L18R, Q22F and Q126H; L18R, Q22W and Q126H; L18R, Q22K and Q126H; L18R, Q22S and Q126H; L18R, Q22V and Q126H; L18R, Q22I and Q126H; L18R Q22Y and Q126H; L18R Q22H and Q126H; L18R Q22R and Q126H; L18R Q22N and Q126H; L18R Q22D and Q126H; and L18R Q22T and Q126H.

5. The polypeptide of any one of claims 1-4 which is PEGylated.

6. The polypeptide of claim 4 wherein the PEG component of such PEGylated polypeptide has a molecular weight of from about 10kD to about 70kD.

7. The polypeptide of claim 6 wherein the PEG component of such PEGylated polypeptide has a molecular weight of from about 40kD.

8. The polypeptide of any one of claims 1-4 wherein the polypeptide is a fusion protein.

9. The polypeptide of Claim 8 wherein the fusion protein comprises an Fe domain.

10. A nucleic acid encoding a polypeptide of any one of claims 1-4 and 8-9.

11. The nucleic acid of claim 10 wherein the nucleic acid is DNA.

12. A recombinant expression vector comprising the nucleic acid of Claim 10 or 11.

13. The vector of claim 12 wherein said vector is a viral vector.

14. The vector of claim 12 wherein said vector is a non-viral vector.

15. A host cell transformed with a vector of any one of claims 13-14.

~~16. — A pharmaceutical formulation comprising a polypeptide of any one of claims 1-9, the nucleic acids of claims 10 or 11 or a vector of claims 12-14.~~

~~17. — A method of treating a mammalian subject suffering from neoplastic disease disorder or condition comprising the administration of a therapeutically effective amount of pharmaceutical formulation of Claim 16.~~

~~18. — The method of claim 17 wherein said method further comprises the administration of a supplementary agent to said subject.~~

~~19. — The method of claim 18 wherein said supplementary agent is selected from the group consisting of chemotherapeutic agents, antibodies, immune checkpoint modulators, TILs, CAR-T cells, and physical methods.~~

~~20. — The method of claim 19 wherein the supplementary agent is an immune checkpoint modulator.~~

~~21. — The method of claim 19 wherein the immune checkpoint modulator is an anti-PD-1 or anti-PD-L1 antibody.~~

~~22. — The method of claim 19 wherein the supplementary agent is an antibody selecte from the group consisting of [fam]-trastuzumab-deruxtecan, enfortumab-vedotin, polatuzumab-vedotin, cemiplimab, moxetumomab-pasudotox, mogamuizumab, tildrakizumab, ibalizumab, durvalumab, inotuzumab, ozogamicin, avelumab, atezolizumab, olaratumab, ixekizumab, aratumumab, elotuzumab, necitumumab, dinutuximab, nivolumab, blinatumomab, pembrolizumab, ramucirumab, siltuximab, obinutuzumab, ado-trastuzumab emtansine, pertuzumab, brentuximab-vedotin, ipilimumab, ofatumumab, certolizumab-pegol, eatumaxomab, panitumumab, bevacizumab, cetuximab, tositumomab-H31, ibritumomab tiuxetan, gemtuzumab, ozogamicin, trastuzumab, infliximab, rituximab, and edrecolomab.~~

~~23. — The method of any one of claims 1-22 wherein the neoplastic disease disorder or condition is selected from the group consisting of: adenomas, fibromas, hemangiomas, hyperplasia, atypia, metaplasia, dysplasia, carcinomas, leukemias, breast cancers, sarcomas, leukemias, lymphomas, genitourinary cancers, ovarian cancers, urethral cancers, bladder cancers, prostate cancers, gastrointestinal cancers, colon cancers, esophageal cancers, stomach cancers, lung cancers, myelomas, pancreatic cancers, liver cancers, kidney cancers, endocrine cancers, skin cancers, gliomas, neuroblastomas, astrocytomas, myelodysplastic disorders, cervical carcinoma in situ, intestinal polyposes, oral leukoplakias, histiocytoses, hyperproliferative sears including keloid sears, respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas,~~

~~breast carcinomas, prostatic carcinomas, endocrine system carcinomas, melanomas, adenocarcinomas, myeloproliferative neoplasms, myeloid and lymphoid disorders with eosinophilia, myeloproliferative/myelodysplastic neoplasms, myelodysplastic syndromes, acute myeloid leukemia and related precursor neoplasms, and acute leukemia of ambiguous lineage, promyeloid leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML), precursor lymphoid neoplasms, mature B-cell neoplasms, mature T-cell neoplasms, Hodgkin's Lymphoma, and immunodeficiency associated lymphoproliferative disorders, lymphoblastic leukemia (ALL) which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HCL) and Waldenstrom's macroglobulinemia (WM), erythroblastic leukemia and acute megakaryoblastic leukemia, malignant lymphomas including, but are not limited to, non-Hodgkins lymphoma and variants thereof, peripheral T-cell lymphomas, adult T-cell leukemia/lymphoma (ATL), cutaneous T-cell lymphoma (CTCL), large granular lymphocytic leukemia (LGL), Hodgkin's disease and Reed-Sternberg disease.~~

~~24. — The method of any one of claims 1-23 comprising administering to said subject a therapeutically effective amount of an hIL2 mutein of sufficient to maintain a serum concentration greater than about 50% of a period of time of at least 24 hours at or above the effective concentration of the IL2 mutein sufficient to promote proliferation of CD3-activated primary human T-cells at or below a serum concentration of such IL2 mutein sufficient to induce activation of T-cells with respect to such IL2 mutein.~~

1. Use of a polypeptide comprising a sequence of formula 1:

(AA1)-(AA2)-(AA3)-(AA4)-(AA5)-(AA6)-(AA7)-(AA8)-
 (AA9)-T10-Q11-L12-Q13-L14-E15- H16-L17-(AA18)-L19-D20-L21-
 (AA22)-M23-I24-L25- N26-G27-I28-N29-N30-Y31-K32-N33-P34-
 (AA35)-L36- T37-(AA38)-(AA39)-L40-T41-F42-K43-F44-Y45-M46-
 P47-K48-K49-A50-T51-E52-L53-K54-(AA55)-L56-Q57-C58-L59-
 E60-E61-E62-L63-K64-P65-L66-E67-E68-(AA69)-L70-N71-L72-
 A73-(AA74)-S75-K76-N77-F78- H79-(AA80)-(AA81)-P82-R83-D84-
 (AA85)-(AA86)-S87- N88-(AA89)-N90-(AA91)-(AA92)-V93-L94-
 E95-L96- (AA97)-G98-S99-E100-T101-T102-F103-(AA104)-C105-
 E106-Y107-A108-(AA109)-E110-T111-A112-(AA113)-I114-V115-
 E116-F117-L118-N119-R120-W121-I122- T123-F124-(AA125)-
 (AA126)-S127-I128-I129- (AA130)-T131-L132-T133

1

in the manufacture of a medicament for treating a human subject suffering from a neoplastic disease,
wherein the polypeptide is to be administered to said subject,

wherein:

AA18 is selected from the group consisting of L, R, G, M, F, E, W, K, Q, S, V, I, Y, H, D and T;

AA22 is selected from the group consisting of Q, E, G, A, L, M, W, K, S, V, I, Y, H, R, N, D, T and
F; and

AA126 is selected from the group consisting of H, M, K, C, D, E, G, I, R and S,

wherein AA18 is not L when AA22 is Q,

and wherein:

AA1 is A or deleted;

AA2 is P or deleted;

AA3 is T, C, A, G, Q, E, N, D, R, K, P or deleted;

AA4 is S or deleted;

AA5 is S or deleted;

AA6 is S or deleted;

AA7 is T or deleted;

AA8 is K or deleted;

AA9 is K or deleted;

AA35 is K or E;

AA38 is R, W or G;

AA39 is M, L or V;

AA55 is H or Y;

AA69 is V or A;

AA74 is Q, P, N, H or S;

AA80 is L, F or V;

AA81 is R, I, D or T;

AA85 is L or V;

AA86 is I or V;

AA89 is I or V;

AA91 is V, R or K

AA92 is I or F;

AA97 is K or Q;

AA104 is M or A;

AA109 is D or C;

AA113 is T or N;

AA125 is C, A or S;

AA130 is S, T, G or R; and

wherein (a) said polypeptide does not comprise the group of amino acid substitutions 80F, 81D, 85V, 86V and 92F; (b) the polypeptide exhibits diminished binding to CD132 relative to wild-type hIL2 of SEQ ID NO:1 and (c) (i) the ratio of pSTAT5 induction in CD25^{pos} T cells relative to pSTAT5 induction in CD25^{neg} T cells upon contacting the CD25^{pos} and CD25^{neg} T cells with the polypeptide, is greater than (ii) the ratio of pSTAT5 induction in CD25^{pos} T cells relative to pSTAT5 induction in CD25^{neg} T cells upon contacting with the CD25^{pos} and CD25^{neg} T cells with wild-type hIL2 of SEQ ID NO:1.

2. The use of claim 1, wherein AA1 is deleted.

3. The use of claim 1, wherein the polypeptide comprises a set of mutations selected from the following sets of mutations:

18R, 22E, and 126H;

18R, 22E, and 126K;

18R, 22E and 126M;

18R and 126H;

22E and 126H;

18A, 22E and 126H;

18M, 22E and 126H;

18F, 22E and 126H;

18W, 22E and 126H;

18K, 22E and 126H;

18Q, 22E and 126H;

18E, 22E and 126H;

18S, 22E and 126H;

18V, 22E and 126H;

18I, 22E and 126H;

18Y, 22E and 126H;

18H, 22E and 126H;

18N, 22E and 126H;

18D, 22E and 126H;

18T, 22E and 126H;
18R, 22G and 126H;
18R, 22A and 126H;
18R, 22L and 126H;
18R, 22M and 126H;
18R, 22F and 126H;
18R, 22W and 126H;
18R, 22K and 126H;
18R, 22S and 126H;
18R, 22V and 126H;
18R, 22I and 126H;
18R, 22Y and 126H;
18R, 22H and 126H;
18R, 22R and 126H;
18R, 22N and 126H;
18R, 22D and 126H; and
18R, 22T and 126H.

4. The use of claim 1, wherein the polypeptide is PEGylated.

5. The use of claim 4, wherein the polypeptide is PEGylated with a PEG having a molecular weight of 10,000 to 50,000 Daltons.

6. The use of claim 1, wherein the administration comprises administration of a nucleic acid encoding the polypeptide.

7. The use of claim 6, wherein the nucleic acid is DNA.

8. The use of claim 6, wherein the nucleic acid is a recombinant expression vector.

9. The use of claim 8, wherein said vector is a viral vector.

10. The use of claim 8, wherein said vector is a non-viral vector.

11. The use of claim 1, wherein a supplementary agent is to be further administered to said subject.

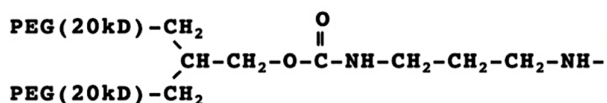
12. The use of claim 11, wherein said supplementary agent is selected from the group consisting of a chemotherapeutic agent, an antibody, an immune checkpoint modulator, tumor infiltrating lymphocytes (TILs), a CAR-T cell, and a physical method.

13. The use of claim 12, wherein the supplementary agent is an immune checkpoint modulator.

14. The use of claim 13, wherein the immune checkpoint modulator is an anti-PD-1 antibody or an anti-PD-L1 antibody.

15. The use of claim 1, wherein the polypeptide comprises SEQ ID NO:8.

16. The use of claim 15, wherein the polypeptide comprises an amino-terminal proline linked to:



17. The use of claim 14, wherein the anti-PD-1 antibody is nivolumab or pembrolizumab.

18. Use of a polypeptide comprising a sequence of formula 1:

(AA1)-(AA2)-(AA3)-(AA4)-(AA5)-(AA6)-(AA7)-(AA8)-
 (AA9)-T10-Q11-L12-Q13-L14-E15- H16-L17-(AA18)-L19-D20-L21-
 (AA22)-M23-I24-L25- N26-G27-I28-N29-N30-Y31-K32-N33-P34-
 (AA35)-L36- T37-(AA38)-(AA39)-L40-T41-F42-K43-F44-Y45-M46-
 P47-K48-K49-A50-T51-E52-L53-K54-(AA55)-L56-Q57-C58-L59-
 E60-E61-E62-L63-K64-P65-L66-E67-E68-(AA69)-L70-N71-L72-
 A73-(AA74)-S75-K76-N77-F78- H79-(AA80)-(AA81)-P82-R83-D84-
 (AA85)-(AA86)-S87- N88-(AA89)-N90-(AA91)-(AA92)-V93-L94-
 E95-L96- (AA97)-G98-S99-E100-T101-T102-F103-(AA104)-C105-
 E106-Y107-A108-(AA109)-E110-T111-A112-(AA113)-I114-V115-
 E116-F117-L118-N119-R120-W121-I122- T123-F124-(AA125)-
 (AA126)-S127-I128-I129- (AA130)-T131-L132-T133

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in the manufacture of a medicament for treating a human subject suffering from a neoplastic disease, wherein

AA18 is selected from the group consisting of L, R, G, M, F, E, W, K, Q, S, V, I, Y, H, D and T;

AA22 is selected from the group consisting of Q, E, G, A, L, M, W, K, S, V, I, Y, H, R, N, D, T and F; and

AA126 is selected from the group consisting of H, M, K, C, D, E, G, I, R and S,

wherein AA18 is not L when AA22 is Q,

and wherein:

AA1 is A or deleted;

AA2 is P or deleted;

AA3 is T, C, A, G, Q, E, N, D, R, K, P or deleted;

AA4 is S or deleted;

AA5 is S or deleted;

AA6 is S or deleted;

AA7 is T or deleted;

AA8 is K or deleted;

AA9 is K or deleted;

AA35 is K;

AA38 is R, W or G;

AA39 is M, L or V;

AA55 is H or Y;

AA69 is V or A;

AA74 is Q, N, H or S;

AA80 is L, F or V;

AA81 is R, I, D or T;
AA85 is L or V;
AA86 is I or V;
AA89 is I or V;
AA91 is V, R or K
AA92 is I or F;
AA97 is K or Q;
AA104 is M or A;
AA109 is D or C;
AA113 is T or N;
AA125 is C, A or S;
AA130 is S, T or R; and

wherein (a) said polypeptide does not comprise the group of amino acid substitutions 80F, 81D, 85V, 86V and 92F; (b) the polypeptide exhibits diminished binding to CD132 relative to wild-type hIL2 of SEQ ID NO:1 and (c) (i) the ratio of pSTAT5 induction in CD25^{pos} T cells relative to pSTAT5 induction in CD25^{neg} T cells upon contacting the CD25^{pos} and CD25^{neg} T cells with the polypeptide, is greater than (ii) the ratio of pSTAT5 induction in CD25^{pos} T cells relative to pSTAT5 induction in CD25^{neg} T cells upon contacting with the CD25^{pos} and CD25^{neg} T cells with wild-type IL2 of SEQ ID NO:1.