

## MUTANT INTERLEUKIN 15 EXPRESSING IMMUNE CELLS

### CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims priority to U.S. Provisional Patent Application No. 63/499, 467 filed May 1, 2023 and U.S. Provisional Patent Application No. 63/502,875 filed May 17, 2023, the contents of both of which are incorporated herein by reference in their entirety as if fully set forth herein.

### REFERENCE TO SEQUENCE LISTING

**[0002]** The Sequence Listing associated with this application is provided in XML format in lieu of a paper copy and is hereby incorporated by reference into the specification. The name of the file containing the Sequence Listing is 3401142.xml. The file is 346,334 bytes, was created on April 29, 2024, and is being submitted electronically via Patent Center.

### FIELD OF THE DISCLOSURE

**[0003]** The present disclosure provides methods and artificial expression constructs for improving persistence or function of an immune cell. Particularly, the methods or artificial expression constructs include a mutated interleukin 15 (IL-15) transgene under the control of a constitutive or inducible expression control sequence to potentiate the function of an immune cell (e.g., recombinant receptor-expressing T cell).

### BACKGROUND OF THE DISCLOSURE

**[0004]** According to the World Health Organization, cancer is a leading cause of death globally, and was responsible for nearly 10 million deaths in 2020.

**[0005]** For many years, the chosen treatments for cancer have been surgery, chemotherapy, and/or radiation therapy. In recent years, more targeted therapies have emerged to specifically target cancer cells by identifying and exploiting specific molecular and/or immunophenotypic changes seen primarily in those cells. For example, many cancer cells preferentially express particular markers on their cellular surfaces and these markers have provided targets for antibody-based therapeutics.

**[0006]** Significant progress has been made in genetically engineering cells of the immune system to target and kill unwanted cell types, such as cancer cells. Many of these immune cells are T cells that have been genetically engineered to express a recombinant receptor, for example, a chimeric antigen receptor (CAR). CAR are proteins including several distinct subcomponents that allow the genetically modified T cells to recognize and kill targeted cell types. The subcomponents

include at least an extracellular component and an intracellular component expressed as a single protein or assembling into a functional unit. The extracellular component includes a binding domain that binds a marker (e.g., an antigen) that is preferentially present on the surface of unwanted cells. When the binding domain binds such markers, the intracellular component signals the T cell to destroy the bound cell. CAR can additionally include a transmembrane domain that can link the extracellular component to the intracellular component.

**[0007]** Other subcomponents that can increase a CAR's function can also be used. For example, spacers provide CAR with additional conformational flexibility, often increasing the binding domain's ability to bind the targeted cell marker, leading to enhanced cytolytic effects. The appropriate length of a spacer within a particular CAR can depend on numerous factors including how close or far a targeted marker is located from the surface of an unwanted cell's membrane.

**[0008]** Although CAR T cells have had substantial success in treating various cancers, challenges remain. For example, CAR T cells can have suboptimal proliferation, functional anergy or exhaustion, impaired memory differentiation, and/or limited persistence limiting their ability to create or maintain remission in patients. Thus, strategies to improve CAR T cell efficacy are needed, especially for scenarios with prolonged antigen exposure that can lead to functional anergy and exhaustion.

**[0009]** Genetic enhancements of CAR T cell potency may improve anti-cancer efficacy but may risk adverse effects, such as uncontrolled cell growth. While intermittent administration of IL-15 to non-human primates is well tolerated and expands memory T cells, continuously delivered high-dose IL-15 is toxic (Berger et al, Blood 114(12):2417).

#### SUMMARY OF THE DISCLOSURE

**[0010]** The present disclosure provides regulated IL-15 secretion to enhance immune cell potency without driving autonomous immune cell growth or severe toxicity. The present disclosure generally relates, in part, to methods and artificial expression constructs including a mutated interleukin 15 (IL-15) transgene under the control of a constitutive or inducible expression control sequence to potentiate the function of an immune cell (e.g., recombinant receptor-expressing T cell). The mutated IL-15 is modified to focus signaling to cells expressing and/or signaling complexes including IL-15R $\alpha$ . In particular embodiments, the mutated IL-15 is modified to restrict signaling to cells expressing and/or signaling complexes including IL-15R $\alpha$ . In particular embodiments, the mutated IL-15 has lower affinity to, or cannot bind to, complexes including IL2R $\beta$  and common gamma receptor without IL-15R $\alpha$ , as compared to complexes including IL2R $\beta$  and common gamma receptor with IL-15R $\alpha$ . In various embodiments, the mutated IL-15 has lower

affinity to the common gamma receptor and/or atypical binding to IL2R $\beta$  compared to wild-type IL-15. In various embodiments, the mutated IL-15 is unable to bind the common gamma receptor. More particularly, the disclosure provides methods or artificial expression constructs that potentiate the function of immune cells by enhancing immune cell proliferation, decreasing antigen-independent interferon-gamma (IFN $\gamma$ ) release, and suppressing tumor growth and uncontrolled immune cell proliferation. Methods of improving persistence (or function) of an adoptive cell therapy (ACT) and methods of treating a subject in need thereof are also provided.

**[0011]** In particular embodiments, an artificial expression construct includes a mutant IL-15 under the control of a constitutive or inducible expression control sequence. In particular embodiments, the mutant IL-15 preferentially binds to an IL15 receptor complex including IL-15R $\alpha$ . In particular embodiments, the mutant IL-15 includes a D to S mutation at position 8 compared to a wild-type IL-15. In particular embodiments, the mutant IL-15 includes the sequence as set forth in SEQ ID NO: 9. In particular embodiments, the mutant IL-15 is encoded by the sequence as set forth in SEQ ID NO: 11. In particular embodiments, the inducible synthetic promoter includes the sequence as set forth in SEQ ID NO: 15. In particular embodiments, the artificial expression construct further includes a recombinant receptor or an exogenous lymphocyte receptor (e.g., T cell receptor; TCR) under the control of a constitutive expression control sequence. In particular embodiments, the recombinant receptor includes a chimeric antigen receptor (CAR), an engineered T cell receptor (eTCR), a dimerizing agent regulated immunoreceptor complex (DARIC), or a hybrid thereof. In particular embodiments, the CAR includes an anti-CD33 CAR. In particular embodiments, the CAR includes an anti-CLL1 CAR. In particular embodiments, the DARIC includes an anti-CD33 DARIC (DARIC33). In particular embodiments, the constitutive expression control sequence includes an MNDU3 promoter or an EF1a promoter. In particular embodiments, the EF1a promoter includes the first intron of a human EF1a gene. In particular embodiments, the EF1a promoter lacks the first intron of a human EF1a gene.

#### BRIEF DESCRIPTION OF THE FIGURES

**[0012]** FIG. 1 shows a schematic of the anti-CD33 dimerizing agent regulated immunoreceptor complex (DARIC33) designs containing constitutively expressed soluble or membrane bound IL-15 variants.

**[0013]** FIG. 2 shows the growth kinetics between T cells transduced with control or IL15 containing lentiviruses.

**[0014]** FIGs. 3A and 3B show the anti-CD33 expression and median fluorescence intensity (MFI) on T cells as measured by flow cytometry.

**[0015]** FIGs. 4A and 4B show the FKBP-rapamycin binding (FRB) percent positive expression and MFI on T cells as measured by flow cytometry.

**[0016]** FIG. 5 shows viral copy number (VCN) for the indicated conditions.

**[0017]** FIG. 6 shows CD4/CD8 staining by flow cytometry for the indicated conditions.

**[0018]** FIG. 7 shows the phenotype of T cells transduced with DARIC33/IL15 constructs or controls.

**[0019]** FIG. 8 shows CD54 median fluorescence intensity as measured by flow cytometry on T cells transduced with the indicated constructs.

**[0020]** FIG. 9 shows IFN $\gamma$  secretion from untransduced T cells or T cells transduced with the indicated constructs, without target cells present.

**[0021]** FIG. 10 shows IFN $\gamma$  secretion from untransduced T cells or T cells transduced with the indicated DARIC33 constructs and co-cultured with the acute myeloid leukemia cell line, MV-4-11.

**[0022]** FIG. 11 shows IFN $\gamma$  secretion from untransduced T cells or T cells transduced with the indicated constructs and co-cultured with CD33-expressing A549 cells (A549-CD33).

**[0023]** FIG. 12 shows IFN $\gamma$  secretion from untransduced T cells or T cells transduced with the indicated constructs and co-cultured with B-cell maturation antigen (BCMA)-expressing A549 cells (A549-BCMA).

**[0024]** FIG. 13 shows A549-CD33 spheroid killing by T cells transduced with the indicated constructs, without AP21967.

**[0025]** FIG. 14 shows A549-CD33 spheroid killing by T cells transduced with the indicated constructs, with AP21967.

**[0026]** FIG. 15 shows A549-BCMA spheroid killing by T cells transduced with the indicated constructs, without AP21967.

**[0027]** FIG. 16 shows A549-BCMA spheroid killing by T cells transduced with the indicated constructs, with AP21967.

**[0028]** FIG. 17 shows a schematic of the DARIC33 designs containing regulatable promoter (iSynPro) expressed soluble or membrane bound IL-15 variants in forward and reverse orientations relative to a MND promoter driven DARIC33 construct.

**[0029]** FIGs. 18A-18C show sIL15 secretion by untransduced T cells or T cells transduced with the indicated constructs in the presence or absence of A549 tumor cells overexpressing BCMA or CD33.



**[0030]** FIGs. 19A and 19B show IFN $\gamma$  secretion by untransduced T cells or T cells transduced with the indicated constructs in the presence or absence of A549 tumor cells overexpressing BCMA or CD33.

**[0031]** FIGs. 20A and 20B show IFN $\gamma$  and IL-2 secretion by untransduced T cells or T cells transduced with the indicated constructs in the presence of HL60 or CD33<sup>low</sup> OCI-AML tumor cells  $\pm$  rapamycin.

**[0032]** FIG. 20C shows IL-15 secretion by untransduced T cells or T cells transduced with the indicated constructs in the absence or presence of HL60 tumor cells  $\pm$  rapamycin.

**[0033]** FIGs. 21A-21C show proliferation of untransduced T cells or T cells transduced with the indicated constructs cultured in the presence of CD33<sup>+</sup> MV4-11 cells and rapamycin, in different cytokine containing medias.

**[0034]** FIG. 22 shows in vivo tumor growth in an NSG mouse model engrafted with CD33<sup>+</sup> MV4-11 tumor cells expressing firefly luciferase and administered T cells ( $10 \times 10^6$ ) transduced with the indicated constructs, without rapamycin present.

**[0035]** FIG. 23 shows in vivo tumor growth in an NSG mouse model engrafted with CD33<sup>+</sup> MV4-11 tumor cells expressing firefly luciferase and administered T cells ( $10 \times 10^6$ ) transduced with the indicated constructs, with rapamycin present.

**[0036]** FIG. 24 shows in vivo tumor growth in an NSG mouse model engrafted with CD33<sup>+</sup> MV4-11 tumor cells expressing firefly luciferase and administered T cells ( $3 \times 10^6$ ) transduced with the indicated constructs, with rapamycin present.

**[0037]** FIGs. 25A and 25B show a schematic of constructs and cells containing a regulatable promoter (iSynPro) expressed soluble IL-15.D8S variant and an eTCR.

**[0038]** FIGs. 26A-26D show in vivo tumor growth in an NSG mouse model engrafted with tumor cells expressing firefly luciferase and administered T cells transduced with the indicated constructs, with or without rapamycin present.

**[0039]** FIG. 27 shows a survival curve of NSG mice engrafted with tumor cells and administered T cells transduced with the indicated constructs, with or without rapamycin present.

#### BRIEF DESCRIPTION OF THE SEQUENCE IDENTIFIERS

**[0040]** SEQ ID NOs: 1-5 set forth the amino acid sequences of exemplary anti-CD33 dimerizing agent regulated immunoreceptor complex (DARIC33) signaling, targeting, and fusion polypeptides.

**[0041]** SEQ ID NO: 6 sets forth the amino acid sequences of an exemplary membrane bound IL15 construct having a CD8  $\alpha$  transmembrane domain.

- [0042] SEQ ID NO: 7 sets forth the amino acid sequences of an exemplary membrane bound IL15 construct having an AMN transmembrane domain.
- [0043] SEQ ID NO: 8 sets forth the amino acid sequence for wild-type IL15.
- [0044] SEQ ID NO: 9 sets forth the amino acid sequence for a mutant IL15 having a D to S mutation at position 8.
- [0045] SEQ ID NO: 10 sets forth the polynucleotide sequence for wild-type IL15.
- [0046] SEQ ID NO: 11 sets forth the polynucleotide sequence for a mutant IL15 having a D to S mutation at position 8.
- [0047] SEQ ID NO: 12 sets forth the polynucleotide sequence for an exemplary MNDU3 promoter.
- [0048] SEQ ID NO: 13 sets forth the polynucleotide sequence for an exemplary EF1 $\alpha$  promoter.
- [0049] SEQ ID NO: 14 sets forth the polynucleotide sequence for an exemplary CMV promoter.
- [0050] SEQ ID NO: 15 sets forth the polynucleotide sequence for an exemplary regulatable promoter (iSynPro).
- [0051] SEQ ID NOs: 16-19 set forth the polynucleotide sequence for exemplary iSynPro promoters operably linked to polynucleotides encoding wild-type IL15 or mutant IL15.D8S in forward and reverse orientations.
- [0052] SEQ ID NOs: 20-40 set forth amino acid sequence for components of DARIC.
- [0053] SEQ ID NO: 41 sets forth the amino acid sequence for processed wild-type IL15.
- [0054] SEQ ID NO: 42 sets forth the amino acid sequence for processed mutant IL15.
- [0055] SEQ ID NO: 43 sets forth the polynucleotide sequence for processed wild-type IL15.
- [0056] SEQ ID NO: 44 sets forth the polynucleotide sequence for processed mutant IL15.
- [0057] SEQ ID NOs: 45-83 set forth exemplary iSynPro promoters.
- [0058] SEQ ID NO: 84 sets forth the amino acid sequence for a minimal CD4 hinge.
- [0059] SEQ ID NO: 85 sets forth the amino acid sequence for a CD3 $\epsilon$  domain.
- [0060] SEQ ID NOs: 86-89 set forth the amino acid sequences of illustrative FRB and FKBP12 polypeptides.
- [0061] SEQ ID NO: 90 sets forth the amino acid sequence of an illustrative CD4 transmembrane domain.
- [0062] SEQ ID NOs: 91 and 92 set forth the amino acid sequences of illustrative truncated intracellular CD4 polypeptides.
- [0063] SEQ ID NOs: 93-95 set forth the CDR amino acid sequences of an illustrative anti-CD33 VHH binder.
- [0064] SEQ ID NO: 96 sets forth the amino acid sequence of an illustrative anti-CD33 VHH binder.

- [0065] SEQ ID NOs: 97-99 set forth the CDR amino acid sequences of an illustrative anti-CLL1 VHH binder.
- [0066] SEQ ID NO: 100 sets forth the amino acid sequence of an illustrative anti-CLL1 VHH binder.
- [0067] SEQ ID NOs: 101 and 102 set forth the amino acid sequences of illustrative signal sequences.
- [0068] SEQ ID NO: 103 sets forth the amino acid sequences of an exemplary engineered T cell receptor (eTCR) signaling component.
- [0069] SEQ ID NO: 104 sets forth the amino acid sequences of an exemplary eTCR targeting component.
- [0070] SEQ ID NO: 105 sets forth the amino acid sequences of an exemplary eTCR fusion polypeptide.
- [0071] SEQ ID NO: 106 sets forth the consensus Kozak sequence.
- [0072] SEQ ID NO: 107 sets forth the polynucleotide sequence for an IL2 minimal promoter.
- [0073] SEQ ID NO: 108 sets forth the amino acid sequence for a CD28 transmembrane domain.
- [0074] SEQ ID NO: 109 sets forth the amino acid sequence for a CD8 $\alpha$  transmembrane domain.
- [0075] SEQ ID NO: 110 sets forth the amino acid sequence for a CD3 $\zeta$  signaling domain.
- [0076] SEQ ID NO: 111 sets forth the amino acid sequence for a 4-1BB signaling domain.
- [0077] In the foregoing sequences, X, if present, refers to any amino acid or the absence of an amino acid.

## DETAILED DESCRIPTION

### A. Overview.

- [0078] According to the World Health Organization, cancer is a leading cause of death globally, and was responsible for nearly 10 million deaths in 2020.
- [0079] For many years, the chosen treatments for cancer have been surgery, chemotherapy, and/or radiation therapy. In recent years, more targeted therapies have emerged to specifically target cancer cells by identifying and exploiting specific molecular and/or immunophenotypic changes seen primarily in those cells. For example, many cancer cells preferentially express particular markers on their cellular surfaces and these markers have provided targets for antibody-based therapeutics.
- [0080] Significant progress has been made in genetically engineering cells of the immune system to target and kill unwanted cell types, such as cancer cells. Many of these immune cells are T cells that have been genetically engineered to express a recombinant receptor, for example, a

chimeric antigen receptor (CAR). CAR are proteins including several distinct subcomponents that allow the genetically modified T cells to recognize and kill targeted cell types. The subcomponents include at least an extracellular component and an intracellular component expressed as a single protein or assembling into a functional unit. The extracellular component includes a binding domain that binds a marker (e.g., an antigen) that is preferentially present on the surface of unwanted cells. When the binding domain binds such markers, the intracellular component signals the T cell to destroy the bound cell. CAR can additionally include a transmembrane domain that can link the extracellular component to the intracellular component.

**[0081]** Other subcomponents that can increase a CAR's function can also be used. For example, spacers provide CAR with additional conformational flexibility, often increasing the binding domain's ability to bind the targeted cell marker, leading to enhanced cytolytic effects. The appropriate length of a spacer within a particular CAR can depend on numerous factors including how close or far a targeted marker is located from the surface of an unwanted cell's membrane.

**[0082]** Although CAR T cells have had substantial success in treating various cancers, challenges remain. For example, CAR T cells can have suboptimal proliferation, functional anergy or exhaustion, impaired memory differentiation, and/or limited persistence limiting their ability to create or maintain remission in patients. Thus, strategies to improve CAR T cell efficacy are needed, especially for scenarios with prolonged antigen exposure that can lead to functional anergy and exhaustion.

**[0083]** Genetic enhancements of CAR T cell potency may improve anti-cancer efficacy but may risk adverse effects, such as uncontrolled cell growth. While intermittent administration of IL-15 to non-human primates is well tolerated and expands memory T cells, continuously delivered high-dose IL-15 is toxic (Berger et al, Blood 114(12):2417). Thus, the present disclosure provides regulated IL-15 secretion to enhance immune cell potency without driving autonomous immune cell growth or severe toxicity.

**[0084]** The present disclosure provides methods and artificial expression constructs including a mutated interleukin 15 (IL-15) transgene under the control of a constitutive or inducible expression control sequence to potentiate the function of an immune cell (e.g., recombinant receptor-expressing T cell). The mutated IL-15 is modified to focus signaling to cells expressing and/or signaling complexes including IL-15R $\alpha$ . In particular embodiments, the mutated IL-15 is modified to restrict signaling to cells expressing and/or signaling complexes including IL-15R $\alpha$ . In particular embodiments, the mutated IL-15 has lower affinity to, or cannot bind to, complexes including IL2R $\beta$  and common gamma receptor without IL-15R $\alpha$ , as compared to complexes including IL2R $\beta$  and common gamma receptor with IL-15R $\alpha$ . In various embodiments, the mutated IL-15 has lower

affinity to the common gamma receptor and/or atypical binding to IL2R $\beta$  compared to wild-type IL-15. In various embodiments, the mutated IL-15 is unable to bind the common gamma receptor. More particularly, the disclosure provides methods or artificial expression constructs that potentiate the function of immune cells by potentiating enhanced immune cell proliferation, decreased antigen-independent interferon-gamma (IFN $\gamma$ ) release, and suppression of tumor growth and uncontrolled immune cell proliferation. Methods of improving persistence (or function) of an adoptive cell therapy (ACT) and methods of treating a subject in need thereof are also provided.

**[0085]** In particular embodiments, a cell or an artificial expression construct includes a mutant IL-15 under the control of a constitutive or inducible expression control sequence. In particular embodiments, the mutant IL-15 preferentially binds to an IL15 receptor complex including IL-15R $\alpha$ . In particular embodiments, the mutant IL-15 includes a D to S mutation at position 8 compared to a processed wild-type IL-15 (SEQ ID NO: 41) or unprocessed wild-type IL-15 (SEQ ID NO: 8). In particular embodiments, the mutant IL-15 includes the sequence as set forth in SEQ ID NO: 9. In particular embodiments, the mutant IL-15 includes the sequence as set forth in SEQ ID NO: 42. In particular embodiments, the mutant IL-15 is encoded by the sequence as set forth in SEQ ID NO: 11. In particular embodiments, the mutant IL-15 is encoded by the sequence as set forth in SEQ ID NO: 44. In particular embodiments, the inducible synthetic promoter includes the sequence as set forth in SEQ ID NO: 15. In particular embodiments, the artificial expression construct further includes a recombinant receptor or exogenous lymphocyte receptor under the control of a constitutive expression control sequence. In particular embodiments, the recombinant receptor includes a chimeric antigen receptor (CAR), an engineered T cell receptor (eTCR), a dimerizing agent regulated immunoreceptor complex (DARIC), or a hybrid thereof. In particular embodiments, an exogenous lymphocyte receptor includes a T cell receptor or a B cell receptor that is introduced into the engineered cell by genetic modification. In particular embodiments, the recombinant receptor includes an anti-CD33 recombinant receptor. In particular embodiments, the recombinant receptor includes an anti-CLL1 recombinant receptor. In particular embodiments, the recombinant receptor includes a CAR. In particular embodiments, the recombinant receptor includes a DARIC. In particular embodiments, the constitutive expression control sequence includes an MNDU3 promoter or an EF1a promoter. In particular embodiments, the EF1a promoter includes the first intron of a human EF1a gene. In particular embodiments, the EF1a promoter lacks the first intron of a human EF1a gene.

**[0086]** Techniques for recombinant (i.e., engineered) DNA, peptide and oligonucleotide synthesis, immunoassays, tissue culture, transformation (e.g., electroporation, lipofection),

enzymatic reactions, purification and related techniques and procedures may be generally performed as described in various general and more specific references in microbiology, molecular biology, biochemistry, molecular genetics, cell biology, virology and immunology as cited and discussed throughout the present specification. See, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 3d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; *Current Protocols in Molecular Biology* (John Wiley and Sons, updated July 2008); *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Greene Pub. Associates and Wiley-Interscience; Glover, *DNA Cloning: A Practical Approach*, vol. I & II (IRL Press, Oxford Univ. Press USA, 1985); *Current Protocols in Immunology* (Edited by: John E. Coligan, Ada M. Kruisbeek, David H. Margulies, Ethan M. Shevach, Warren Strober 2001 John Wiley & Sons, NY, NY); *Real-Time PCR: Current Technology and Applications*, Edited by Julie Logan, Kirstin Edwards and Nick Saunders, 2009, Caister Academic Press, Norfolk, UK; Anand, *Techniques for the Analysis of Complex Genomes*, (Academic Press, New York, 1992); Guthrie and Fink, *Guide to Yeast Genetics and Molecular Biology* (Academic Press, New York, 1991); *Oligonucleotide Synthesis* (N. Gait, Ed., 1984); *Nucleic Acid The Hybridization* (B. Hames & S. Higgins, Eds., 1985); *Transcription and Translation* (B. Hames & S. Higgins, Eds., 1984); *Animal Cell Culture* (R. Freshney, Ed., 1986); Perbal, *A Practical Guide to Molecular Cloning* (1984); *Next-Generation Genome Sequencing* (Janitz, 2008 Wiley-VCH); *PCR Protocols (Methods in Molecular Biology)* (Park, Ed., 3rd Edition, 2010 Humana Press); *Immobilized Cells And Enzymes* (IRL Press, 1986); the treatise, *Methods In Enzymology* (Academic Press, Inc., N.Y.); *Gene Transfer Vectors For Mammalian Cells* (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); Harlow and Lane, *Antibodies*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1998); *Immunochemical Methods In Cell And Molecular Biology* (Mayer and Walker, eds., Academic Press, London, 1987); *Handbook Of Experimental Immunology*, Volumes I-IV (D. M. Weir and CC Blackwell, eds., 1986); Roitt, *Essential Immunology*, 6th Edition, (Blackwell Scientific Publications, Oxford, 1988); *Current Protocols in Immunology* (Q. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach and W. Strober, eds., 1991); *Annual Review of Immunology*; as well as monographs in journals such as *Advances in Immunology*.

B. Definitions.

**[0087]** Prior to setting forth this disclosure in more detail, it may be helpful to an understanding thereof to provide definitions of certain terms to be used herein.

**[0088]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention

belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of particular embodiments, preferred embodiments of compositions, methods and materials are described herein. For the purposes of the present disclosure, the following terms are defined below.

**[0089]** The articles “a,” “an,” and “the” are used herein to refer to one or to more than one (i.e., to at least one, or to one or more) of the grammatical object of the article. By way of example, “an element” means one element or one or more elements.

**[0090]** The use of the alternative (e.g., “or”) should be understood to mean either one, both, or any combination thereof of the alternatives.

**[0091]** “And/or” should be understood to mean either one, or both of the alternatives.

**[0092]** In one embodiment, a range, e.g., 1 to 5 refers to each numerical value encompassed by the range. For example, in one non-limiting and merely illustrative embodiment, the range “1 to 5” is equivalent to the expression 1, 2, 3, 4, 5; or 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, or 5.0; or 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, or 5.0. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

**[0093]** “Substantially” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that is 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher compared to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length. In one embodiment, “substantially the same” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that produces an effect, e.g., a physiological effect, that is approximately the same as a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

**[0094]** Reference throughout this specification to “one embodiment,” “an embodiment,” “a particular embodiment,” “a related embodiment,” “a certain embodiment,” “an additional

embodiment,” or “a further embodiment” or combinations thereof means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment. Thus, the appearances of the foregoing phrases in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments. It is also understood that the positive recitation of a feature in one embodiment, serves as a basis for excluding the feature in a particular embodiment.

**[0095]** Additional definitions are set forth throughout this disclosure.

C. Mutated IL-15 Polypeptides.

**[0096]** In particular embodiments, the mutated IL-15 preferentially binds to an IL15 receptor complex including IL-15R $\alpha$ , compared to an IL15 receptor complex without IL-15R $\alpha$ . In particular embodiments, the mutated IL-15 includes a D to S mutation compared to wild-type IL-15. In particular embodiments, wild-type IL-15 includes the sequence as set forth in SEQ ID NO: 8 and/or is encoded by the sequence as set forth in SEQ ID NO: 10. In particular embodiments, wild-type IL-15 includes the sequence as set forth in SEQ ID NO: 41 and/or is encoded by the sequence as set forth in SEQ ID NO: 43. In particular embodiments, mutated IL-15 includes the sequence as set forth in SEQ ID NO: 9 or a sequence having at least 90%, 95%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 9. In particular embodiments, mutated IL-15 includes the sequence as set forth in SEQ ID NO: 42 or a sequence having at least 90%, 95%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 42. In particular embodiments, mutated IL-15 is encoded by the sequence as set forth in SEQ ID NO: 11 or a sequence having at least 90%, 95%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 11. In particular embodiments, mutated IL-15 is encoded by the sequence as set forth in SEQ ID NO: 44 or a sequence having at least 90%, 95%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 44. Herein, the mutated IL-15 is also referred to as a mutant IL-15.

D. Recombinant Receptor.

**[0097]** In particular embodiments, a recombinant receptor is or includes a binding domain that binds a target antigen, wherein the recombinant receptor is expressed by a cell following the artificial introduction of a nucleic acid encoding the recombinant receptor into the cell. The recombinant receptor can be, e.g., a CAR, an engineered T cell receptor (eTCR), a dimerizing agent-regulated immunoreceptor complex (DARIC), or a hybrid thereof.

**[0098]** As described previously, CAR include several distinct subcomponents that allow genetically modified cells to recognize and kill unwanted cells, such as cancer cells. The



subcomponents include at least an extracellular component and an intracellular component. The extracellular component includes a binding domain that specifically binds a target antigen that is preferentially present on the surface of cells or the area thereof. When the binding domain binds such antigens, the intracellular component activates the cell (e.g., an immune effector cell) to destroy the bound cell. CARs additionally include a transmembrane domain that directly or indirectly links the extracellular component to the intracellular component, and other subcomponents that can increase the CAR's function. For example, the inclusion of a spacer region and/or one or more linker sequences can allow the CAR to have additional conformational flexibility, often increasing the binding domain's ability to bind the target antigen.

**[0099]** Many considerations associated with CAR apply to eTCR as well. eTCR disclosed herein include a binding domain that binds a target antigen (e.g., an scFv) linked to the C $\alpha$  and/or C $\beta$  chains of a TCR. A TCR is a heterodimeric fusion protein that typically includes an  $\alpha$  and  $\beta$  chain. Each chain includes a variable region (V $\alpha$  and V $\beta$ ) and a constant region (C $\alpha$  and C $\beta$ ). In particular embodiments, an eTCR does not include the native TCR variable region but does include the native TCR constant region. In particular embodiments, the eTCR includes an scFv as the variable region of either the  $\alpha$  or  $\beta$  chain. In particular embodiments, the eTCR includes an scFv as the variable region of both the  $\alpha$  and  $\beta$  chain. In particular embodiments, eTCR include a C $\alpha$  and/or C $\beta$  chain sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to an amino acid sequence of a known or identified TCR C $\alpha$  or C $\beta$ .

**[0100]** In particular embodiments, a TCR or eTCR lack a substantive intracellular domain. Rather, the TCR or eTCR is associated (or associates) with CD3 dimers to activate the downstream signaling machinery. The CD3 dimers can be made up of CD3 $\epsilon$ , CD3 $\gamma$ , CD3 $\delta$ , and CD3 $\zeta$  chains.

**[0101]** For particular examples of TCR that can be used within the context of the current disclosure, see, for example, WO2018/129270; WO2017/112944; WO2011/039507; US 8,008,438; US2016/0083449; US2015/0246959; Stromnes, et al. (2015) Cancer cell 28(5): 638-652; Kobayashi, et al. (2013) Nature Medicine 19: 1542-1546; Varela-Rohena, et al. (2008) Nature Medicine. 14(12): 1390-1395; and Robbins et al. (2008) The Journal of Immunology 180(9): 6116-6131.

**[0102]** Additionally, in particular embodiments, the TCRs and eTCRs described herein include amino acid substitutions that improve expression, stability, and/or functional avidity. In various embodiments, the TCRs and eTCRs include a minimally murinized TCR $\alpha$  chain and a minimally murinized TCR $\beta$  chain. In various embodiments, a TCR $\alpha$  chain transmembrane domain includes one or more hydrophobic amino acid substitutions. See, for example, WO2021/195503. In

particular embodiments, an eTCR includes modified TCRs. In particular embodiments, an eTCR includes a minimally murinized TCR.

**[0103]** Likewise, considerations associated with CAR apply to DARIC as well. The present disclosure provides methods and compositions for multimerizing components of a DARIC such that the DARIC is primed for signaling. In particular embodiments, a DARIC includes a first fusion protein (or first portion of a recombinant receptor) including a first multimerization domain and a second fusion protein (or second portion of a recombinant receptor) including a second multimerization domain and an intracellular component, wherein a dimerizing agent binds the first and second multimerization domains such that the first and second fusion proteins multimerize to form a DARIC ready for activation (i.e., primed for signaling).

**[0104]** In particular embodiments, “primed for signaling”, “priming for signaling”, “primes for signaling”, and similar phrases thereof (e.g., “priming a DARIC for signaling”) refers to the reconfiguration of the components of the DARIC such that a fusion protein including the binding domain and a fusion protein including the intracellular component are functionally coupled such that activation or downstream signaling can occur within the engineered cell upon binding a target antigen. In some places herein, a DARIC is referred to as activated or active when it is primed for signaling. In some embodiments, a DARIC does not include a binding domain. In some embodiments, a DARIC not including a binding domain includes an intracellular component on each of the first and second fusion proteins, wherein signaling occurs upon multimerization.

**[0105]** In particular embodiments, a DARIC can include a dimer, trimer, or higher order multimer formed by at least two different proteins, including at least one protein having a binding domain specific for a target and/or one protein having an intracellular component, such as an intracellular signaling domain, a co-stimulatory domain, or a co-receptor domain. The DARIC is primed for signaling when a dimerizing agent(s) brings together at least two of the proteins and the associated proteins together. In certain embodiments, the DARIC includes at least an intracellular component that allows transmission of or transmits an intracellular signal. In other embodiments, the DARIC includes a binding domain.

**[0106]** In particular embodiments, a DARIC includes a targeting component and a signaling component. In particular embodiments, the targeting component is the fusion protein including at least a binding domain and a multimerization domain. The targeting component can additionally include a linker, a spacer, and/or a transmembrane domain. In particular embodiments, the signaling component is the fusion protein including at least an intracellular component (e.g., effector domains, co-stimulatory domains) and a multimerization domain. The signaling component can additionally include a linker, a spacer, and/or a transmembrane domain.

**[0107]** For additional information on DARIC or similar recombinant receptors that can be used within the context of the current disclosure, see, for example, WO2023/108158, WO2023/196996A2, WO2023/196997A2, and WO2020/227474A1.

**[0108]** In particular embodiments, recombinant receptors include an extracellular component including a binding domain, an intracellular component including signaling domains, and a transmembrane domain. Each of these subcomponents is not mutually exclusive and there may be some overlap. For example, the transmembrane domain may extend into either or both of the extracellular and intracellular compartments. The intracellular and/or extracellular components can also extend into the transmembrane domain. Each of these subcomponents is described in more detail in the following section subheadings.

**[0109]** D1. Binding Domains.

**[0110]** A "binding domain" refers to a protein, polypeptide, oligopeptide, peptide or other molecule that possesses the ability to specifically recognize and bind to a target (e.g., CD19, CD20, CD33, CLL1 and/or other target antigen).

**[0111]** Binding domains useful in the instant disclosure include those known in the art or as described herein, or those generated by a variety of methods known in the art (see, e.g., U.S. Patent Nos. 6,291,161 and 6,291,158). For example, binding domains may be identified by screening a Fab phage library for Fab fragments that specifically bind to a target of interest (see Hoet *et al.*, *Nat. Biotechnol.* 23:344, 2005). Additionally, traditional strategies for hybridoma development, such as using a target antigen as an immunogen in convenient systems (e.g., mice, HuMAb mouse®, TC mouse™, KM-mouse®, llamas, sheep, chicken, rats, hamsters, rabbits, etc.), can be used to develop anti-target antibodies having target-specific binding domains of interest.

**[0112]** Sources of further binding domains include target-specific antibody variable domains from various species (which can be formatted as antibodies, single chain variable fragments (scFvs), single-domain antibodies (sdAbs), fragment antigen binding regions (Fabs), or soluble heavy chain variable (VH) domain, single chain single domain antibodies (VHH), or domain antibodies), including human, rodent, avian, and ovine. Additional sources of binding domains include variable domains of antibodies from other species, such as camelid (from camels, dromedaries, or llamas (Ghahroudi *et al.*, *FEBS Letters* 414:521, 1997; Vincke *et al.*, *J. Biol. Chem.* 284:3273, 2009; and Hamers-Casterman *et al.*, *Nature* 363:446, 1993; and Nguyen *et al.*, *J. Mol. Biol.* 275:413, 1998), nurse sharks (Roux *et al.*, *Proc. Nat'l. Acad. Sci. (USA)* 95:11804, 1998), spotted ratfish (Nguyen *et al.*, *Immunogenetics* 54:39, 2002), or lamprey (Herrin *et al.*, *Proc. Nat'l. Acad. Sci. (USA)* 105:2040, 2008 and Alder *et al.*, *Nature Immunol.* 9:319, 2008). These antibodies can apparently

form antigen-binding regions using only heavy chain variable region, *i.e.*, these functional antibodies are homodimers of heavy chains only (referred to as “heavy chain antibodies”) (Jespers *et al.*, *Nat. Biotechnol.* 22:1161, 2004; Cortez-Retamozo *et al.*, *Cancer Res.* 64:2853, 2004; Baral *et al.*, *Nature Med.* 12:580, 2006, and Barthelemy *et al.*, *J. Biol. Chem.* 283:3639, 2008).

**[0113]** Other alternative sources of target-specific binding domains includes sequences that encode random peptide libraries or sequences that encode an engineered diversity of amino acids in loop regions of alternative non-antibody scaffolds, such as fibrinogen domains (see, *e.g.*, Weisel *et al.* (1985) *Science* 230:1388), Kunitz domains (see, *e.g.*, US Patent No. 6,423,498), ankyrin repeat proteins (also known as DARPins; Binz *et al.*, *J. Mol. Biol.* 332:489, 2003 and Binz *et al.*, *Nat. Biotechnol.* 22:575, 2004), fibronectin binding domains (also known as adnectins or monobodies; Richards *et al.*, *J. Mol. Biol.* 326:1475, 2003; Parker *et al.*, *Protein Eng. Des. Sel.* 18:435, 2005 and Hackel *et al.*, *J. Mol. Biol.* 381:1238, 2008), cysteine-knot miniproteins (Vita *et al.*, *Proc. Nat'l. Acad. Sci. (USA)* 92:6404, 1995; Martin *et al.*, *Nat. Biotechnol.* 21:71, 2002 and Huang *et al.*, *Structure* 13:755, 2005), tetratricopeptide repeat domains (Main *et al.*, *Structure* 11:497, 2003 and Cortajarena *et al.*, *ACS Chem. Biol.* 3:161, 2008), leucine-rich repeat domains (Stumpp *et al.*, *J. Mol. Biol.* 332:471, 2003), anticalins (Skerra, *FEBS J.* 275:2677, 2008), lipocalin domains (see, *e.g.*, PCT Publication No. WO 2006/095164, Beste *et al.*, *Proc. Nat'l. Acad. Sci. (USA)* 96:1898, 1999 and Schönfeld *et al.*, *Proc. Nat'l. Acad. Sci. (USA)* 106:8198, 2009), armadillo repeat proteins (ArmRPs; Varadamsetty *et al.*, *J. Mol. Biol.* 424:68, 2012), diabodies (Manzke *et al.*, *Int. J. Cancer* 82:700, 1999), repebodies (Lee *et al.*, *Proc. Nat'l. Acad. Sci. U.S.A.* 109: 3299, 2012), minibodies (Hu *et al.*, *Cancer Res.* 56:3055, 1996), cyclotides (Craik *et al.*, *J. Mol. Biol.* 294:1327, 1999), V-like domains (see, *e.g.*, US Patent Application Publication No. 2007/0065431), C-type lectin domains (Zelensky and Gready, *FEBS J.* 272:6179, 2005; Beavil *et al.*, *Proc. Nat'l. Acad. Sci. (USA)* 89:753, 1992 and Sato *et al.*, *Proc. Nat'l. Acad. Sci. (USA)* 100:7779, 2003), mAb<sup>2</sup> or Fcab<sup>TM</sup> (see, *e.g.*, PCT Publication Nos. WO 2007/098934; WO 2006/072620), or the like (Nord *et al.*, *Protein Eng.* 8:601, 1995; Nord *et al.*, *Nat. Biotechnol.* 15:772, 1997; Nord *et al.*, *Eur. J. Biochem.* 268:4269, 2001; and Binz *et al.* (2005) *Nat. Biotechnol.* 23:1257, 2005).

**[0114]** In further embodiments, a binding domain is specific for a target that is an antigen associated with a cancer (*e.g.*, solid malignancy, hematologic malignancy), an inflammatory disease, an autoimmune disease, or a graft versus host disease. Exemplary target antigens include, alpha folate receptor (FR $\alpha$ ),  $\alpha_v\beta_6$  integrin, ADGRE2, BACE2, B cell maturation antigen (BCMA), B7-H3 (CD276), B7-H4, B7-H6, CA19.9, carbonic anhydrase IX (CAIX), CCR1, CD7,

CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CD244, carcinoembryonic antigen (CEA), C-type lectin-like molecule-1 (CLL1), CD2 subset 1 (CS-1), CLDN6, cMET, chondroitin sulfate proteoglycan 4 (CSPG4), CLDN18.2, cutaneous T cell lymphoma-associated antigen 1 (CTAGE1), DLL3, epidermal growth factor receptor (EGFR), epidermal growth factor receptor variant III (EGFRvIII), EGFR806, epithelial glycoprotein 2 (EGP2), epithelial glycoprotein 40 (EGP40), EPHB2, ERBB4, epithelial cell adhesion molecule (EPCAM), ephrin type-A receptor 2 (EPHA2), fibroblast activation protein (FAP), Fc Receptor Like 5 (FCRL5), fetal acetylcholinesterase receptor (AChR), FLT3, FN, FN-EDB, FRBeta, ganglioside G2 (GD2), ganglioside G3 (GD3), Glypican-3 (GPC3), EGFR family including ErbB2 (HER2), HER2p95, EGFRv3, IL-10R $\alpha$ , IL-13R $\alpha$ 2, Kappa, cancer/testis antigen 2 (LAGE-1A), K-Ras, K-Ras G12C, K-Ras G12D, K-Ras G12V, Lambda, Lewis-Y (LeY), L1 cell adhesion molecule (L1-CAM), LILRB2, LY6G6GD, melanoma antigen recognized by T cells 1 (MelanA or MART1), Mesothelin (MSLN), MMP10, MUC1, MUC16, MHC class I chain related proteins A (MICA), MHC class I chain related proteins B (MICB), neural cell adhesion molecule (NCAM), prostate stem cell antigen (PSCA), prostate-specific membrane antigen (PSMA), receptor tyrosine kinase-like orphan receptor 1 (ROR1), synovial sarcoma, X breakpoint 2 (SSX2), Survivin, tumor associated glycoprotein 72 (TAG72), transmembrane activator and CAML interactor (TACI), tumor endothelial marker 1 (TEM1/CD248), tumor endothelial marker 7-related (TEM7R), TIM3, trophoblast glycoprotein (TPBG), UL16-binding protein (ULBP) 1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, and vascular endothelial growth factor receptor 2 (VEGFR2). Additional illustrative binding domains can be found in WO2023196996A2, WO2023196997A2, and WO2017172981A2.

**[0115]** In some embodiments, the one or more antigen-binding domains bind CD19, CD20, CD22, CD33, CD79A, CD79B, B7H3, Muc16, Her2, EGFR, FN-EDB, CLDN18.2, DLL3, FLT3, CLL1, CD123, or BCMA. In some embodiments, the one or more antigen-binding domains bind CD33, CLL1, CD19, CD20, CD22, CD79A, CD79B, or BCMA. In some embodiments, the one or more antigen-binding domains bind CD33 and/or CLL1. In particular embodiments, the binding domain binds CD33. In particular embodiments, the binding domain binds CLL1. In particular embodiments, the binding domain binds CD33 and CLL1. In particular embodiments, the binding domain is an anti-CD33 VHH antibody, an anti-CD33 scFv, or an anti-CD33 sdAb. In particular embodiments, the binding domain is an anti-CLL1 VHH antibody, an anti-CLL1 scFv, or an anti-CLL1 sdAb.

**[0116]** D2. Intracellular Components.

**[0117]** An intracellular component of a recombinant receptor includes one or more intracellular

signaling, co-stimulatory, or co-receptor domains that transmit or allow the transmission of an intracellular signal. In particular embodiments, the intracellular component generates a signal that promotes an immune effector function of a recombinant receptor modified cell. In particular embodiments, the intracellular component generates a stimulatory and/or co-stimulatory signal based on ligand binding. Examples of immune effector function include cytolytic activity and helper activity, including the secretion of cytokines. Intracellular component signals can also lead to immune cell proliferation, activation, differentiation, and the like.

**[0118]** The intracellular effector domains of a recombinant receptor are responsible for activation of the cell in which the recombinant receptor is expressed. "Effector domain" is thus meant to include any portion of the intracellular domain sufficient to transduce an activation signal. An effector domain can directly or indirectly promote a biological or physiological response in a cell when receiving the appropriate signal. In certain embodiments, an effector domain is part of a protein or protein complex that receives a signal when bound, or it binds directly to a target molecule, which triggers a signal from the effector domain. An effector domain may directly promote a cellular response when it contains one or more signaling domains or motifs, such as an immunoreceptor tyrosine-based activation motif (ITAM). In other embodiments, an effector domain will indirectly promote a cellular response by associating with one or more other proteins that directly promote a cellular response, such as co-stimulatory domains.

**[0119]** Effector domains can provide for activation of at least one function of a modified cell upon binding to the cellular marker expressed by a cancer cell. Activation of the modified cell can include one or more of differentiation, proliferation and/or activation or other effector functions. In particular embodiments, an effector domain can include an intracellular signaling component including a T cell receptor and a co-stimulatory domain which can include the cytoplasmic sequence from co-receptor or co-stimulatory molecule.

**[0120]** An effector domain can include one, two, three or more intracellular signaling components (e.g., receptor signaling domains, cytoplasmic signaling sequences), co-stimulatory domains, or combinations thereof. Exemplary effector domains include signaling and stimulatory domains selected from: 4-1BB (CD137), CARD11, CD3 $\gamma$ , CD3 $\delta$ , CD3 $\epsilon$ , CD3 $\zeta$ , CD27, CD28, CD79A, CD79B, DAP10, FcR $\alpha$ , FcR $\beta$  (Fc $\epsilon$ R1b), FcR $\gamma$ , Fyn, HVEM (LIGHTR), ICOS, LAG3, LAT, Lck, LRP, NKG2D, NOTCH1, pT $\alpha$ , PTCH2, OX40, ROR2, Ryk, SLAMF1, Slp76, TCR $\alpha$ , TCR $\beta$ , TRIM, Wnt, Zap70, or any combination thereof. In particular embodiments, exemplary effector domains include signaling and co-stimulatory domains selected from: CD86, Fc $\gamma$ RIIa, DAP12, CD30, CD40, PD-1, 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, SLAMF7, NKp80

(KLRF1), CD127, CD160, CD19, CD4, CD8 $\alpha$ , CD8 $\beta$ , IL2R $\beta$ , IL2R $\gamma$ , IL7R $\alpha$ , ITGA4, VLA1, CD49a, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, GADS, PAG/Cbp, NKp44, NKp30, NKp46, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, or TLR9. In particular embodiments, the effector domain includes a CD3 $\zeta$  signaling domain. In particular embodiments, CD3 $\zeta$  signaling domain includes the sequence as set forth in SEQ ID NO: 110.

**[0121]** Intracellular signaling component sequences that act in a stimulatory manner may include iTAMs. Examples of iTAMs including primary cytoplasmic signaling sequences include those derived from CD3 $\gamma$ , CD3 $\delta$ , CD3 $\epsilon$ , CD3 $\zeta$ , CD5, CD22, CD66d, CD79a, CD79b, and common FcR $\gamma$  (FCER1G), Fc $\gamma$ R1a, FcR $\beta$  (Fc $\epsilon$  Rib), DAP10, and DAP12. In particular embodiments, variants of CD3 $\zeta$  retain at least one, two, three, or all ITAM regions.

**[0122]** In particular embodiments, an effector domain includes a cytoplasmic portion that associates with a cytoplasmic signaling protein, wherein the cytoplasmic signaling protein is a lymphocyte receptor or signaling domain thereof, a protein including a plurality of ITAMs, a co-stimulatory domain, or any combination thereof.

**[0123]** Additional examples of intracellular signaling components include the cytoplasmic sequences of the CD3 $\zeta$  chain, and/or co-receptors that act in concert to initiate signal transduction following binding domain engagement. In particular embodiments, more than just the cytoplasmic part of the sequence can be included in the intracellular component. For example, the intracellular component can include the transmembrane domain or a portion thereof of the same molecule.

**[0124]** A co-stimulatory domain is a domain whose activation can be required for an efficient lymphocyte response to cellular marker binding. Some molecules are interchangeable as intracellular signaling components or co-stimulatory domains. Examples of costimulatory domains include CD27, CD28, 4-1BB (CD 137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds with CD83. For example, CD27 co-stimulation has been demonstrated to enhance expansion, effector function, and survival of immune effector cells in vitro and augments human T cell persistence and anti-cancer activity in vivo (Song et al. Blood. 2012; 119(3):696-706). Further examples of such co-stimulatory domain molecules include CDS, ICAM-1, GITR, BAFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160, CD19, CD4, CD8 $\alpha$ , CD8 $\beta$ , IL2R $\beta$ , IL2R $\gamma$ , IL7R $\alpha$ , ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f,

ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), NKG2D, CEACAM1, CRTAM, Ly9 (CD229), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, and CD19a. In particular embodiments, the co-stimulatory domain includes a 4-1BB signaling domain. In particular embodiments, the 4-1BB signaling domain includes the sequence as set forth in SEQ ID NO: 111.

**[0125]** Intracellular components may also include one or more of a protein of a Wnt signaling pathway (e.g., LRP, Ryk, or ROR2), NOTCH signaling pathway (e.g., NOTCH1, NOTCH2, NOTCH3, or NOTCH4), Hedgehog signaling pathway (e.g., PTCH or SMO), receptor tyrosine kinases (RTKs) (e.g., epidermal growth factor (EGF) receptor family, fibroblast growth factor (FGF) receptor family, hepatocyte growth factor (HGF) receptor family, insulin receptor (IR) family, platelet-derived growth factor (PDGF) receptor family, vascular endothelial growth factor (VEGF) receptor family, tropomyosin receptor kinase (Trk) receptor family, ephrin (Eph) receptor family, AXL receptor family, leukocyte tyrosine kinase (LTK) receptor family, tyrosine kinase with immunoglobulin-like and EGF-like domains 1 (TIE) receptor family, receptor tyrosine kinase-like orphan (ROR) receptor family, discoidin domain (DDR) receptor family, rearranged during transfection (RET) receptor family, tyrosine-protein kinase-like (PTK7) receptor family, related to receptor tyrosine kinase (RYK) receptor family, or muscle specific kinase (MuSK) receptor family); G-protein-coupled receptors, GPCRs (Frizzled or Smoothened); serine/threonine kinase receptors (BMPR or TGFR); or cytokine receptors (IL1R, IL2R, IL7R, or IL15R).

**[0126]** In particular embodiments, the intracellular component includes a 4-1BB signaling domain, a CD3 $\zeta$  signaling domain, a CD3 $\epsilon$  signaling domain, or a CD4 signaling domain. In particular embodiments, the intracellular component includes a 4-1BB signaling domain. In particular embodiments, the intracellular component includes a CD3 $\zeta$  signaling domain. In particular embodiments, the intracellular component includes a CD3 $\epsilon$  signaling domain. In particular embodiments, the intracellular component includes a CD4 signaling domain. In particular embodiments, the CD4 signaling domain is a truncated intracellular polypeptide.

**[0127]** D3. Transmembrane Domains.

**[0128]** A recombinant receptor can be designed to include a transmembrane domain. A transmembrane domain can anchor a recombinant receptor to a cell membrane. A transmembrane domain can include one or more additional amino acids adjacent to the transmembrane region, e.g., one or more amino acids associated with the extracellular region of the protein from which the transmembrane was derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12,



13, 14, 15 amino acids, or more of the extracellular region) and/or one or more additional amino acids associated with the intracellular region of the protein from which the transmembrane protein is derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 amino acids, or more of the intracellular region). In particular embodiments, the transmembrane domain may be from the same protein that an intracellular component signaling domain, costimulatory domain, hinge domain, or co-receptor is derived from. In particular embodiments, the transmembrane domain is not derived from the same protein that any other domain of a recombinant receptor is derived from. In particular embodiments, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of or to minimize interactions with other domains in the recombinant receptor.

**[0129]** In particular embodiments, a transmembrane domain has a three-dimensional structure that is thermodynamically stable in a cell membrane, and generally ranges in length from 15 to 30 amino acids. The structure of a transmembrane domain can include an alpha helix, a beta barrel, a beta sheet, a beta helix, or any combination thereof.

**[0130]** The transmembrane domain can be derived either from a natural and/or a synthetic source. When the source is natural, the transmembrane domain can be derived from any membrane-bound or transmembrane protein. Transmembrane domains can include at least the transmembrane region(s) of the  $\alpha$ ,  $\beta$  or  $\zeta$  chain of a T-cell receptor, CD28, CD27, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22; CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, and TLR9. In particular embodiments, a transmembrane domain may include at least the transmembrane region(s) of, e.g., KIRDS2, OX40, CD2, CD27, LFA-1 (CD 11a, CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRP1), NKp44, NKp30, NKp46, CD160, CD19, IL2R $\beta$ , IL2R $\gamma$ , IL7R  $\alpha$ , ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CDI Id, ITGAE, CD103, ITGAL, CDI Ia, ITGAM, CDI Ib, ITGAX, CDI Ic, ITGB1, CD29, ITGB2, CD18, ITGB7, TNFR2, DNAM1(CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9(CD229), , PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKG2D, or NKG2C. In particular embodiments, a variety of human hinges can be employed as well including the human Ig (immunoglobulin) hinge (e.g., an IgG4 hinge, an IgD hinge), a GS linker (e.g., a GS linker described herein), a KIR2DS2 hinge or a CD8a hinge. In particular embodiments, the recombinant receptor includes a CD28 transmembrane domain, a CD4 transmembrane domain, or a CD8 $\alpha$  transmembrane domain. In particular embodiments, the CD28 transmembrane domain includes the sequence as set forth in SEQ ID NO: 108. In particular

embodiments, the CD4 transmembrane domain includes the sequence as set forth in SEQ ID NO: 90. In particular embodiments, the CD8 $\alpha$  transmembrane domain includes the sequence as set forth in SEQ ID NO: 109.

**[0131]** In particular embodiments, the transmembrane domain can include predominantly hydrophobic residues such as leucine and valine. In particular embodiments, the transmembrane domain can include a triplet of phenylalanine, tryptophan and valine found at each end of the transmembrane domain. In particular embodiments, a CD28, CD4, or CD8 hinge is juxtaposed on the extracellular side of the transmembrane domain.

**[0132]** D4. Linkers and Spacers.

**[0133]** A linker within a recombinant receptor can be any portion of a recombinant receptor that serves to connect two subcomponents or domains of the recombinant receptor. In particular embodiments, linkers can provide flexibility for different components of the recombinant receptor. Linkers in the context of linking VH and VL of antibody derived binding domains of scFv are described above. Linkers can also include spacer regions and junction amino acids.

**[0134]** Spacer regions are a type of linker region that are used to create appropriate distances and/or flexibility from other linked components.

**[0135]** In particular embodiments, the length of a spacer region can be customized for individual purposes. For example, a spacer region can be customized for individual cellular markers on targeted cells to optimize cell recognition and destruction following recombinant receptor binding. In certain examples, the spacer can be of a length that provides for increased responsiveness of a recombinant receptor expressing cell following antigen binding, as compared to in the absence of the spacer. In particular embodiments, a spacer region length can be selected based upon the location of a cellular marker epitope, affinity of a binding domain for the epitope, and/or the ability of the recombinant receptor modified cells to destroy target cells *ex vivo* and/or *in vivo* in response to cellular marker recognition. Spacer regions can also allow for high expression levels in recombinant receptor modified cells. In particular embodiments, an extracellular spacer region of a recombinant receptor is located between a transmembrane domain and the extracellular binding domain.

**[0136]** Exemplary spacers include those having 10 to 250 amino acids, 10 to 200 amino acids, 10 to 150 amino acids, 10 to 100 amino acids, 10 to 50 amino acids, or 10 to 25 amino acids. In particular embodiments, a spacer region is 12 amino acids, 20 amino acids, 21 amino acids, 26 amino acids, 27 amino acids, 45 amino acids, or 50 amino acids. In particular embodiments, a long spacer is greater than 119 amino acids, an intermediate spacer is 13-119 amino acids, and a short spacer is 10-12 amino acids.

**[0137]** In particular embodiments, a spacer region includes an immunoglobulin hinge region. An immunoglobulin hinge region may be a wild-type immunoglobulin hinge region or an altered wild-type immunoglobulin hinge region. In particular embodiments, an immunoglobulin hinge region is a human immunoglobulin hinge region. An immunoglobulin hinge region may be an IgG, IgA, IgD, IgE, or IgM hinge region. An IgG hinge region may be an IgG1, IgG2, IgG3, or IgG4 hinge region. In particular embodiments, the spacer region can include all or a portion of a hinge region sequence from IgG1, IgG2, IgG3, IgG4 or IgD alone or in combination with all or a portion of a CH2 region; all or a portion of a CH3 region; or all or a portion of a CH2 region and all or a portion of a CH3 region. A “wild type immunoglobulin hinge region” refers to a naturally occurring upper and middle hinge amino acid sequences interposed between and connecting the CH1 and CH2 domains (for IgG, IgA, and IgD) or interposed between and connecting the CH1 and CH3 domains (for IgE and IgM) found in the heavy chain of an antibody.

**[0138]** Exemplary spacers include IgG4 hinge alone, IgG4 hinge linked to CH2 and CH3 domains, or IgG4 hinge linked to the CH3 domain. Hinge regions can be modified to avoid undesirable structural interactions such as dimerization with unintended partners. Other examples of hinge regions that can be used in recombinant receptor described herein include the hinge region present in extracellular regions of type 1 membrane proteins, such as CD8 $\alpha$ , CD4, CD28, and CD7, which may be wild-type or variants thereof.

**[0139]** In particular embodiments, a spacer region includes a hinge region of a type II C-lectin interdomain (stalk) region or a cluster of differentiation (CD) molecule stalk region. A “stalk region” of a type II C-lectin or CD molecule refers to the portion of the extracellular domain of the type II C-lectin or CD molecule that is located between the C-type lectin-like domain (CTLD; e.g., similar to CTLD of natural killer cell receptors) and the hydrophobic portion (transmembrane domain). For example, the extracellular domain of human CD94 (GenBank Accession No. AAC50291.1) corresponds to amino acid residues 34–179, but the CTLD corresponds to amino acid residues 61–176, so the stalk region of the human CD94 molecule includes amino acid residues 34–60, which are located between the hydrophobic portion (transmembrane domain) and CTLD (see Boyington et al., *Immunity* 10:15, 1999; for descriptions of other stalk regions, see *also* Beavil et al., *Proc. Nat'l. Acad. Sci. USA* 89:153, 1992; and Figdor et al., *Nat. Rev. Immunol.* 2:11, 2002). These type II C-lectin or CD molecules may also have junction amino acids between the stalk region and the transmembrane region or the CTLD. In another example, the 233 amino acid human NKG2A protein (UniProt ID P26715.1) has a hydrophobic portion (transmembrane domain) ranging from amino acids 71–93 and an extracellular domain ranging from amino acids 94–233. The CTLD includes amino acids 119–231 and the stalk region includes amino acids 99–

116, which may be flanked by additional junction amino acids. Other type II C-lectin or CD molecules, as well as their extracellular ligand-binding domains, stalk regions, and CTLDs are known in the art (see, e.g., GenBank Accession Nos. NP 001993.2; AAH07037.1; NP 001773.1; AAL65234.1; CAA04925.1; for the sequences of human CD23, CD69, CD72, NKG2A, and NKG2D and their descriptions, respectively).

**[0140]** Linkers can be flexible, rigid, or semi-rigid, depending on the desired function of the linker. Linkers can include junction amino acids. For example, in particular embodiments, linkers provide flexibility and room for conformational movement between different components of a recombinant receptor. Commonly used flexible linkers include Gly-Ser linkers. In particular embodiments, the linker sequence includes sets of glycine and serine repeats such as from one to ten repeats of  $(\text{Gly}_x\text{Ser}_y)_n$ , wherein x and y are independently an integer from 0 to 10 provided that x and y are not both 0 and wherein n is an integer of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10). Particular examples include GG, GS, SG, SS, GSS, SSG, GSG, SGS, SGG, G4S, 2xG4S, 3xG4S, 4xG4S, 5xG4S, and any combination thereof, wherein "G4" indicates four Gly amino acids. Linkers can be used to connect components of an scFv such as linkers described in Whitlow et al. (Protein Eng. 6(8):989-95, 1993).

**[0141]** D5. Multimerization Domains.

**[0142]** A "multimerization domain" refers to a molecule that preferentially interacts or associates with another molecule directly or via a dimerizing agent, wherein the interaction of the different multimerization domains substantially contribute to or efficiently promote multimerization (*i.e.*, the formation of a dimer, trimer, or multipartite complex, which may be a homodimer, heterodimer, homotrimer, heterotrimer, homomultimer, heteromultimer).

**[0143]** In particular embodiments, multimerization domains will associate using a dimerizing agent. In particular embodiments, the dimerizing agent is rapamycin or an analog thereof. For example, the first and second multimerization domains are a pair selected from a FKBP binding protein (FKBP) multimerization domain and a FKBP-rapamycin binding (FRB) multimerization domain, or variants thereof. FRB domains are polypeptide regions (protein "domains") that are capable of forming a tripartite complex with an FKBP protein and rapamycin or rapalog thereof. FRB domains are present in a number of naturally occurring proteins, including mTOR proteins (also referred to in the literature as FRAP, RAPT1, or RAFT) from human and other species; yeast proteins including Tor1 and Tor2; and a *Candida* FRAP homolog. Information concerning the nucleotide sequences, cloning, and other aspects of these proteins is known in the art. For example, a protein sequence accession number for a human mTOR is GenBank Accession No. L34075.1 (Brown *et al.*, *Nature* 369:756, 1994).

**[0144]** In particular embodiments, the first and second multimerization domains localize extracellularly when the first and second portions of a recombinant receptor are expressed. In particular embodiments, the first and second multimerization domains localize intracellularly when the first and second portions of a recombinant receptor are expressed.

**[0145]** In particular embodiments, “FKBP-rapamycin binding (FRB) multimerization domain” refers to an FRB polypeptide. FRB domains for use in the recombinant receptor of this disclosure generally contain at least 85 to 100 amino acid residues. In certain embodiments, an FRB amino acid sequence for use in recombinant receptor of this disclosure will include a 93 amino acid sequence Ile-2021 through Lys -2113 and a mutation of T2098L (T82L is equivalent position in 93 amino acid FRB polypeptide), with reference to GenBank Accession No. L34075.1. An FRB domain for use in recombinant receptor of this disclosure will be capable of binding to a complex of an FKBP protein bound to rapamycin or an analog thereof of this disclosure. In certain embodiments, a peptide sequence of an FRB domain includes (a) a naturally occurring peptide sequence spanning at least the indicated 93 amino acid region of human mTOR or corresponding regions of homologous proteins; (b) a variant of a naturally occurring FRB in which up to ten amino acids, or 1 to 5 amino acids or 1 to 3 amino acids, or in some embodiments just one amino acid, of the naturally-occurring peptide have been deleted, inserted, or substituted; or (c) a peptide encoded by a nucleic acid molecule capable of selectively hybridizing to a DNA molecule encoding a naturally occurring FRB domain or by a DNA sequence which would be capable, but for the degeneracy of the genetic code, of selectively hybridizing to a DNA molecule encoding a naturally occurring FRB domain. In particular embodiments, an FRB polypeptide binds to an FKBP polypeptide through a bridging factor, thereby forming a ternary complex.

**[0146]** Particular embodiments utilize the FRB sequence as set forth in SEQ ID NO: 86 and particular embodiments utilize the sequence as set forth in SEQ ID NO: 87.

**[0147]** In particular embodiments, “FK506 binding protein (FKBP) multimerization domain” refers to an FKBP polypeptide. FKBP are the cytosolic receptors for macrolides, such as FK506, FK520 and rapamycin, and are highly conserved across species lines. For the purpose of this disclosure, FKBP are proteins or protein domains that are capable of binding to rapamycin or to an analog thereof and further forming a tripartite complex with an FRB-containing protein or recombinant receptor. An FKBP domain may also be referred to as a “rapamycin binding domain”. Information concerning the nucleotide sequences, cloning, and other aspects of various FKBP species is known in the art (see, e.g., Staendart *et al.*, *Nature* 346:671, 1990 (human FKBP12); Kay, *Biochem. J.* 314:361, 1996). Homologous FKBP proteins in other mammalian species, in yeast, and in other organisms are also known in the art and may be used in the recombinant receptor

disclosed herein. The size of FKBP domains for use in the disclosure varies, depending on which FKBP protein is employed. An FKBP domain of a recombinant receptor of this disclosure will be capable of binding to rapamycin or an analog thereof and participating in a tripartite complex with an FRB-containing protein (as may be determined by any means, direct or indirect, for detecting such binding).

**[0148]** The peptide sequence of an FKBP domain of an FKBP recombinant receptor of the disclosure includes (a) a naturally occurring FKBP peptide sequence, preferably derived from the human FKBP12 protein (GenBank Accession No. AAA58476.1) or a peptide sequence derived therefrom, from another human FKBP, from a murine or other mammalian FKBP, or from some other animal, yeast or fungal FKBP; (b) a variant of a naturally occurring FKBP sequence in which up to ten amino acids, or 1 to 5 amino acids or 1 to 3 amino acids, or in some embodiments just one amino acid, of the naturally-occurring peptide have been deleted, inserted, or substituted; or (c) a peptide sequence encoded by a nucleic acid molecule capable of selectively hybridizing to a DNA molecule encoding a naturally occurring FKBP or by a DNA sequence which would be capable, but for the degeneracy of the genetic code, of selectively hybridizing to a DNA molecule encoding a naturally occurring FKBP. In particular embodiments, the FKBP polypeptide is an FKBP12 polypeptide or an FKBP12 polypeptide including an F36V mutation. In particular embodiments, an FKBP polypeptide contemplated herein binds to an FRB polypeptide through a bridging factor, thereby forming a ternary complex.

**[0149]** In particular embodiments, FKBP includes the sequence as set forth in SEQ ID NO: 88. In particular embodiments, FKBP includes the sequence as set forth in SEQ ID NO: 89.

**[0150]** A “bridging factor” refers to a molecule that associates with and that is disposed between two or more multimerization domains. In particular embodiments, multimerization domains substantially contribute to or efficiently promote formation of a polypeptide complex only in the presence of a bridging factor. In particular embodiments, multimerization domains do not contribute to or do not efficiently promote formation of a polypeptide complex in the absence of a bridging factor. Illustrative examples of bridging factors suitable for use in particular embodiments contemplated herein include AP21967, rapamycin (sirolimus) or a rapalog thereof, coumermycin or a derivative thereof, gibberellin or a derivative thereof, abscisic acid (ABA) or a derivative thereof, methotrexate or a derivative thereof, cyclosporin A or a derivative thereof, FKCsA or a derivative thereof, trimethoprim (Tmp)-synthetic ligand for FKBP (SLF) or a derivative thereof, or any combination thereof.

**[0151]** Other multimerization domain pairs include FKBP and calcineurin, FKBP and cyclophilin, FKBP and bacterial DHFR, calcineurin and cyclophilin, PYL1 and ABI1, or GIB1 and GAI, or

variants thereof.

**[0152]** In particular embodiments, the first multimerization domain is an FRB multimerization domain and the second multimerization domain is an FKBP multimerization domain. In particular embodiments, the first multimerization domain is an FKBP multimerization domain and the second multimerization domain is an FRB multimerization domain. In particular embodiments, the dimerizing agent/bridging factor is a rapamycin and/or analog thereof.

**[0153]** In certain embodiments, the first and second multimerization domains are the same or different.

**[0154]** A “dimerizing agent” refers to any molecule capable of binding to a first multimerization domain and second multimerization domain, thus bringing together the two multimerization domains and any constituents thereby attached to the multimerization domain.

**[0155]** In particular embodiments, the dimerizing agent is rapamycin (sold under the brand name Rapamune® (Amgen, Thousand Oaks, CA) and also known as sirolimus). Rapamycin analogs (rapalogs) can also be used. Exemplary rapamycin analogs include those disclosed in U.S. Patent No. 6,649,595, which describes various rapalog structures. In certain embodiments, a dimerizing agent is a rapalog with substantially reduced immunosuppressive effect as compared to rapamycin. A “substantially reduced immunosuppressive effect” refers to a rapalog having at least less than 0.1 to 0.005 times the immunosuppressive effect observed or expected for an equimolar amount of rapamycin, as measured either clinically or in an appropriate *in vitro* (e.g., inhibition of T cell proliferation) or *in vivo* surrogate of human immunosuppressive activity. Alternatively, “substantially reduced immunosuppressive effect” refers to a rapalog having an EC<sub>50</sub> value in such an *in vitro* assay that is at least 10 to 250 times larger than the EC<sub>50</sub> value observed for rapamycin in the same assay. Other exemplary rapalogs include everolimus, novolimus, pimecrolimus, ridaforolimus, tacrolimus, temsirolimus, umirolimus, zotarolimus, rimiducid (AP1903), AP20187 (other names: 2,2'-[[2-[(dimethylamino)methyl]-1,3-propanediyl]bis[imino(2-oxo-2,1-ethanediyl)oxy-3,1-phenylene[(1R)-3-(3,4-dimethoxyphenyl)propylidene]]] ester; (2S,2'S)-1-[(2S)-1-oxo-2-(3,4,5-trimethoxyphenyl)butyl]-2-piperidinecarboxylic acid B/B Homodimerizer), AP21967 (other names: C16-(S)-7-methylindolerapamycin; C16-AiRap) and BPC015.

**[0156]** In particular embodiments, dimerizing agents include rapamycin (sirolimus) or a rapalog thereof, coumermycin or a derivative thereof, gibberellin or a derivative thereof, abscisic acid (ABA) or a derivative thereof, methotrexate or a derivative thereof, cyclosporin A or a derivative thereof, FKCsA or a derivative thereof, trimethoprim (Tnp)-synthetic ligand for FKBP (SLF) or a

derivative thereof, or any combination thereof.

E. Polynucleotides.

**[0157]** “Constitutive expression control sequence” refers to a promoter, enhancer, or promoter/enhancer that continually or continuously allows for transcription of an operably linked sequence. A constitutive expression control sequence may be a “ubiquitous” promoter, enhancer, or promoter/enhancer that allows expression in a wide variety of cell and tissue types or a “cell specific,” “cell type specific,” “cell lineage specific,” or “tissue specific” promoter, enhancer, or promoter/enhancer that allows expression in a restricted variety of cell and tissue types, respectively.

**[0158]** “Promoter” refers to a recognition site of a polynucleotide (DNA or RNA) to which an RNA polymerase binds. An RNA polymerase initiates and transcribes polynucleotides operably linked to the promoter. In particular embodiments, promoters operative in mammalian cells include an AT-rich region located 25 to 30 bases upstream from the site where transcription is initiated and/or another sequence found 70 to 80 bases upstream from the start of transcription, a CNCAAT region where N may be any nucleotide.

**[0159]** “Enhancer” refers to a segment of DNA which contains sequences capable of providing enhanced transcription and in some instances can function independent of their orientation relative to another control sequence. An enhancer can function cooperatively or additively with promoters and/or other enhancer elements. “Promoter/enhancer” refers to a segment of DNA which contains sequences capable of providing both promoter and enhancer functions.

**[0160]** Illustrative ubiquitous expression control sequences suitable for use in particular embodiments include, but are not limited to, a cytomegalovirus (CMV) immediate early promoter, a viral simian virus 40 (SV40) (e.g., early or late), a Moloney murine leukemia virus (MoMLV) LTR promoter, a Rous sarcoma virus (RSV) LTR, a herpes simplex virus (HSV) (thymidine kinase) promoter, H5, P7.5, and P11 promoters from vaccinia virus, an elongation factor 1-alpha (EF1a) promoter, early growth response 1 (EGR1), ferritin H (FerH), ferritin L (FerL), Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), eukaryotic translation initiation factor 4A1 (EIF4A1), heat shock 70kDa protein 5 (HSPA5), heat shock protein 90kDa beta, member 1 (HSP90B1), heat shock protein 70kDa (HSP70),  $\beta$ -kinesin ( $\beta$ -KIN), the human ROSA 26 locus (Irions et al., Nature Biotechnology 25, 1477 - 1482 (2007)), a Ubiquitin C promoter (UBC), a phosphoglycerate kinase-1 (PGK) promoter, a cytomegalovirus enhancer/chicken  $\beta$ -actin (CAG) promoter, a  $\beta$ -actin promoter and a myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer-binding site substituted (MND) U3 promoter (Haas et al. Journal of Virology. 2003;77(17): 9439-9450).



**[0161]** In one embodiment, an artificial expression construct includes an MNDU3 promoter. In one embodiment, a vector includes an MNDU3 promoter.

**[0162]** In one embodiment, an artificial expression construct includes EF1a promoter including the first intron of a human EF1a gene. In one embodiment, a vector includes an EF1a promoter including the first intron of a human EF1a gene.

**[0163]** In one embodiment, an artificial expression construct includes an EF1a promoter that lacks the first intron of a human EF1a gene. In one embodiment, a vector includes an EF1a promoter that lacks the first intron of a human EF1a gene.

**[0164]** “Conditional expression” may refer to any type of conditional expression including, but not limited to, inducible expression; repressible expression; expression in cells or tissues having a particular physiological, biological, or disease state, etc. This definition is not intended to exclude cell type or tissue specific expression. Certain embodiments provide conditional expression of a polynucleotide-of-interest, e.g., expression is controlled by subjecting a cell, tissue, organism, etc., to a treatment or condition that causes the polynucleotide to be expressed or that causes an increase or decrease in expression of the polynucleotide encoded by the polynucleotide-of-interest.

**[0165]** In some alternatives, an inducible synthetic promoter is used, wherein the inducible synthetic promoter includes a first sequence encoding a transcription factor response element; and a second sequence encoding a promoter sequence, optionally, wherein said inducible synthetic promoter includes one or more of SEQ ID NOs: 45-83. In some alternatives, the inducible synthetic promoter is inducible by chimeric antigen receptor activation. In some alternatives, the inducible synthetic promoter is inducible by binding of the chimeric antigen receptor to a ligand. In some alternatives, the inducible synthetic promoter is inducible by interaction with CD3/CD28. In some alternatives, the CD3/CD28 are conjugated on beads. In some alternatives, the inducible synthetic promoter is inducible by a chemical. In some alternatives, the chemical is PMA or Ionomycin. In some alternatives, the promoter sequence includes an IL2 minimal promoter sequence. In particular embodiments, the IL2 minimal promoter sequence includes the sequence as set forth in SEQ ID NO: 107. In some alternatives, the first sequence in the inducible synthetic promoter includes a sequence as set forth in any one of SEQ ID NOs: 45-83. In some alternatives, the inducible synthetic promoter includes a sequence that has at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity or has a sequence identity within a range between any two aforementioned percentages to a sequence as set forth in any one of SEQ ID NOs: 45-83. In some alternatives, the transcription factor response element includes E2F1, EGR1, HIF1A, NFAT, LEF1, SP1, PU.1, NFkB, JUN, FOS, and/or STAT4. In

particular embodiments, the inducible synthetic promoter includes a sequence as set forth in any of SEQ ID NOs: 45-83 and an IL2 minimal promoter. In particular embodiments, the inducible synthetic promoter includes the sequence as set forth in SEQ ID NO: 15.

**[0166]** Additional examples of inducible promoters/systems include, but are not limited to, steroid-inducible promoters such as promoters for genes encoding glucocorticoid or estrogen receptors (inducible by treatment with the corresponding hormone), metallothionine promoter (inducible by treatment with various heavy metals), MX-1 promoter (inducible by interferon), the “GeneSwitch” mifepristone-regulatable system (Sirin et al., 2003, *Gene*, 323:67), the cumate inducible gene switch (WO 2002/088346), tetracycline-dependent regulatory systems, etc.

**[0167]** “Operably linked” refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. In one embodiment, “operably linked” refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter and/or enhancer) and a second polynucleotide sequence, e.g., a polynucleotide-of-interest, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

#### F. Genetically-Modified Cells.

**[0168]** In various embodiments, cells are modified to include and/or express the polypeptides and/or polynucleotides contemplated herein. In particular embodiments, the cells are for use in the treatment of a disease or disorder (e.g., cancer or autoimmune disease or disorder). Cells may be non-genetically modified to express one or more of the polypeptides contemplated herein, or in particular preferred embodiments, cells may be genetically modified to express one or more of the polypeptides contemplated herein. “Genetically engineered” or “genetically modified” refers to the addition of extra genetic material in the form of DNA or RNA into the total genetic material in a cell. “Genetically modified cells,” “modified cells,” and “non-natural” are used interchangeably in particular embodiments.

**[0169]** In particular embodiments, an artificial expression construct contemplated herein, is introduced and expressed in cells (e.g., lymphocytes or immune effector cells) to improve the efficacy, function, and/or persistence of the cells. In particular embodiments, one or more artificial expression constructs are introduced and expressed in cells that have been redirected to a target cell by virtue of co-expressing a recombinant receptor or exogenous lymphocyte receptor.

**[0170]** In some embodiments, the persistence is increased compared to a similar cell or population of cells expressing an exogenous wild-type IL15. In some embodiments, the improved function includes decreased antigen-independent IFN $\gamma$  release compared to a similar cell or population of cells expressing an exogenous wild-type IL15. In some embodiments, the improved

function includes increased proliferation compared to a similar cell or population of cells expressing an exogenous wild-type IL15.

**[0171]** An “immune effector cell,” is any cell of the immune system that has one or more effector functions (e.g., cytotoxic cell killing activity, secretion of cytokines, induction of antibody-dependent cellular cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC)). The illustrative immune effector cells contemplated herein include T lymphocytes, including but not limited to cytotoxic T cells (CTLs; CD8+ T cells), TILs, and helper T cells (HTLs; CD4+ T cells. In a particular embodiment, the cells include  $\alpha\beta$  T cells. In a particular embodiment, the cells include  $\gamma\delta$  T cells. In one embodiment, immune effector cells include natural killer (NK) cells. In one embodiment, immune effector cells include natural killer T (NKT) cells. In particular embodiments, immune cell and immune effector cell are used interchangeably.

**[0172]** Immune effector cells can be autologous/autogeneic (“self”) or non-autologous (“non-self,” e.g., allogeneic, syngeneic or xenogeneic). “Autologous” refers to cells from the same subject. “Allogeneic” refers to cells of the same species that differ genetically to the cell in comparison. “Syngeneic” refers to cells of a different subject that are genetically identical to the cell in comparison. “Xenogeneic” refers to cells of a different species to the cell in comparison. In preferred embodiments, the cells are human autologous immune effector cells.

**[0173]** Illustrative immune effector cells suitable for introducing an artificial expression construct contemplated herein include T lymphocytes. “T cell” or “T lymphocyte” are art-recognized and include thymocytes, immature T lymphocytes, mature T lymphocytes, resting T lymphocytes, or activated T lymphocytes. A T cell can be a T helper (Th) cell, for example a T helper 1 (Th1) or a T helper 2 (Th2) cell. The T cell can be a helper T cell (HTL; CD4+ T cell) CD4+ T cell, a cytotoxic T cell (CTL; CD8+ T cell), CD4+CD8+ T cell, CD4-CD8- T cell, or any other subset of T cells. Other illustrative populations of T cells suitable for use in particular embodiments include naïve T cells and memory T cells. For example, populations of T cells suitable for use in particular embodiments include naïve T cells (TN), T memory stem cells (TSCM), central memory T cells (TCM), effector memory T cells (TEM), and effector T cells (TEFF).

**[0174]** As would be understood by the skilled person, other cells may also be used as immune effector cells including an artificial expression construct and/or expressing a mutant IL15 polypeptide contemplated herein. In particular embodiments, immune effector cells also include NK cells, NKT cells, neutrophils, and macrophages. Immune effector cells also include progenitors of effector cells wherein such progenitor cells can be induced to differentiate into immune effector cells in vivo or in vitro. Thus, in particular embodiments, immune effector cells include progenitors of immune effectors cells such as hematopoietic stem cells (HSCs) contained

within the CD34+ population of cells derived from cord blood, bone marrow or mobilized peripheral blood which upon administration in a subject differentiate into mature immune effector cells, or which can be induced in vitro to differentiate into mature immune effector cells.

**[0175]** “CD34+ cell” refers to a cell expressing the CD34 protein on its cell surface. “CD34” refers to a cell surface glycoprotein (e.g., sialomucin protein) that often acts as a cell-cell adhesion factor and is involved in T cell entrance into lymph nodes. The CD34+ cell population contains hematopoietic stem cells (HSC), which upon administration to a patient differentiate and contribute to all hematopoietic lineages, including T cells, NK cells, NKT cells, neutrophils and cells of the monocyte/macrophage lineage.

**[0176]** Methods for making the immune effector cells which include an artificial expression construct and/or express a mutant IL15 polypeptide contemplated herein are provided in particular embodiments. In one embodiment, the method includes transfecting or transducing immune effector cells isolated from an individual such that the immune effector cells include one or more of the artificial expression constructs contemplated herein. In one embodiment, the method includes transfecting or transducing immune effector cells isolated from an individual such that the immune effector cells express i) a mutant IL15 polypeptide and ii) a recombinant receptor or exogenous lymphocyte receptor. In certain embodiments, the immune effector cells are isolated from an individual and genetically modified without further manipulation in vitro. Such cells can then be directly re-administered into the individual. In further embodiments, the immune effector cells are first activated and stimulated to proliferate in vitro prior to being genetically modified. In this regard, the immune effector cells may be cultured before and/or after being genetically modified.

**[0177]** In particular embodiments, the cells are human cells. In particular embodiments, prior to in vitro manipulation or genetic modification of the immune effector cells described herein, the source of cells is obtained from a subject. In particular embodiments, the modified immune effector cells include T cells. In particular embodiments, the immune effector cells are genetically modified *in vivo*.

**[0178]** T cells can be obtained from a number of sources including, but not limited to, peripheral blood mononuclear cells, bone marrow, lymph nodes tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled person, such as sedimentation, e.g., FICOLL® (Cytiva Sweden AB, Sweden) separation.

**[0179]** In other embodiments, an isolated or purified population of T cells is used. In some embodiments, after isolation of peripheral blood mononuclear cells (PBMC), both cytotoxic and helper T lymphocytes can be sorted into naïve, memory, and effector T cell subpopulations either before or after activation, expansion, and/or genetic modification.

**[0180]** In one embodiment, an isolated or purified population of T cells expresses one or more of the markers including, but not limited to a CD3+, CD4+, CD8+, or a combination thereof.

**[0181]** In certain embodiments, the T cells are isolated from an individual and first activated and stimulated to proliferate in vitro prior to being modified to include an artificial expression construct and/or express a mutant IL15 polypeptide contemplated herein.

**[0182]** In order to achieve sufficient therapeutic doses of T cell compositions, T cells are often subjected to one or more rounds of stimulation, activation and/or expansion. In particular embodiments, T cells can be activated and expanded generally using methods as described, for example, in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; and 6,867,041, each of which is incorporated herein by reference in its entirety. In particular embodiments, T cells are activated and expanded for 6 hours, 12 hours, 18 hours or 24 hours prior to introduction of vectors or polynucleotides encoding an artificial expression construct contemplated herein, optionally in combination with a recombinant receptor or exogenous lymphocyte receptor.

**[0183]** In one embodiment, T cells are activated at the same time that they are modified.

**[0184]** In various embodiments, a method of generating an immune effector cell includes activating a population of cells including T cells and expanding the population of T cells. T cell activation can be accomplished by providing a primary stimulation signal through the T cell TCR/CD3 complex and by providing a secondary costimulation signal through an accessory molecule, e.g., CD28.

**[0185]** The TCR/CD3 complex may be stimulated by contacting the T cell with a suitable CD3 binding agent, e.g., a CD3 ligand or an anti-CD3 monoclonal antibody. Illustrative examples of CD3 antibodies include, but are not limited to, OKT3, G19-4, BC3, and 64.1.

**[0186]** In addition to the primary stimulation signal provided through the TCR/CD3 complex, induction of T cell responses requires a second, costimulatory signal. In particular embodiments, a CD28 binding agent can be used to provide a costimulatory signal. Illustrative examples of CD28 binding agents include but are not limited to: natural CD28 ligands, e.g., a natural ligand for CD28 (e.g., a member of the B7 family of proteins, such as B7-1(CD80) and B7-2 (CD86); and anti-

CD28 monoclonal antibody or fragment thereof capable of crosslinking the CD28 molecule, e.g., monoclonal antibodies 9.3, B-T3, XR-CD28, KOLT-2, 15E8, 248.23.2, and EX5.3D10.

**[0187]** In one embodiment, the molecule providing the primary stimulation signal, for example a molecule which provides stimulation through the TCR/CD3 complex and the costimulatory molecule are coupled to the same surface.

**[0188]** In certain embodiments, binding agents that provide stimulatory and costimulatory signals are localized on the surface of a cell. This can be accomplished by transfecting or transducing a cell with a nucleic acid encoding the binding agent in a form suitable for its expression on the cell surface or alternatively by coupling a binding agent to the cell surface.

**[0189]** In another embodiment, the molecule providing the primary stimulation signal, for example a molecule which provides stimulation through the TCR/CD3 complex and the costimulatory molecule are displayed on antigen presenting cells.

**[0190]** In one embodiment, the molecule providing the primary stimulation signal, for example a molecule which provides stimulation through the TCR/CD3 complex and the costimulatory molecule are provided on separate surfaces.

**[0191]** In a certain embodiment, one of the binding agents that provides stimulatory and costimulatory signals is soluble (provided in solution) and the other agent(s) is provided on one or more surfaces. In a particular embodiments, the binding agents that provide stimulatory and costimulatory signals are both provided in a soluble form (provided in solution). In various embodiments, the methods for making T cells contemplated herein include activating T cells with soluble anti-CD3 and/or soluble anti-CD28 antibodies, or fragments thereof. In various embodiments, the methods for making T cells contemplated herein include activating T cells with surface bound anti-CD3 and/or surface bound anti-CD28 antibodies, or fragments thereof. In various embodiments, the methods for making T cells contemplated herein include activating T cells with bead-bound anti-CD3 and/or bead-bound anti-CD28 antibodies, or fragments thereof.

**[0192]** In one embodiment, expanding immune cells (e.g., T cells) activated by the methods contemplated herein further includes culturing a population of cells including immune cells for several hours (3 hours) to 7 days to 28 days or any hourly integer value in between. In another embodiment, the immune cell composition may be cultured for 14 days. In a particular embodiment, immune cells are cultured for 21 days. In another embodiment, the immune cell compositions are cultured for 2-3 days. Several cycles of stimulation/activation/expansion may also be desired.

**[0193]** In particular embodiments, conditions appropriate for immune cell (e.g., T cell) culture include an appropriate media (e.g., Minimal Essential Media or RPMI Media 1640 or, X-vivo 15,

(Lonza)) and one or more factors necessary for proliferation and viability including, but not limited to serum (e.g., fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN- $\gamma$ , IL-4, IL-7, IL-21, GM-CSF, IL-10, IL-12, IL-15, TGF $\beta$ , and TNF- $\alpha$  or any other additives suitable for the growth of cells known to the skilled artisan.

**[0194]** Further illustrative examples of cell culture media include, but are not limited to RPMI 1640, Clicks, AIM-V, DMEM, MEM,  $\alpha$ -MEM, IMDM, F-12, X-Vivo 15, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of immune cells.

**[0195]** Antibiotics, e.g., penicillin and streptomycin, are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (e.g., 37° C) and atmosphere (e.g., air plus 5% CO<sub>2</sub>).

**[0196]** In particular embodiments, PBMCs or isolated immune cells are contacted with a stimulatory agent and costimulatory agent, such as anti-CD3 and anti-CD28 antibodies, generally attached to a bead or other surface, in a culture medium with appropriate cytokines, such as IL-2, IL-7, and/or IL-15.

**[0197]** In other embodiments, artificial antigen-presenting cells (aAPC) are made by engineering K562, U937, 721.221, T2, and C1R cells to have stable expression and secretion of a variety of costimulatory molecules and cytokines. In a particular embodiment, K32 or U32 aAPCs are used to direct the display of one or more antibody-based stimulatory molecules on the aAPC cell surface. Populations of T cells can be expanded by aAPCs expressing a variety of costimulatory molecules including, but not limited to, CD137L (4-1BBL), CD134L (OX40L), and/or CD80 or CD86. Finally, the aAPCs provide an efficient platform to expand genetically modified immune cells and to maintain CD28 expression on CD8 T cells. aAPCs provided in WO 03/057171 and US2003/0147869 are hereby incorporated by reference in their entirety.

**[0198]** In a particular embodiment, an artificial expression construct or polynucleotide encoding a mutant IL15 is introduced into the population of immune cells. In a particular embodiment, an artificial expression construct or polynucleotide encoding a mutant IL15 is introduced into a population of immune cells that express a recombinant receptor or exogenous lymphocyte receptor. In particular embodiments, an artificial expression construct or polynucleotide encoding i) a mutant IL15 and ii) a recombinant receptor or exogenous lymphocyte receptor is introduced into a population of immune cells. The polynucleotides may be introduced into the T cells by

microinjection, transfection, lipofection, heat-shock, electroporation, transduction, gene gun, microinjection, DEAE-dextran-mediated transfer, and the like.

**[0199]** “Vector” refers to a nucleic acid molecule capable of transferring or transporting another nucleic acid molecule. The transferred nucleic acid is generally linked to, e.g., inserted into, the vector nucleic acid molecule. A vector may include sequences that direct autonomous replication in a cell or may include sequences sufficient to allow integration into host cell DNA. In particular embodiments, non-viral vectors are used to deliver one or more polynucleotides contemplated herein to an immune cell.

**[0200]** Illustrative examples of non-viral vectors include, but are not limited to mRNA, plasmids (e.g., DNA plasmids or RNA plasmids), transposons, cosmids, and bacterial artificial chromosomes. Other non-viral vectors are discussed above.

**[0201]** Illustrative methods of non-viral delivery of polynucleotides contemplated in particular embodiments include, but are not limited to: electroporation, sonoporation, lipofection, microinjection, biolistics, virosomes, liposomes, immunoliposomes, nanoparticles, polycation or lipid:nucleic acid conjugates, naked DNA, artificial virions, DEAE-dextran-mediated transfer, gene gun, and heat-shock.

**[0202]** Illustrative examples of non-viral / polynucleotide delivery systems suitable for use in particular embodiments contemplated in particular embodiments include, but are not limited to those provided by Amaxa Biosystems, Maxcyte, Inc., BTX Molecular Delivery Systems, and Copernicus Therapeutics Inc. Lipofection reagents are sold commercially (e.g., Transfectam™ and Lipofectin™). Cationic and neutral lipids that are suitable for efficient receptor-recognition lipofection of polynucleotides have been described in the literature. See e.g., Liu et al. (2003) Gene Therapy. 10:180–187; and Balazs et al. (2011) Journal of Drug Delivery. 2011:1-12. Antibody-targeted, bacterially derived, non-living nanocell-based delivery is also contemplated in particular embodiments.

**[0203]** In various embodiments, the polynucleotide is an mRNA that is introduced into a cell in order to transiently express a desired polypeptide. “Transient” refers to expression of a non-integrated transgene for a period of hours, days or weeks, wherein the period of time of expression is less than the period of time for expression of the polynucleotide if integrated into the genome or contained within a stable plasmid replicon in the cell.

**[0204]** In particular embodiments, viral vectors are used to deliver one or more polynucleotides contemplated herein to an immune cell (e.g., T cell).



**[0205]** Illustrative examples of viruses useful as vectors include, without limitation, retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpesvirus (e.g., herpes simplex virus), poxvirus, baculovirus, papillomavirus, and papovavirus (e.g., SV40).

**[0206]** In one embodiment, polynucleotides are introduced into an immune cell by AAV transduction. In one embodiment, polynucleotides are introduced into an immune cell by retroviral transduction. In one embodiment, polynucleotides are introduced into an immune cell by lentiviral transduction. In one embodiment, polynucleotides are introduced into an immune cell by adenovirus transduction. In one embodiment, polynucleotides are introduced into an immune cell by herpes simplex virus transduction. In one embodiment, polynucleotides are introduced into an immune cell by vaccinia virus transduction.

**[0207]** Additional illustrative examples of expression vectors include, but are not limited to, pCIneo vectors (Promega) for expression in mammalian cells; pLenti4/V5-DEST™, pLenti6/V5-DEST™, and pLenti6.2/V5-GW/lacZ (Invitrogen) for lentivirus-mediated gene transfer and expression in mammalian cells. In particular embodiments, coding sequences of polypeptides disclosed herein can be ligated into such expression vectors for the expression of the polypeptides in mammalian cells.

**[0208]** In particular embodiments, the vector is an episomal vector or a vector that is maintained extrachromosomally. “Episomal” refers to a vector that is able to replicate without integration into host’s chromosomal DNA and without gradual loss from a dividing host cell also meaning that said vector replicates extrachromosomally or episomally.

**[0209]** The “control elements” or “regulatory sequences” present in an artificial expression vector are those non-translated regions of the vector—origin of replication, selection cassettes, promoters, enhancers, translation initiation signals (Shine Dalgarno sequence or Kozak sequence) introns, a polyadenylation sequence, 5' and 3' untranslated regions—which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including ubiquitous promoters and inducible promoters may be used.

**[0210]** In particular embodiments, vectors include, but are not limited to expression vectors and viral vectors, and will include exogenous, endogenous, or heterologous control sequences such as promoters and/or enhancers. An “endogenous” control sequence is one which is naturally linked with a given gene in the genome. An “exogenous” control sequence is one which is placed in juxtaposition to a gene by means of genetic manipulation (i.e., molecular biological techniques) such that transcription of that gene is directed by the linked enhancer/promoter. A “heterologous”

control sequence is an exogenous sequence that is from a different species than the cell being genetically manipulated.

**[0211]** Conditional expression can also be achieved by using a site-specific DNA recombinase. According to certain embodiments the vector includes at least one (typically two) site(s) for recombination mediated by a site-specific recombinase. "Recombinase" or "site specific recombinase" include excisive or integrative proteins, enzymes, co-factors or associated proteins that are involved in recombination reactions involving one or more recombination sites (e.g., two, three, four, five, seven, ten, twelve, fifteen, twenty, thirty, fifty, etc.), which may be wild-type proteins (see Landy, *Current Opinion in Biotechnology* 3:699-707 (1993)), or mutants, derivatives (e.g., fusion proteins containing the recombination protein sequences or fragments thereof), fragments, and variants thereof. Illustrative examples of recombinases suitable for use in particular embodiments include, but are not limited to: Cre, Int, IHF, Xis, Flp, Fis, Hin, Gin,  $\Phi$ C31, Cin, Tn3 resolvase, TndX, XerC, XerD, TnpX, Hjc, Gin, SpCCE1, and ParA.

**[0212]** The vectors may include one or more recombination sites for any of a wide variety of site-specific recombinases. It is to be understood that the target site for a site-specific recombinase is in addition to any site(s) required for integration of a vector, e.g., a retroviral vector or lentiviral vector. "Recombination sequence," "recombination site," or "site specific recombination site" refer to a particular nucleic acid sequence to which a recombinase recognizes and binds.

**[0213]** For example, one recombination site for Cre recombinase is loxP which is a 34 base pair sequence including two 13 base pair inverted repeats (serving as the recombinase binding sites) flanking an 8 base pair core sequence (see FIG. 1 of Sauer, B., *Current Opinion in Biotechnology* 5:521-527 (1994)). Other exemplary loxP sites include, but are not limited to: lox511 (Hoess et al., 1996; Bethke and Sauer, 1997), lox5171 (Lee and Saito, 1998), lox2272 (Lee and Saito, 1998), m2 (Langer et al., 2002), lox71 (Albert et al., 1995), and lox66 (Albert et al., 1995).

**[0214]** Suitable recognition sites for the FLP recombinase include, but are not limited to: FRT (McLeod, et al., 1996), F1, F2, F3 (Schlake and Bode, 1994), F4, F5 (Schlake and Bode, 1994), FRT(LE) (Senecoff et al., 1988), FRT(RE) (Senecoff et al., 1988).

**[0215]** Other examples of recognition sequences are the attB, attP, attL, and attR sequences, which are recognized by the recombinase enzyme  $\lambda$  Integrase, e.g.,  $\phi$ C31. The  $\phi$ C31 SSR mediates recombination only between the heterotypic sites attB (34 bp in length) and attP (39 bp aposiength) (Groth et al., 2000). attB and attP, named for the attachment sites for the phage integrase on the bacterial and phage genomes, respectively, both contain imperfect inverted repeats that are likely bound by  $\phi$ C31 aposidimers (Groth et al., 2000). The product sites, attL and attR, are effectively inert to further  $\phi$ C31-mediated recombination (Belteki et al., 2003),

making the reaction irreversible. For catalyzing insertions, it has been found that attB-bearing DNA inserts into a genomic attP site more readily than an attP site into a genomic attB site (Thyagarajan et al., 2001; Belteki et al., 2003). Thus, typical strategies position by homologous recombination an attP-bearing “docking site” into a defined locus, which is then partnered with an attB-bearing incoming sequence for insertion.

**[0216]** An “internal ribosome entry site” or “IRES” refers to an element that promotes direct internal ribosome entry to the initiation codon, such as ATG, of a cistron (a protein encoding region), thereby leading to the cap-independent translation of the gene. See, e.g., Jackson et al., 1990. Trends Biochem Sci 15(12):477-83) and Jackson and Kaminski. 1995. RNA 1(10):985-1000. In particular embodiments, vectors include one or more polynucleotides-of-interest that encode one or more polypeptides. In particular embodiments, to achieve efficient translation of each of the plurality of polypeptides, the polynucleotide sequences can be separated by one or more IRES sequences or polynucleotide sequences encoding self-cleaving polypeptides. In one embodiment, the IRES used in polynucleotides contemplated herein is an EMCV IRES.

**[0217]** In particular embodiments, artificial expression constructs can include a polynucleotide that encodes a self-cleaving polypeptide. Exemplary self-cleaving polypeptides include 2A peptide from porcine teschovirus-1 (P2A), *Thosea asigna* virus (T2A), equine rhinitis A virus (E2A), foot-and-mouth disease virus (F2A), or variants thereof. Further exemplary nucleic acid and amino acid sequences of 2A peptides are set forth in, for example, Kim *et al.* (PLOS One 6:e18556 (2011)). In particular embodiments, cells are genetically modified to include a self-cleaving polypeptide. In particular embodiments, the sequence encoding the self-cleaving polypeptide is between the sequence encoding the extracellular component of a recombinant receptor and the sequence encoding the intracellular component of a recombinant receptor. In particular embodiments, the sequence encoding the self-cleaving polypeptide is between the sequence encoding the mutated IL-15 and the sequence encoding the extracellular component of a recombinant receptor. In particular embodiments, the sequence encoding the self-cleaving polypeptide is between the sequence encoding the mutated IL-15 and the sequence encoding the intracellular component of a recombinant receptor.

**[0218]** “Kozak sequence” refers to a short nucleotide sequence that greatly facilitates the initial binding of mRNA to the small subunit of the ribosome and increases translation. The consensus Kozak sequence is set forth in SEQ ID NO: 106, where R is a purine (A or G) (Kozak, 1986. Cell. 44(2):283-92, and Kozak, 1987. Nucleic Acids Res. 15(20):8125-48). In particular embodiments, the vectors include polynucleotides that have a consensus Kozak sequence and that encode a desired polypeptide.

**[0219]** Elements directing the efficient termination and polyadenylation of the exogenous nucleic acid transcripts increases exogenous gene expression. Transcription termination signals are generally found downstream of the polyadenylation signal. In particular embodiments, vectors include a polyadenylation sequence 3' of a polynucleotide encoding a polypeptide to be expressed. "PolyA site" or "polyA sequence" denotes a DNA sequence which directs both the termination and polyadenylation of the nascent RNA transcript by RNA polymerase II. Polyadenylation sequences can promote mRNA stability by addition of a polyA tail to the 3' end of the coding sequence and thus, contribute to increased translational efficiency. Cleavage and polyadenylation is directed by a poly(A) sequence in the RNA. The core poly(A) sequence for mammalian pre-mRNAs has two recognition elements flanking a cleavage-polyadenylation site. Typically, an almost invariant AAUAAA hexamer lies 20-50 nucleotides upstream of a more variable element rich in U or GU residues. Cleavage of the nascent transcript occurs between these two elements and is coupled to the addition of up to 250 adenosines to the 5' cleavage product. In particular embodiments, the core poly(A) sequence is an ideal polyA sequence (e.g., AATAAA, ATTAAA, AGTAAA). In particular embodiments, the poly(A) sequence is an SV40 polyA sequence, a bovine growth hormone polyA sequence (BGHpA), a rabbit  $\beta$ -globin polyA sequence (r $\beta$ gpA), variants thereof, or another suitable exogenous or endogenous polyA sequence known in the art.

**[0220]** In some embodiments, a polynucleotide or cell harboring the polynucleotide utilizes a suicide gene, including an inducible suicide gene to reduce the risk of direct toxicity and/or uncontrolled proliferation. In specific aspects, the suicide gene is not immunogenic to the host harboring the polynucleotide or cell. A certain example of a suicide gene that may be used is caspase-9 or caspase-8 or cytosine deaminase. Caspase-9 can be activated using a specific chemical inducer of dimerization (CID).

**[0221]** Other control features can include tag cassettes, transduction markers, or selection cassettes.

**[0222]** Tag cassettes and transduction markers can be used to activate, promote proliferation of, detect, enrich for, isolate, track, deplete and/or eliminate genetically modified cells *in vitro*, *in vivo* and/or *ex vivo*. "Tag cassette" refers to a unique synthetic peptide sequence affixed to, fused to, or that is part of an expressed molecule (e.g., recombinant receptor or chemokine receptor), to which a cognate binding molecule (e.g., ligand, antibody, or other binding partner) is capable of binding where the binding property can be used to activate, promote proliferation of, detect, enrich for, isolate, track, deplete and/or eliminate the tagged protein and/or cells expressing the tagged protein. Transduction markers can serve the same purposes but are derived from naturally

occurring molecules and are often expressed using a skipping element (or self-cleaving polypeptide) that separates the transduction marker from the rest of the expressed molecule.

**[0223]** Exemplary tags include His tag, Flag tags, Xpress tag, Avi tag, Calmodulin binding peptide (CBP) tag, Polyglutamate tag, HA tags, Myc tag, Strep tag (which refers to the original STREP® tag, STREP® tag II (IBA Institut für Bioanalytik, Germany); see, e.g., US 7,981,632), Softag 1, Softag 3, and V5. See FIG. 6 for exemplary sequences.

**[0224]** Conjugate binding molecules that specifically bind tag sequences disclosed herein are commercially available. For example, His tag antibodies are commercially available from suppliers including Life Technologies, Pierce Antibodies, and GenScript. Flag tag antibodies are commercially available from suppliers including Pierce Antibodies, GenScript, and Sigma-Aldrich. Xpress tag antibodies are commercially available from suppliers including Pierce Antibodies, Life Technologies, and GenScript. Avi tag antibodies are commercially available from suppliers including Pierce Antibodies, IsBio, and Genecopoeia. Calmodulin tag antibodies are commercially available from suppliers including Santa Cruz Biotechnology, Abcam, and Pierce Antibodies. HA tag antibodies are commercially available from suppliers including Pierce Antibodies, Cell Signal, and Abcam. Myc tag antibodies are commercially available from suppliers including Santa Cruz Biotechnology, Abcam, and Cell Signal. Strep tag antibodies are commercially available from suppliers including Abcam, Iba, and Qiagen.

**[0225]** In particular embodiments, the transduction marker can include any cell surface displayed marker that can be detected with an antibody that binds to that marker and allows sorting of cells that have the marker. In particular embodiments, the transduction marker can include the magnetic sortable marker streptavidin binding peptide (SBP) displayed at the cell surface by a truncated Low Affinity Nerve Growth Receptor (LNGFRF) and one-step selection with streptavidin-conjugated magnetic beads (Matheson et al. (2014) PloS one 9(10): e111437) or a truncated human epidermal growth factor receptor (EGFR) (tEGFR or EGFRt; see Wang et al., Blood 118: 1255, 2011), a truncated CD19 (tCD19 or CD19t; see Budde et al., Blood 122: 1660, 2013); a truncated HER2 protein (Her2tG); an ECD of human CD34; and/or RQR8 which combines target epitopes from CD34 (see Fehse et al, Mol. Therapy 1( 5 Pt 1); 448–456, 2000) and CD20 antigens (see Philip et al, Blood 124: 1277–1278). In particular embodiments, cells are genetically modified to express EGFRt.

**[0226]** Transduction markers can include any suitable fluorescent protein including: blue fluorescent proteins (e.g., BFP, eBFP, eBFP2); cyan fluorescent proteins (e.g., eCFP, Cerulean, CyPet); green fluorescent proteins (e.g., GFP-2, tagGFP, turboGFP, eGFP,); orange fluorescent proteins (e.g., mOrange, mKO, Kusabira-Orange); red fluorescent proteins (e.g., mKate, mPlum,

DsRed monomer, mCherry, mRFP1, DsRed-Express); yellow fluorescent proteins (e.g., YFP, eYFP, Citrine, Venus); and any other suitable fluorescent proteins, including, for example, firefly luciferase.

**[0227]** In particular embodiments, a selection cassette provides for positive selection or negative selection of a desired cell population. Negative selection is when several cell types are removed, leaving the cell type of interest. Positive selection involves targeting the desired cell population to only retain desired cells.

**[0228]** A selection cassette can encode proteins that (a) confer resistance to antibiotics or other toxins, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*. Any number of selection systems may be used to recover transformed cells. In particular embodiments, a positive selection cassette includes resistance genes to neomycin, hygromycin, ampicillin, puromycin, phleomycin, zeomycin, blasticidin, or viomycin. In particular embodiments, a selection cassette includes the DHFR (dihydrofolate reductase) gene or DHFR double mutant (DHFRdm) gene providing resistance to methotrexate (MTX), the MGMT P140K gene responsible for the resistance to O<sup>6</sup>BG/BCNU, the HPRT (Hypoxanthine phosphoribosyl transferase) gene responsible for the transformation of specific bases present in the HAT selection medium (aminopterin, hypoxanthine, thymidine) or other genes for detoxification with respect to some drugs. In particular embodiments, the selection agent includes neomycin, hygromycin, puromycin, phleomycin, zeomycin, blasticidin, viomycin, ampicillin, O<sup>6</sup>BG/BCNU, MTX, tetracycline, aminopterin, hypoxanthine, thymidine kinase, DHFR, Gln synthetase, or ADA.

**[0229]** In particular embodiments, the selection cassette includes DHFRdm. In particular embodiments, the method does not require a selection cassette to acquire highly purified cell populations.

**[0230]** In particular embodiments, negative selection cassettes include a gene for transformation of a substrate present in the culture medium into a toxic substance for the cell that expresses the gene. These molecules include detoxification genes of diphtheria toxin (DTA) (Yagi *et al.*, *Anal Biochem.* 214(1):77-86, 1993; Yanagawa *et al.*, *Transgenic Res.* 8(3):215-221, 1999), the kinase thymidine gene of the Herpes virus (HSV TK) sensitive to the presence of ganciclovir or FIAU. The HPRT gene may also be used as a negative selection by addition of 6-thioguanine (6TG) into the medium. and for all positive and negative selections, a poly A transcription termination sequence from different origins, the most classical being derived from SV40 poly A, or a eukaryotic gene poly A (bovine growth hormone, rabbit  $\beta$ -globin, etc.).

**[0231]** Viral vectors including polynucleotides contemplated in particular embodiments can be delivered in vivo by administration to an individual patient, typically by systemic administration (e.g., intravenous, intraperitoneal, intramuscular, subdermal, or intracranial infusion) or topical application, as described below. Alternatively, vectors can be delivered to cells ex vivo, such as cells explanted from an individual patient (e.g., mobilized peripheral blood, lymphocytes, bone marrow aspirates, tissue biopsy, etc.) or universal donor hematopoietic stem cells, followed by reimplantation of the cells into a patient.

G. Compositions and Formulations.

**[0232]** In particular embodiments, genetically modified cells can be harvested from a culture medium and washed and concentrated into a carrier in a therapeutically-effective amount to prepare a formulation. In particular embodiments, pharmaceutically-acceptable carrier solutions are well-known to those of skill in the art, as is the development of suitable dosing and treatment regimens for using the particular compositions and formulations described herein in a variety of treatment regimens, including e.g., enteral and parenteral, e.g., intravascular, intravenous, intrarterial, intraosseously, intraventricular, intracerebral, intracranial, intraspinal, intrathecal, and intramedullary administration and formulation. It would be understood by the skilled artisan that particular embodiments contemplated herein may include other formulations, such as those that are well known in the pharmaceutical art, and are described, for example, in Remington: The Science and Practice of Pharmacy, volume I and volume II. 22nd Edition. Edited by Loyd V. Allen Jr. Philadelphia, PA: Pharmaceutical Press; 2012, which is incorporated by reference herein, in its entirety.

**[0233]** Exemplary carriers include saline, buffered saline, physiological saline, water, Hanks' solution, Ringer's solution, Normosol-R (Abbott Labs), PLASMA-LYTE A® (Baxter Laboratories, Inc., Morton Grove, IL), and combinations thereof.

**[0234]** In particular embodiments, carriers can be supplemented with human serum albumin (HSA) or other human serum components or fetal bovine serum. In particular embodiments, a carrier for infusion includes buffered saline with 5% HSA or dextrose. Additional isotonic agents include polyhydric sugar alcohols including trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol, or mannitol.

**[0235]** Carriers can include buffering agents, such as citrate buffers, succinate buffers, tartrate buffers, fumarate buffers, gluconate buffers, oxalate buffers, lactate buffers, acetate buffers, phosphate buffers, histidine buffers, and/or trimethylamine salts.

**[0236]** Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which helps to prevent cell adherence to container walls. Typical

stabilizers can include polyhydric sugar alcohols; amino acids, such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, and threonine; organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinositol, galactitol, glycerol, and cyclitols, such as inositol; PEG; amino acid polymers; sulfur-containing reducing agents, such as urea, glutathione, thiocetic acid, sodium thioglycolate, thioglycerol, alpha-monothioglycerol, and sodium thiosulfate; low molecular weight polypeptides (i.e., <10 residues); proteins such as HSA, bovine serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides such as xylose, mannose, fructose and glucose; disaccharides such as lactose, maltose and sucrose; trisaccharides such as raffinose, and polysaccharides such as dextran.

**[0237]** Where necessary or beneficial, formulations can include a local anesthetic such as lidocaine to ease pain at a site of injection.

**[0238]** Exemplary preservatives include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalkonium halides, hexamethonium chloride, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol.

**[0239]** Therapeutically effective amounts of cells within formulations can be greater than  $10^2$  cells, greater than  $10^3$  cells, greater than  $10^4$  cells, greater than  $10^5$  cells, greater than  $10^6$  cells, greater than  $10^7$  cells, greater than  $10^8$  cells, greater than  $10^9$  cells, greater than  $10^{10}$  cells, or greater than  $10^{11}$ .

**[0240]** In formulations disclosed herein, cells are generally in a volume of a liter or less, 500 ml or less, 250 ml or less or 100 ml or less. Hence the density of administered cells is typically greater than  $10^4$  cells/ml,  $10^7$  cells/ml or  $10^8$  cells/ml.

**[0241]** In particular embodiments, formulations can include at least one genetically modified cell type (e.g., modified T cells, NK cells, or stem cells). Formulations can include different types of genetically-modified cells (e.g., T cells, NK cells, and/or stem cells in combination).

**[0242]** Different types of genetically-modified cells or cell subsets (e.g., modified T cells, NK cells, and/or stem cells) can be provided in different ratios e.g., a 1:1:1 ratio, 2:1:1 ratio, 1:2:1 ratio, 1:1:2 ratio, 5:1:1 ratio, 1:5:1 ratio, 1:1:5 ratio, 10:1:1 ratio, 1:10:1 ratio, 1:1:10 ratio, 2:2:1 ratio, 1:2:2 ratio, 2:1:2 ratio, 5:5:1 ratio, 1:5:5 ratio, 5:1:5 ratio, 10:10:1 ratio, 1:10:10 ratio, 10:1:10 ratio, etc. These ratios can also apply to numbers of cells expressing the same or different expressed molecule (e.g., mutated IL-15 and/or recombinant receptor) components. If only two of the cell types are combined or only 2 combinations of expressed molecule components are included within a formulation, the ratio can include any 2-number combination that can be created from the



3 number combinations provided above. In embodiments, the combined cell populations are tested for efficacy and/or cell proliferation *in vitro*, *in vivo* and/or *ex vivo*, and the ratio of cells that provides for efficacy and/or proliferation of cells is selected. Particular embodiments include genetically-modified cells expressing mutated IL-15.

**[0243]** The cell-based formulations disclosed herein can be prepared for administration by, *e.g.*, injection, infusion, perfusion, or lavage. The formulations can further be formulated for intravenous, intradermal, intraarterial, intranodal, intralymphatic, intraperitoneal, intralesional, intraprostatic, intravaginal, intrarectal, intrathecal, intratumoral, intramuscular, intravesicular, bone marrow, and/or subcutaneous injection.

**[0244]** Targeted vectors and/or nanoparticles can also be used to genetically-modify immune cells *in vivo* or *ex vivo*. Vectors that can be used to deliver artificial expression constructs (encoding mutated IL-15 and/or a recombinant receptor) to cells are described elsewhere herein, and numerous vectors are known in the art.

**[0245]** Exemplary cell-targeted nanoparticles include a cell targeting ligand (*e.g.*, CD3, CD4, CD8, CD34) on the surface of the nanoparticle wherein the cell targeting ligand results in selective uptake of the nanoparticle by a selected cell type. The nanoparticle then delivers gene modifying components that result in expression of the mutated IL-15 and/or the recombinant receptor.

**[0246]** Exemplary nanoparticles include liposomes (microscopic vesicles including at least one concentric lipid bilayer surrounding an aqueous core), liposomal nanoparticles (a liposome structure used to encapsulate another smaller nanoparticle within its core); and lipid nanoparticles (liposome-like structures that lack the continuous lipid bilayer characteristic of liposomes). Other polymer-based nanoparticles can also be used as well as porous nanoparticles constructed from any material capable of forming a porous network. Exemplary materials include metals, transition metals and metalloids (*e.g.*, lithium, magnesium, zinc, aluminum and silica).

**[0247]** For *in vivo* delivery and cellular uptake, nanoparticles can have a neutral or negatively-charged coating and a size of 130 nm or less. Dimensions of the nanoparticles can be determined using, *e.g.*, conventional techniques, such as dynamic light scattering and/or electron microscopy. In particular embodiments, the nanoparticles can be those described in WO2014153114, WO2017181110, and WO201822672.

**[0248]** Therapeutically effective amounts of vectors and/or nanoparticles within formulations can range from 0.1 to 5 µg/kg or from 0.5 to 1 µg /kg. In other examples, a dose can include 1 µg /kg, 30 µg /kg, 90 µg/kg, 150 µg/kg, 500 µg/kg, 750 µg/kg, 0.1 to 5 mg/kg or from 0.5 to 1 mg/kg. In other examples, a dose can include 1 mg/kg, 10 mg/kg, 30 mg/kg, 50 mg/kg, 70 mg/kg, 100 mg/kg, 300 mg/kg, 500 mg/kg, 700 mg/kg, 1000 mg/kg or more.

**[0249]** The methods for administering the vector and/or nanoparticle compositions contemplated in particular embodiments include any method which is effective to result in modified immune effector cells.

H. Methods of Use.

**[0250]** Methods disclosed herein include treating subjects (humans, non-human primates, veterinary animals (dogs, cats, reptiles, birds, etc.) livestock (horses, cattle, goats, pigs, chickens, etc.) and research animals (monkeys, rats, mice, fish, etc.)) with formulations disclosed herein. Treating subjects includes delivering therapeutically effective amounts. Therapeutically effective amounts include those that provide effective amounts, prophylactic treatments and/or therapeutic treatments.

**[0251]** An "effective amount" is the amount of a formulation necessary to result in a desired physiological change in the subject. For example, an effective amount can provide an immunogenic anti-cancer or anti-infection effect. Effective amounts are often administered for research purposes. Effective amounts disclosed herein can cause a statistically significant effect in an animal model or in vitro assay relevant to the assessment of a cancer or infection's development or progression. An immunogenic formulation can be provided in an effective amount, wherein the effective amount stimulates an immune response.

**[0252]** A "prophylactic treatment" includes a treatment administered to a subject who does not display signs or symptoms of a cancer or infection or displays only early signs or symptoms of a cancer or infection such that treatment is administered for the purpose of diminishing or decreasing the risk of developing the cancer or infection further. Thus, a prophylactic treatment functions as a preventative treatment against a target antigen-expressing cancer or infection. In particular embodiments, prophylactic treatments reduce, delay, or prevent metastasis from a primary a cancer tumor site from occurring. In particular embodiments, prophylactic treatments reduce, delay, or prevent infection from a bacteria, virus, fungi, parasite, or arthropod.

**[0253]** A "therapeutic treatment" includes a treatment administered to a subject who displays symptoms or signs of a cancer or infection and is administered to the subject for the purpose of diminishing or eliminating those signs or symptoms of the cancer or infection. The therapeutic treatment can reduce, control, or eliminate the presence or activity of the cancer or infection and/or reduce control or eliminate side effects of the cancer or infection.

**[0254]** Function as an effective amount, prophylactic treatment or therapeutic treatment are not mutually exclusive, and in particular embodiments, administered dosages may accomplish more than one treatment type.

**[0255]** In particular embodiments, therapeutically effective amounts provide anti-cancer effects.

Anti-cancer effects include a decrease in the number of cancer cells, decrease in the number of metastases, a decrease in tumor volume, an increase in life expectancy, induced chemo- or radiosensitivity in cancer cells, inhibited angiogenesis near cancer cells, inhibited cancer cell proliferation, inhibited tumor growth, prevented or reduced metastases, prolonged subject life, reduced cancer-associated pain, and/or reduced relapse or re-occurrence of cancer following treatment. In particular embodiments, the cancer is a hematological malignancy. In particular embodiments, the cancer is a solid cancer or tumor.

**[0256]** A "tumor" is a swelling or lesion formed by an abnormal growth of cells (called neoplastic cells or tumor cells). A "tumor cell" is an abnormal cell that grows by a rapid, uncontrolled cellular proliferation and continues to grow after the stimuli that initiated the new growth cease. Tumors show partial or complete lack of structural organization and functional coordination with the normal tissue, and usually form a distinct mass of tissue, which may be benign, pre-malignant or malignant.

**[0257]** Examples of hematological malignancies that can be treated with the methods and artificial expression constructs disclosed herein include leukemia, lymphoma, or multiple myeloma. In particular embodiments, the leukemia includes acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), chronic myelomonocytic leukemia (CMML), or myeloproliferative neoplasms (MPNs). In particular embodiments, the leukemia includes AML. In particular embodiments, lymphoma includes non-Hodgkin lymphoma and Hodgkin lymphoma. In particular embodiments, multiple myeloma includes light chain myeloma, non-secretory myeloma, solitary plasmacytoma, extramedullary plasmacytoma, monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma (SMM), immunoglobulin D (IgD) myeloma, or immunoglobulin E (IgE) myeloma.

**[0258]** Examples of solid cancers that can be treated with the methods and artificial expression constructs disclosed herein include lung cancer, squamous cell carcinoma, colorectal cancer, pancreatic cancer, breast cancer, thyroid cancer, bladder cancer, cervical cancer, esophageal cancer, ovarian cancer, gastric cancer, endometrial cancer, or brain cancer. In particular embodiments, the lung cancer is non-small cell lung carcinoma. In particular embodiments, the brain cancer includes gliomas, glioblastomas, or oligodendrogliomas.

**[0259]** For administration, therapeutically effective amounts (also referred to herein as doses) can be initially estimated based on results from in vitro assays and/or animal model studies. Such information can be used to more accurately determine useful doses in subjects of interest. The actual dose amount administered to a particular subject can be determined by a physician, veterinarian or researcher taking into account parameters such as physical and physiological

factors including target, body weight, severity of condition, type of cancer or infection, stage of cancer or infection, previous or concurrent therapeutic interventions, idiopathy of the subject and route of administration.

**[0260]** Therapeutically effective amounts of cell-based formulations can include  $10^4$  to  $10^9$  cells/kg body weight, or  $10^3$  to  $10^{11}$  cells/kg body weight. Therapeutically effective amounts to administer can include greater than  $10^2$  cells, greater than  $10^3$  cells, greater than  $10^4$  cells, greater than  $10^5$  cells, greater than  $10^6$  cells, greater than  $10^7$  cells, greater than  $10^8$  cells, greater than  $10^9$  cells, greater than  $10^{10}$  cells, or greater than  $10^{11}$ .

**[0261]** Therapeutically effective amounts of vectors and/or nanoparticles within formulations can range from 0.1 to 5  $\mu\text{g/kg}$  or from 0.5 to 1  $\mu\text{g/kg}$ . In other examples, a dose can include 1  $\mu\text{g/kg}$ , 30  $\mu\text{g/kg}$ , 90  $\mu\text{g/kg}$ , 150  $\mu\text{g/kg}$ , 500  $\mu\text{g/kg}$ , 750  $\mu\text{g/kg}$ , 0.1 to 5  $\text{mg/kg}$  or from 0.5 to 1  $\text{mg/kg}$ . In other examples, a dose can include 1  $\text{mg/kg}$ , 10  $\text{mg/kg}$ , 30  $\text{mg/kg}$ , 50  $\text{mg/kg}$ , 70  $\text{mg/kg}$ , 100  $\text{mg/kg}$ , 300  $\text{mg/kg}$ , 500  $\text{mg/kg}$ , 700  $\text{mg/kg}$ , 1000  $\text{mg/kg}$  or more.

**[0262]** Therapeutically effective amounts can be achieved by administering single or multiple doses during the course of a treatment regimen (e.g., daily, every other day, every 3 days, every 4 days, every 5 days, every 6 days, weekly, every 2 weeks, every 3 weeks, monthly, every 2 months, every 3 months, every 4 months, every 5 months, every 6 months, every 7 months, every 8 months, every 9 months, every 10 months, every 11 months or yearly). In particular embodiments, the treatment protocol may be dictated by a clinical trial protocol or an FDA-approved treatment protocol.

**[0263]** Therapeutically effective amounts can be administered by, e.g., injection, infusion, perfusion, or lavage. Routes of administration can include intravesical, intravenous, intradermal, intraarterial, intraparenteral, intranodal, intralymphatic, intraperitoneal, intralesional, intraprostatic, intravaginal, intrarectal, topical, intrathecal, intratumoral, intramuscular, or subcutaneous administration.

**[0264]** In certain embodiments, formulations and/or compositions are administered to a patient in conjunction with (e.g., before, simultaneously or following) any number of relevant treatment modalities. In particular embodiments, cells may be used in combination with chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAM PATH, anti-CD3 antibodies or other antibody therapies, cytoxin, fludarabine, cyclosporin, FK506, rapamycin, mycoplienolic acid, steroids, FR901228, cytokines, and irradiation.

**[0265]** In some embodiments, the chemotherapeutic agent is administered at the same time or within one week after the administration of the engineered cell or artificial expression construct.

In other embodiments, the chemotherapeutic agent is administered from 1 to 4 weeks or from 1 week to 1 month, 1 week to 2 months, 1 week to 3 months, 1 week to 6 months, 1 week to 9 months, or 1 week to 12 months after the administration of the engineered cell or nucleic acid. In other embodiments, the chemotherapeutic agent is administered at least 1 month before administering the cell or nucleic acid. In some embodiments, the methods further include administering two or more chemotherapeutic agents.

[0266] In additional embodiments, the formulations and/or compositions disclosed herein can be administered with an anti-inflammatory agent. Anti-inflammatory agents or drugs include steroids, glucocorticoids, nonsteroidal anti-inflammatory drugs (NSAIDS).

[0267] In certain embodiments, the formulations and/or compositions described herein are administered in conjunction with a cytokine. “Cytokine” refers to proteins released by one cell population that act on another cell as intercellular mediators. Examples of cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormones such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor (HGF); fibroblast growth factor (FGF); prolactin; placental lactogen; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors (NGFs) such as NGF-beta; platelet-growth factor; transforming growth factors (TGFs) such as TGF-alpha and TGF-beta; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-alpha, beta, and - gamma; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte- macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-I, IL- 1 alpha, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-I I, IL-12; IL-15, a tumor necrosis factor such as TNF-alpha or TNF-beta; and other polypeptide factors including LIF and kit ligand (KL). Cytokines include proteins from natural sources or from recombinant cell culture, and biologically active equivalents of the native sequence cytokines.

I. Sequence Listing.

| SEQ ID NO: | Description                           | Sequence                                                                                                                                                                                                                                  |
|------------|---------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1          | Daric33 signaling polypeptide without | ILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQ<br>TLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYH<br>VFRISKASAGTGSDIYWAPLAGTCGVLLLSLVITMHKRGRKKLL<br>YIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADA<br>PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK |

|   |                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|---|------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|   | signal peptide                                       | NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST<br>ATKDTYDALHMQALPPR                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| 2 | Daric33 signaling polypeptide with signal peptide    | MALPVTALLPLALLLHAARPGSILWHEMWHEGLEEASRLYFGER<br>NVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWC<br>RKYMKSGNVKDLLQAWDLYYHVFRISKASAGTGSDIYIWAPLAG<br>TCGVLLLSLVITMHKRGGRKKLLYIFKQPFMRPVQTTQEEDGCSCR<br>FPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEY<br>DVLDKRRGRDPENGGKPRRKNPQEGLYNELQKDKMAEAYSEIG<br>MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR                                                                                                                                                                                                                                                                                                                                                |
| 3 | Daric33 targeting polypeptide without signal peptide | EVQLVESGGGEVQPGGSLRLSCAASRSSGIDVMGWYRQAPGKE<br>RELVAEISGVGDTNYAASLADRFTVSRDNAKNTVYLQMSSLRAED<br>TAVYYCNAHSFLDLVGAWGQGTLVTVKPGGGGSGVQVETISPG<br>DGRTFPKRGQTCVVHYTGMELEDGKKFDSSRDNRNPKFKMLGKQ<br>EVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLV<br>FDVELLKLEGGRMALIVLGGVAGLLFIGLGIFFCVRCRHRRRQ                                                                                                                                                                                                                                                                                                                                                                                        |
| 4 | Daric33 targeting polypeptide with signal peptide    | METDTLLLWVLLLWVPGSTGEVQLVESGGGEVQPGGSLRLSCAA<br>SRSSGIDVMGWYRQAPGKERELVAEISGVGDTNYAASLADRFTV<br>SRDNAKNTVYLQMSSLRAEDTAVYYCNAHSFLDLVGAWGQGT<br>LVTVKPGGGGSGVQVETISPGDGRTFPKRGQTCVVHYTGMELEDG<br>KKFDSSRDNRNPKFKMLGKQEVIRGWEEGVAQMSVGQRAKLTIS<br>PDYAYGATGHPGIIPPHATLVFDVELLKLEGGRMALIVLGGVAGLL<br>LFIGLGIFFCVRCRHRRRQ                                                                                                                                                                                                                                                                                                                                                               |
| 5 | Daric33 fusion                                       | MALPVTALLPLALLLHAARPGSILWHEMWHEGLEEASRLYFGER<br>NVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWC<br>RKYMKSGNVKDLLQAWDLYYHVFRISKASAGTGSDIYIWAPLAG<br>TCGVLLLSLVITMHKRGGRKKLLYIFKQPFMRPVQTTQEEDGCSCR<br>FPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEY<br>DVLDKRRGRDPENGGKPRRKNPQEGLYNELQKDKMAEAYSEIG<br>MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRSGSGAT<br>NFSLLKQAGDVEENPGPSMETDTLLLWVLLLWVPGSTGEVQLVE<br>SGGGEVQPGGSLRLSCAASRSSGIDVMGWYRQAPGKERELVAE<br>ISGVGDTNYAASLADRFTVSRDNAKNTVYLQMSSLRAEDTAVYY<br>CNAHSFLDLVGAWGQGTLVTVKPGGGGSGVQVETISPGDGRTF<br>PKRGQTCVVHYTGMELEDGKKFDSSRDNRNPKFKMLGKQEVIRG<br>WEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVEL<br>LKLEGGRMALIVLGGVAGLLFIGLGIFFCVRCRHRRRQ |
| 6 | mIL15.CD8t m                                         | MALPVTALLPLALLLHAARPNWVNISDLKKIEDLIQSMHIDATLY<br>TESDVHPSCKVTAMKCFLELQVISLESGDASIHDTVENLIILANNS<br>LSSNGNVTESGCKECEEELEEKNIFEQLQSFVHIVQMFINTSTTPA<br>PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 7 | mIL15.AMNt m                                         | MALPVTALLPLALLLHAARPNWVNISDLKKIEDLIQSMHIDATLY<br>TESDVHPSCKVTAMKCFLELQVISLESGDASIHDTVENLIILANNS<br>LSSNGNVTESGCKECEEELEEKNIFEQLQSFVHIVQMFINTSGGGG<br>SVWGSSAAGLAGGVAALVLLALLVLLVAPPLL                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| 8 | sIL15.WT a.a. seq                                    | MRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSAGLPKTEA<br>NWWNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLE<br>LQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEEELEE<br>KNIFEQLQSFVHIVQMFINTS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |

|    |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
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| 9  | sIL15.D8S<br>a.a. seq | MRISKPHLRISISIQCYLCLLLNSHFLTEAGIHVFILGCFSA GLPKTEA<br>NWWNVISLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLE<br>LQVISLESGDASIHDTVENLILANNSLSSNGNVTESGCKECEEELE<br>KNIKEFLQSFVHIVQMFINTS                                                                                                                                                                                                                                                                                                                                                                       |
| 10 | sIL15.WT<br>n.a. seq  | ATGCGGATCAGCAAGCCCCACCTGCGGAGCATCAGCATCCAG<br>TGCTACCTGTGCCTGCTGCTGAACAGCCACTTCCTGACCGAG<br>GCCGGCATCCACGTGTTTCATCCTGGGCTGCTTCAGCGCCGGC<br>CTGCCCCAAGACCGAGGCCAACTGGGTGAACGTGATCAGCGAC<br>CTGAAGAAGATCGAGGACCTGATCCAGAGCATGCACATCGAC<br>GCCACCCTGTACACCGAGAGCGACGTGCACCCCAGCTGCAAA<br>GTCACCGCCATGAAGTGCTTCCTGCTGGAGCTGCAGGTGATC<br>AGCCTGGAGAGCGGCGACGCCAGCATCCACGACACCGTGGA<br>GAACCTGATCATCCTGGCCAACAACAGCCTGAGCAGCAACGG<br>CAACGTGACCGAGAGCGGCTGCAAGGAGTGCGAGGAGCTGG<br>AGGAGAAGAATCAAGGAGTTCCTGCAGAGCTTCGTGCACAT<br>CGTGCAGATGTTTCATCAACACCAGC |
| 11 | sIL15.D8S<br>n.a. seq | ATGCGGATCAGCAAGCCCCACCTGCGGAGCATCAGCATCCAG<br>TGCTACCTGTGCCTGCTGCTGAACAGCCACTTCCTGACCGAG<br>GCCGGCATCCACGTGTTTCATCCTGGGCTGCTTCAGCGCCGGC<br>CTGCCCCAAGACCGAGGCCAACTGGGTGAACGTGATCAGCAGC<br>CTGAAGAAGATCGAGGACCTGATCCAGAGCATGCACATCGAC<br>GCCACCCTGTACACCGAGAGCGACGTGCACCCCAGCTGCAAA<br>GTCACCGCCATGAAGTGCTTCCTGCTGGAGCTGCAGGTGATC<br>AGCCTGGAGAGCGGCGACGCCAGCATCCACGACACCGTGGA<br>GAACCTGATCATCCTGGCCAACAACAGCCTGAGCAGCAACGG<br>CAACGTGACCGAGAGCGGCTGCAAGGAGTGCGAGGAGCTGG<br>AGGAGAAGAATCAAGGAGTTCCTGCAGAGCTTCGTGCACAT<br>CGTGCAGATGTTTCATCAACACCAGC |
| 12 | MND<br>promoter       | TTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCACC<br>TG TAGGTTTGGCAAGCTAGGATCAAGGTTAGGAACAGAGAGA<br>CAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTC<br>CTGCCCCGGCTCAGGGCCAAGAACAGTTGGAACAGCAGAATA<br>TGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGG<br>CTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCCGCC<br>TCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCC<br>AAGGACCTGAAATGACCCTGTGCCTTATTTGAACAAACCAATC<br>AGTTCGCTTCTCGCTTCTGTTTCGCGCGCTTCTGCTCCCCGAGC<br>TCAATAAAAGAGCCCA                                                                                                |
| 13 | sEF1a<br>promoter     | AGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCA<br>CAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACC<br>GGTGCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGAT<br>GTCGTGTA CTGGCTCCGCTTTTTCCCGAGGGTGGGGGAGAA<br>CCGTATATAAGTGCAAGTAGTCGCCGTGAACGTTCTTTTCGCA<br>ACGGGTTTGCCGCCAGAACACA                                                                                                                                                                                                                                                                                          |
| 14 | CMV<br>promoter       | TTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCAT<br>ATATGGAGTTCGCGGTTACATAACTTACGGTAAATGGCCCGCC<br>TGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAAT<br>GACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGA<br>CGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAG<br>TACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGT                                                                                                                                                                                                                                                                |

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|    |                          | CAATGACGGTAAATGGCCCCGCTGGCATTATGCCCAGTACATG<br>ACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGT<br>CATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAAT<br>GGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTC<br>CACCCCATTTGACGTCAATGGGAGTTTGTTTTGGCACCAAATC<br>AACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGAC<br>GCAAATGGGCGGTAGGCGTGACGGTGGGAGGTCTATATAAG<br>CAGAGCTCGTTTAGTGAACC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| 15 | iSynPro                  | TGAATGAGTCACATCGATCTCCGCCCCCTCTTCGAGGGGGC<br>GGGGTCGAGGAGGAAAACTCGAATGAGTCACATCGACCCTT<br>TGATCTTCGAGGGGACTTTCCGGGGTGAGCAAGCGTGACAA<br>GTCCACGTATGACCCGACCGACGATATCGAAGCCTACGCGCT<br>GAACGCCAGCCCCGATCGACCCGCCCCCTCGATTTCCAAGA<br>AATCGAATGACATCATCTTTCGAATGACATCATCTTTCGAGGG<br>GACTTTCTCGAACTTCTTCGAGGGGACTTTCTCGAGGGGA<br>CTTTCCTCGAGGAGGAAAACTCGAGTAGAGTCTAGACTCTAC<br>ATTTTGACACCCCCATAATATTTTCCAGAATTAACAGTATAAAT<br>TGCATCTCTTGTTCAAGAGTTCCTATCACTCTCTTTAATCACT<br>ACTCACAGTAACCTCAACTCCTG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 16 | iSynPro.IL15<br>.Rev     | GCTGGTGTTGATGAACATCTGCACGATGTGCACGAAGCTCTGC<br>AGGAACTCCTTGATGTTCTTCTCCTCCAGCTCCTCGCACTCCT<br>TGCAGCCGCTCTCGGTACGTTGCCGTTGCTGCTCAGGCTGT<br>TGTTGGCCAGGATGATCAGGTTCTCCACGGTGCTGCTGATGC<br>TGGCGTCGCCGCTCTCCAGGCTGATCACCTGCAGCTCCAGCA<br>GGAAGCACTTCATGGCGGTACCTTGCAGCTGGGGTGACGT<br>CGCTCTCGGTGTACAGGGTGGCGTCGATGTGCATGCTCTGGA<br>TCAGGTCTCGATCTTCTTCAGGTCGCTGATCACGTTACCCCA<br>GTTGGCCTCGGTCTTGGGCAGGCCGGCGCTGAAGCAGCCCA<br>GGATGAACACGTGGATGCCGGCCTCGGTGAGGAAGTGGCTGT<br>TCAGCAGCAGGCACAGGTAGCACTGGATGCTGATGCTCCGCA<br>GGTGGGGCTTGCTGATCCGCATGGTGGCGTCCGGACAGGAG<br>TTGAGGTTACTGTGAGTAGTGATTAAGAGAGTGATAGGGAAC<br>TCTTGAACAAGAGATGCAATTTATACTGTTAATTCTGGAAAAAT<br>ATTATGGGGGTGTCAAAATGTAGAGTCTAGACTCTACTCGAGT<br>TTTTCTCCTCGAGGAAAGTCCCCTCGAGGAAAGTCCCCTCGA<br>AGGAAGTTTCGAGGAAAGTCCCCTCGAAAGATGATGTCATTGCA<br>AAGATGATGTCAATTCGATTTCTTGAAATCGAGGGGGCGGGGT<br>CGATCGGGGCTGGCGTTCAGCGCGTAGGCTTCGATATCGTCG<br>GTCGGGTCATACGTGGACTTGTACGCTTGCTCCACCCCGGA<br>AAGTCCCCTCGAAGATCAAAGGGTCGATGTGACTCATTGAGT<br>TTTTCTCCTCGACCCCGCCCCCTCGAAGAGGGGGCGGAGAT<br>CGATGTGACTCATTGCA |
| 17 | iSynPro.IL15<br>.D8S.Rev | GCTGGTGTTGATGAACATCTGCACGATGTGCACGAAGCTCTGC<br>AGGAACTCCTTGATGTTCTTCTCCTCCAGCTCCTCGCACTCCT<br>TGCAGCCGCTCTCGGTACGTTGCCGTTGCTGCTCAGGCTGT<br>TGTTGGCCAGGATGATCAGGTTCTCCACGGTGCTGCTGATGC<br>TGCGCTCGCCGCTCTCCAGGCTGATCACCTGCAGCTCCAGCA<br>GGAAGCACTTCATGGCGGTACCTTGCAGCTGGGGTGACGT<br>CGCTCTCGGTGTACAGGGTGGCGTCGATGTGCATGCTCTGGA<br>TCAGGTCTCGATCTTCTTCAGGCTGCTGATCACGTTACCCCA<br>GTTGGCCTCGGTCTTGGGCAGGCCGGCGCTGAAGCAGCCCA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |



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|----|--------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |                          | GGATGAACACGTGGATGCCGGCCTCGGTTCAGGAAGTGGCTGT<br>TCAGCAGCAGGCACAGGTAGCACTGGATGCTGATGCTCCGCA<br>GGTGGGGCTTGCTGATCCGCATGGTGGCGTCCGGACAGGAG<br>TTGAGGTTACTGTGAGTAGTGATTAAGAGAGTAGAGGAAAC<br>TCTTGAACAAGAGATGCAATTTATACTGTTAATTCGGAAAAAT<br>ATTATGGGGGTGTCAAAATGTAGAGTCTAGACTCTACTCGAGT<br>TTTTCTCCTCGAGGAAAGTCCCCTCGAGGAAAGTCCCCTCGA<br>AGGAAGTTCGAGGAAAGTCCCCTCGAAAGATGATGTCATTCTGA<br>AAGATGATGTCATTCTGATTTCTTGAAATCGAGGGGGCGGGGT<br>CGATCGGGGCTGGCGTTCAGCGCGTAGGCTTCGATATCGTCG<br>GTCGGGTCATACGTGGACTTGTACGCTTGCTCCACCCCGGA<br>AAGTCCCCTCGAAGATCAAAGGGTCGATGTGACTCATTCTGAGT<br>TTTTCTCCTCGACCCCGCCCCCTCGAAGAGGGGGCGGAGAT<br>CGATGTGACTCATTCTGA                                                                                                                                                                                                                                                                                                                                                                                                                                |
| 18 | iSynPro.IL15<br>.Fwd     | TCGAATGAGTCACATCGATCTCCGCCCCCTCTTCGAGGGGGC<br>GGGGTCGAGGAGGAAAACTCGAATGAGTCACATCGACCCTT<br>TGATCTTCGAGGGGACTTTCCGGGGTGGAGCAAGCGTGACAA<br>GTCCACGTATGACCCGACCGACGATATCGAAGCCTACGCGCT<br>GAACGCCAGCCCCGATCGACCCCGCCCCCTCGATTTCCAAGA<br>AATCGAATGACATCATCTTTTGAATGACATCATCTTTTCGAGGG<br>GACTTTCTCGAACTTCCTTCGAGGGGACTTTCTTCGAGGGGA<br>CTTTCCTCGAGGAGGAAAACTCGAGTAGAGTCTAGACTCTAC<br>ATTTTGACACCCCATATAATTTTTCCAGAATTAACAGTATAAAT<br>TGCATCTCTTGTTCAAGAGTTCCTATCACTCTCTTTAATCACT<br>ACTCACAGTAACCTCAACTCCTGTCCGGACGCCACCATGCGG<br>ATCAGCAAGCCCCACCTGCGGAGCATCAGCATCCAGTGCTAC<br>CTGTGCCTGCTGCTGAACAGCCACTTCCTGACCGAGGCCGGC<br>ATCCACGTGTTTCATCCTGGGCTGCTTCAGCGCCGGCCTGCCC<br>AAGACCGAGGCCAACTGGGTGAACGTGATCAGCGACCTGAAG<br>AAGATCGAGGACCTGATCCAGAGCATGCACATCGACGCCACC<br>CTGTACACCGAGAGCGACGTGCACCCAGCTGCAAAGTCAAC<br>GCCATGAAGTGCTTCCTGCTGGAGCTGCAGGTGATCAGCCTG<br>GAGAGCGGCGACGCCAGCATCCACGACACCGTGGAGAACCT<br>GATCATCCTGGCCAACAACAGCCTGAGCAGCAACGGCAACGT<br>GACCGAGAGCGGCTGCAAGGAGTGCGAGGAGCTGGAGGAGA<br>AGAACATCAAGGAGTTCCTGCAGAGCTTCGTGCACATCGTGCA<br>GATGTTTCATCAACACCAGC |
| 19 | iSynPro.IL15<br>.D8S.Fwd | TCGAATGAGTCACATCGATCTCCGCCCCCTCTTCGAGGGGGC<br>GGGGTCGAGGAGGAAAACTCGAATGAGTCACATCGACCCTT<br>TGATCTTCGAGGGGACTTTCCGGGGTGGAGCAAGCGTGACAA<br>GTCCACGTATGACCCGACCGACGATATCGAAGCCTACGCGCT<br>GAACGCCAGCCCCGATCGACCCCGCCCCCTCGATTTCCAAGA<br>AATCGAATGACATCATCTTTTGAATGACATCATCTTTTCGAGGG<br>GACTTTCTCGAACTTCCTTCGAGGGGACTTTCTTCGAGGGGA<br>CTTTCCTCGAGGAGGAAAACTCGAGTAGAGTCTAGACTCTAC<br>ATTTTGACACCCCATATAATTTTTCCAGAATTAACAGTATAAAT<br>TGCATCTCTTGTTCAAGAGTTCCTATCACTCTCTTTAATCACT<br>ACTCACAGTAACCTCAACTCCTGTCCGGACGCCACCATGCGG<br>ATCAGCAAGCCCCACCTGCGGAGCATCAGCATCCAGTGCTAC<br>CTGTGCCTGCTGCTGAACAGCCACTTCCTGACCGAGGCCGGC<br>ATCCACGTGTTTCATCCTGGGCTGCTTCAGCGCCGGCCTGCCC                                                                                                                                                                                                                                                                                                                                                                                                       |

|    |                                           |                                                                                                                                                                                                                                                                                                                                                                                                   |
|----|-------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |                                           | AAGACCGAGGCCAACTGGGTGAACGTGATCAGCAGCCTGAAG<br>AAGATCGAGGACCTGATCCAGAGCATGCACATCGACGCCACC<br>CTGTACACCGAGAGCGACGTGCACCCAGCTGCAAAGTCACC<br>GCCATGAAGTGCTTCCTGCTGGAGCTGCAGGTGATCAGCCTG<br>GAGAGCGGCGACGCCAGCATCCACGACACCGTGGAGAACCT<br>GATCATCCTGGCCAACAACAGCCTGAGCAGCAACGGCAACGT<br>GACCGAGAGCGGCTGCAAGGAGTGCGAGGAGCTGGAGGAGA<br>AGAACATCAAGGAGTTCCTGCAGAGCTTCGTGCACATCGTGCA<br>GATGTTCATCAACACCAGC |
| 20 | anti-CD33.1<br>DARIC<br>binding<br>domain | METDTLLLWVLLLWPGSTGQVTLRESGGGLVQAGGSLRLSCKG<br>SGRAINTYAMGWFRQAPGKEREFVAAISWNGGHTRYADSVQGR<br>FAISRDNADNTMYLQMNSLKPEDTAVYHCAAYS DYHRIATMEAD<br>ADSWGQGTQVTVSSGGGGSGVQVETISPGDGRTF PKRGQTCVV<br>HYTGMLLEDGKKFDSSRDNRNPKFKF MLGKQEVIRGWEEGVAQMS<br>VGQRAKLTISPDIAYGATGHPGIIPPHATLVFDVELLKLEGGRMAL<br>IVLGGVAGLLFI GLGIFFCVRCRHRRRQ                                                                  |
| 21 | anti-CD33.2<br>DARIC<br>binding<br>domain | METDTLLLWVLLLWPGSTGEVQLVESGGGEVQPGGSLRLSCAA<br>SGRTFSGYIMGWFRQAPGKERELVARISGNNLSTEYAESVKGRF<br>TISRDNAKNTLYLQMSSLRAEDTAVYYCAA EYDYSSGDFVYWGQ<br>GTLVTVKPGGGGSGVQVETISPGDGRTF PKRGQTCVVHYTGML<br>EDGKKFDSSRDNRNPKFKF MLGKQEVIRGWEEGVAQMSVGQRAK<br>LTISPDIAYGATGHPGIIPPHATLVFDVELLKLEGGRMALIVLGGVA<br>GLLLFI GLGIFFCVRCRHRRRQ                                                                       |
| 22 | anti-CD33.3<br>DARIC<br>binding<br>domain | METDTLLLWVLLLWPGSTGEVQLVESGGGEVQPGGSLRLSCAA<br>SGSGFSASLMSWHRQAPGKQRDLVASITRDGRANYVESVKGRF<br>TISRDNAKNTLYLQMSSLRAEDTAVYYCHAYSFDYPIRSYWGQGT<br>LVTVKPGGGGSGVQVETISPGDGRTF PKRGQTCVVHYTGMLLED<br>GKKFDSSRDNRNPKFKF MLGKQEVIRGWEEGVAQMSVGQRAKLT<br>ISPDIAYGATGHPGIIPPHATLVFDVELLKLEGGRMALIVLGGVAGL<br>LLFI GLGIFFCVRCRHRRRQ                                                                         |
| 23 | anti-CD33.4<br>DARIC<br>binding<br>domain | METDTLLLWVLLLWPGSTGEVQLVESGGGEVQPGGSLRLSCAA<br>SGFTFSSYAMGWFRQAPGKGREWWAAITTS GDTTYAESVKGR<br>FTISRDNAKNTVYLQMSSLRAEDTAVYYCAA HGGGVIDYWGQG<br>TLVTVKPGGGGSGVQVETISPGDGRTF PKRGQTCVVHYTGMLLED<br>GKKFDSSRDNRNPKFKF MLGKQEVIRGWEEGVAQMSVGQRAKLT<br>ISPDIAYGATGHPGIIPPHATLVFDVELLKLEGGRMALIVLGGVAGL<br>LLFI GLGIFFCVRCRHRRRQ                                                                         |
| 24 | anti-CD33.5<br>DARIC<br>binding<br>domain | METDTLLLWVLLLWPGSTGEVQLVESGGGEVQPGGSLRLSCAA<br>SGSIFSI SIMGWYRQAPGKERELVASTTSSGTTNYVESVKGRFTIS<br>RDNAKNTLYLQMSSLRAEDTAVYYCHAYIATTTDRGYRGYWGQG<br>TLVTVKPGGGGSGVQVETISPGDGRTF PKRGQTCVVHYTGMLLED<br>GKKFDSSRDNRNPKFKF MLGKQEVIRGWEEGVAQMSVGQ2RAKL<br>TISPDIAYGATGHPGIIPPHATLVFDVELLKLEGGRMALIVLGGVA<br>GLLLFI GLGIFFCVRCRHRRRQ                                                                    |
| 25 | anti-CD33.6<br>DARIC<br>binding<br>domain | METDTLLLWVLLLWPGSTGQVQLVQSGGGLVQAGGSLRLSCA<br>ASGSISSYNVVGWYRQLSGNERGGRTMVAQINAYGDTNYANAV<br>VGRFTISRDDAKNTVYLHMSNLKPEDTG VYYCNGQRMLENYTYR<br>DQSWGQGTQVTVKPGGGGSGVQVETISPGDGRTF PKRGQTCV<br>VHYTGMLLEDGKKFDSSRDNRNPKFKF MLGKQEVIRGWEEGVAQM                                                                                                                                                       |

|    |                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|----|--------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |                                            | SVGQRAKLISPDYAYGATGHPGIIPPHATLVFDVELLKLEGGRMALIVLGGVAGLLFIGLGIFFCVRCRHRRRQ                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| 26 | anti-CD33.7<br>DARIC<br>binding<br>domain  | METDTLLLWVLLLWPGSTGQVQLVQSGGGLVQAGGSLTSCAA<br>SRSSGIDVMGWYRQAPGKERELVAEISGVGDTNYAASLADRFTV<br>SRDNAKNTVYLLQMKNLKPEDTAVYYCNAHSFLDLVGAWGQGTQ<br>VTVKPGGGGSGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDG<br>KKFDSSRDNRNKPFFKMLGKQEVIRGWEEGVAQMSVGQRAKLIS<br>PDYAYGATGHPGIIPPHATLVFDVELLKLEGGRMALIVLGGVAGLL<br>LFIGLGIFFCVRCRHRRRQ                                                                                                                                                                                                                                                                                                                                                                            |
| 27 | anti-CD33.8<br>DARIC<br>binding<br>domain  | METDTLLLWVLLLWPGSTGEVQLVQSGGGSVQVGGSLRLSCA<br>ASGSTLNIDHIGWYRQAPGKERELVGVISSGAGPNYADSVKGRFT<br>ISRDNAKNTVYLLQMNSLKPEDTAVYNCNAWIDYGSGLPQNYWG<br>QGTQVTVKPGGGGSGVQVETISPGDGRTFPKRGQTCVVHYTGM<br>LEDGKKFDSSRDNRNKPFFKMLGKQEVIRGWEEGVAQMSVGQRA<br>KLISPDYAYGATGHPGIIPPHATLVFDVELLKLEGGRMALIVLGGV<br>AGLLFIGLGIFFCVRCRHRRRQ                                                                                                                                                                                                                                                                                                                                                                        |
| 28 | anti-CD33.9<br>DARIC<br>binding<br>domain  | METDTLLLWVLLLWPGSTGEVQLVESGGGEVQPGGSLRLSCAA<br>SRSSGIDVMGWYRQAPGKERELVAEISGVGDTNYAASLADRFTV<br>SRDNAKNTVYLLQMSSLRAEDTAVYYCNAHSFLDLVGAWGQGT<br>VTVKPGGGGSGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDG<br>KKFDSSRDNRNKPFFKMLGKQEVIRGWEEGVAQMSVGQRAKLIS<br>PDYAYGATGHPGIIPPHATLVFDVELLKLEGGRMALIVLGGVAGLL<br>LFIGLGIFFCVRCRHRRRQ                                                                                                                                                                                                                                                                                                                                                                            |
| 29 | anti-CD33.10<br>DARIC<br>binding<br>domain | METDTLLLWVLLLWPGSTGEVQLVESGGGEVQPGGSLRLSCAA<br>SGSTLNIDHIGWYRQAPGKERELVGVISSGAGPNYAESVKGRFTI<br>SRDNAKNTVYLLQMSSLRAEDTAVYYCNAWIDYGSGLPQNYWGQ<br>GTLVTVKPGGGGSGVQVETISPGDGRTFPKRGQTCVVHYTGML<br>EDGKKFDSSRDNRNKPFFKMLGKQEVIRGWEEGVAQMSVGQRAK<br>LTISPDYAYGATGHPGIIPPHATLVFDVELLKLEGGRMALIVLGGVA<br>GLLLFIGLGIFFCVRCRHRRRQ                                                                                                                                                                                                                                                                                                                                                                      |
| 30 | anti-CD33.1<br>DARIC<br>fusion<br>protein  | MALPVTALLPLALLLHAARPGSILWHEMWHEGLEEASRLYFGER<br>NVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWC<br>RKYMKSGNVKDLLQAWDLYYHVFRISKASAGTGSDIYIWAPLAG<br>TCGVLLLSLVITMHKRGGRKKLLYIFKQPFMRPVQTTQEEDGCSCR<br>FPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEY<br>DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG<br>MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRSMSGAT<br>NFSLLKQAGDVEENPGPSMETDTLLLWVLLLWPGSTGQVTLRE<br>SGGGLVQAGGSLRLSCKGSGRAINTYAMGWFRQAPGKEREFVA<br>AISWNGGHTRYADSVQGRFAISRDNADNTMYLQMNSLKPEDTAV<br>YHCAAYSDYHRIATMEADADSWGQGTQVTVSSGGGSGVQVET<br>ISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDNRNKPFFKML<br>GKQEVIRGWEEGVAQMSVGQRAKLISPDYAYGATGHPGIIPPHA<br>TLVFDVELLKLEGGRMALIVLGGVAGLLFIGLGIFFCVRCRHRRR<br>Q |
| 31 | anti-CD33.2<br>DARIC<br>fusion<br>protein  | MALPVTALLPLALLLHAARPGSILWHEMWHEGLEEASRLYFGER<br>NVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWC<br>RKYMKSGNVKDLLQAWDLYYHVFRISKASAGTGSDIYIWAPLAG<br>TCGVLLLSLVITMHKRGGRKKLLYIFKQPFMRPVQTTQEEDGCSCR<br>FPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEY                                                                                                                                                                                                                                                                                                                                                                                                                                                  |

|    |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
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|    |                                           | DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG<br>MKGERRRGKGHDLGYQGLSTATKDTYDALHMQALPPRSGSGAT<br>NFSLLKQAGDVEENPGPSMETDTLLLWLLLWPGSTGEVQLVE<br>SGGGEVQPGGSLRLSCAASGRFTSGYIMGWFRQAPGKERELVA<br>RISGNNLSTEYAESVKGRFTISRDNANTLYLQMSSLRAEDTAVY<br>YCAAEDYSSGDFVYWGQGTTLVTVKPGGGGSGVQVETISPGDG<br>RTFPKRQTCVVHYTGMLLEDGKKFDSSDRDNKPFKMLGKQEVI<br>RGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDV<br>ELLKLEGGRMALIVLGGVAGLLLFIGLGIFFCVRCRHRRRQ                                                                                                                                                                                                                                                |
| 32 | anti-CD33.3<br>DARIC<br>fusion<br>protein | MALPVTALLPLALLHAARPGSILWHEMWHEGLEEASRLYFGER<br>NVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWC<br>RKYMKSGNVKDLLQAWDLYYHVFRISKASAGTGSDIYWAPLAG<br>TCGVLLLSLVITMHKRGKRKLLYIFKQPFMRPVQTTQEEDGCSCR<br>FPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEY<br>DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG<br>MKGERRRGKGHDLGYQGLSTATKDTYDALHMQALPPRSGSGAT<br>NFSLLKQAGDVEENPGPSMETDTLLLWLLLWPGSTGEVQLVE<br>SGGGEVQPGGSLRLSCAASGSGFSASLMSWHRQAPGKQRDV<br>ASITRDGRANYVESVKGRFTISRDNANTLYLQMSSLRAEDTAVY<br>YCHAYSFDYPIRSYWGQGTTLVTVKPGGGGSGVQVETISPGDGRT<br>FPKRQTCVVHYTGMLLEDGKKFDSSDRDNKPFKMLGKQEVIRG<br>WEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVEL<br>LKLEGGRMALIVLGGVAGLLLFIGLGIFFCVRCRHRRRQ     |
| 33 | anti-CD33.4<br>DARIC<br>fusion<br>protein | MALPVTALLPLALLHAARPGSILWHEMWHEGLEEASRLYFGER<br>NVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWC<br>RKYMKSGNVKDLLQAWDLYYHVFRISKASAGTGSDIYWAPLAG<br>TCGVLLLSLVITMHKRGKRKLLYIFKQPFMRPVQTTQEEDGCSCR<br>FPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEY<br>DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG<br>MKGERRRGKGHDLGYQGLSTATKDTYDALHMQALPPRSGSGAT<br>NFSLLKQAGDVEENPGPSMETDTLLLWLLLWPGSTGEVQLVE<br>SGGGEVQPGGSLRLSCAASGFTFSSYAMGWFRQAPGKGREWW<br>AAITSGDTTYAESVKGRFTISRDNANTVYLQMSSLRAEDTAV<br>YYCAAHRGGGVIDYWGQGTTLVTVKPGGGGSGVQVETISPGDGR<br>TFPKRQTCVVHYTGMLLEDGKKFDSSDRDNKPFKMLGKQEVIR<br>GWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVE<br>LLKLEGGRMALIVLGGVAGLLLFIGLGIFFCVRCRHRRRQ      |
| 34 | anti-CD33.5<br>DARIC<br>fusion<br>protein | MALPVTALLPLALLHAARPGSILWHEMWHEGLEEASRLYFGER<br>NVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWC<br>RKYMKSGNVKDLLQAWDLYYHVFRISKASAGTGSDIYWAPLAG<br>TCGVLLLSLVITMHKRGKRKLLYIFKQPFMRPVQTTQEEDGCSCR<br>FPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEY<br>DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG<br>MKGERRRGKGHDLGYQGLSTATKDTYDALHMQALPPRSGSGAT<br>NFSLLKQAGDVEENPGPSMETDTLLLWLLLWPGSTGEVQLVE<br>SGGGEVQPGGSLRLSCAASGSIFSISIMGWYRQAPGKERELVAS<br>TTSSGTTNYVESVKGRFTISRDNANTLYLQMSSLRAEDTAVYYC<br>HAYIATTTDRGYRGYWGQGTTLVTVKPGGGGSGVQVETISPGDG<br>RTFPKRQTCVVHYTGMLLEDGKKFDSSDRDNKPFKMLGKQEVI<br>RGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDV<br>ELLKLEGGRMALIVLGGVAGLLLFIGLGIFFCVRCRHRRRQ |

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| 35 | anti-CD33.6<br>DARIC<br>fusion<br>protein | MALPVTALLPLALLLHAARPGSILWHEMWHEGLEEASRLYFGER<br>NVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWC<br>RKYMKSGNVKDLLQAWDLYYHVFRISKASAGTGSDIYIWAPLAG<br>TCGVLLLSLVITMHKRGKRKLLYIFKQPFMRPVQTTQEEDGCSCR<br>FPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEY<br>DVLDKRRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG<br>MKGERRRGKGGHDGLYQGLSTATKDTYDALHMQUALPPRSGSGAT<br>NFSLLKQAGDVEENPGPSMETDTLLLWLLLWPGSTGQVQLVQ<br>SGGGLVQAGGSLRLSCAASGSISSYNVVGWYRQLSGNERGGRT<br>MVAQINAYGDTNYANAVVGRFTISRDDAKNTVYLHMSNLKPEDT<br>GVYYCNGQRMLENYTYRDQSWGQGTQVTVKPGGGGSGVQVET<br>ISPGDGRTFPKRQTCVVHYTGMLEDGKKFDSSRDNRNPKFKFML<br>GKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHA<br>TLVFDVELLKLEGGRMALIVLGGVAGLLFIGLGIFFCVRCRHRRR<br>Q |
| 36 | anti-CD33.7<br>DARIC<br>fusion<br>protein | MALPVTALLPLALLLHAARPGSILWHEMWHEGLEEASRLYFGER<br>NVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWC<br>RKYMKSGNVKDLLQAWDLYYHVFRISKASAGTGSDIYIWAPLAG<br>TCGVLLLSLVITMHKRGKRKLLYIFKQPFMRPVQTTQEEDGCSCR<br>FPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEY<br>DVLDKRRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG<br>MKGERRRGKGGHDGLYQGLSTATKDTYDALHMQUALPPRSGSGAT<br>NFSLLKQAGDVEENPGPSMETDTLLLWLLLWPGSTGQVQLVQ<br>SGGGLVQAGGSLTLSCAASRSSGIDVMGWYRQAPGKERELVAEI<br>SGVGDTNYAASLADRFTVSRDNAKNTVYLQMKNLKPEDTAVYYC<br>NAHSFLDLVGAWGQGTQVTVKPGGGGSGVQVETISPGDGRTFP<br>KRQTCVVHYTGMLEDGKKFDSSRDNRNPKFKFMLGKQEVIRGW<br>EEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLK<br>LEGGRMALIVLGGVAGLLFIGLGIFFCVRCRHRRRQ             |
| 37 | anti-CD33.8<br>DARIC<br>fusion<br>protein | MALPVTALLPLALLLHAARPGSILWHEMWHEGLEEASRLYFGER<br>NVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWC<br>RKYMKSGNVKDLLQAWDLYYHVFRISKASAGTGSDIYIWAPLAG<br>TCGVLLLSLVITMHKRGKRKLLYIFKQPFMRPVQTTQEEDGCSCR<br>FPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEY<br>DVLDKRRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG<br>MKGERRRGKGGHDGLYQGLSTATKDTYDALHMQUALPPRSGSGAT<br>NFSLLKQAGDVEENPGPSMETDTLLLWLLLWPGSTGEVQLVQ<br>SGGGSVQVGGSRLRLSCAASGSTLNIDHIGWYRQAPGKERELVGV<br>ISSGAGPNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYNC<br>NAWIDYGSGLPQNYWGQGTQVTVKPGGGGSGVQVETISPGDG<br>RTFPKRQTCVVHYTGMLEDGKKFDSSRDNRNPKFKFMLGKQEVI<br>RGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDV<br>ELLKLEGGRMALIVLGGVAGLLFIGLGIFFCVRCRHRRRQ        |
| 38 | anti-CD33.9<br>DARIC<br>fusion<br>protein | MALPVTALLPLALLLHAARPGSILWHEMWHEGLEEASRLYFGER<br>NVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWC<br>RKYMKSGNVKDLLQAWDLYYHVFRISKASAGTGSDIYIWAPLAG<br>TCGVLLLSLVITMHKRGKRKLLYIFKQPFMRPVQTTQEEDGCSCR<br>FPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEY<br>DVLDKRRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG<br>MKGERRRGKGGHDGLYQGLSTATKDTYDALHMQUALPPRSGSGAT<br>NFSLLKQAGDVEENPGPSMETDTLLLWLLLWPGSTGEVQLVE                                                                                                                                                                                                                                                                                                     |

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|    |                                            | SGGGEVQPGGSLRLSCAASRSSGIDVMGWYRQAPGKERELVAE<br>ISGVGDTNYAASLADRFTVSRDNAKNTVYLQMSSLRAEDTAVYY<br>CNAHSFLDLVGAWGQGT LVT VKPGGGGSGVQVETISPGDGRTF<br>PKRGQTCVVHYTG MLEDGKKFDSSDRNKP FKFMLGKQEVIRG<br>WEEGVAQMSVGQRAKLTISP DYAYGATGHPGIIPPHATLVFDVEL<br>LKLEGGRMALIVLGGVAGLLLFIGLGIFFCVRCRHRRRQ                                                                                                                                                                                                                                                                                                                                                                                                     |
| 39 | anti-CD33.10<br>DARIC<br>fusion<br>protein | MALPVTALLPLALLLHAARPGSILWHEMWHEGLEEASRLYFGER<br>NVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQEWC<br>RKYMKSGNVKDLLQAWDLYYHVFRISKASAGTGSDIYIWAPLAG<br>TCGVLLLSLVITMHKRGGRKKLLYIFKQPFMRPVQTTQEEDGCSCR<br>FPEEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEY<br>DVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG<br>MKGERRRGKGHDGLYQGLSTATKDTYDALHMQUALPPRSGSGAT<br>NFSLLKQAGDVEENPGPSMETDTLLLWLLLWPGSTGEVQLVE<br>SGGGEVQPGGSLRLSCAASGSTLNIDHIGWYRQAPGKERELVGV<br>ISSGAGPNYAESVKGRFTISR DNAKNTVYLQMSSLRAEDTAVYYC<br>NAWDYGSGLPQNYWGQGT LVT VKPGGGGSGVQVETISPGDGR<br>TFPKRGQTCVVHYTG MLEDGKKFDSSDRNKP FKFMLGKQEVIR<br>GWEEGVAQMSVGQRAKLTISP DYAYGATGHPGIIPPHATLVFDVE<br>LLKLEGGRMALIVLGGVAGLLLFIGLGIFFCVRCRHRRRQ |
| 40 | anti-CD33<br>DARIC<br>signaling<br>domain  | MALPVTALLPLALLLHAARPGSILWHEMWHEGLEEASRLYFGER<br>NVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQEWC<br>RKYMKSGNVKDLLQAWDLYYHVFRISKASAGTGSDIYIWAPLAG<br>TCGVLLLSLVITMHKRGGRKKLLYIFKQPFMRPVQTTQEEDGCSCR<br>FPEEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEY<br>DVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG<br>MKGERRRGKGHDGLYQGLSTATKDTYDALHMQUALPPR                                                                                                                                                                                                                                                                                                                                                      |
| 41 | processed<br>sIL15.WT<br>a.a. seq          | NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLE<br>LQVISLES GDASIHTVENLILANNSLSSNGNVTESGCKECEEELE<br>KNIKEFLQSFVHVHVMFINTS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 42 | processed<br>sIL15.D8S<br>a.a. seq         | NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLE<br>LQVISLES GDASIHTVENLILANNSLSSNGNVTESGCKECEEELE<br>KNIKEFLQSFVHVHVMFINTS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 43 | processed<br>sIL15.WT<br>n.a. seq          | AACTGGGTGAACGTGATCAGCGACCTGAAGAAGATCGAGGAC<br>CTGATCCAGAGCATGCACATCGACGCCACCCTGTACACCGAG<br>AGCGACGTGCACCCAGCTGCAAAGTCACCGCCATGAAGTGC<br>TTCCTGCTGGAGCTGCAGGTGATCAGCCTGGAGAGCGGCGAC<br>GCCAGCATCCACGACACCGTGGAGAACCTGATCATCCTGGCC<br>AACAAACAGCCTGAGCAGCAACGGCAACGTGACCGAGAGCGGC<br>TGCAAGGAGTGCGAGGAGCTGGAGGAGAAGAACATCAAGGAG<br>TTCCTGCAGAGCTTCGTGCACATCGTGCAGATGTTTCATCAACA<br>CCAGC                                                                                                                                                                                                                                                                                                       |
| 44 | processed<br>sIL15.D8S<br>n.a. seq         | AACTGGGTGAACGTGATCAGCAGCCTGAAGAAGATCGAGGAC<br>CTGATCCAGAGCATGCACATCGACGCCACCCTGTACACCGAG<br>AGCGACGTGCACCCAGCTGCAAAGTCACCGCCATGAAGTGC<br>TTCCTGCTGGAGCTGCAGGTGATCAGCCTGGAGAGCGGCGAC<br>GCCAGCATCCACGACACCGTGGAGAACCTGATCATCCTGGCC<br>AACAAACAGCCTGAGCAGCAACGGCAACGTGACCGAGAGCGGC<br>TGCAAGGAGTGCGAGGAGCTGGAGGAGAAGAACATCAAGGAG                                                                                                                                                                                                                                                                                                                                                                |

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|    |       | TTCCTGCAGAGCTTCGTGCACATCGTGCAGATGTTTCATCAACA<br>CCAGC                                                                                                                                                                                                                                                                                                                                                                                    |
| 45 | S1-17 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAGGA<br>GGAAAACTCGATGTGACTCATTCTGAAGATCAAAGGGTCGAGG<br>AAAGTCCCCTCGACCCTTTGATCTTGAATGACATCATCTTTCC<br>AGGAAAGTCCCCTCGAGGGGACTTTCTCGAGGGGACTTTCC<br>TCGAATGAGTCACATCGATCTCCGCCCCCTCTTCGAGCGCCAA<br>ATCGAGGACGTGATCGAGTAGAGTCTAGACTCTACATTTTGAC<br>ACCCCAT                                                                                                                                           |
| 46 | S1-37 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAGGG<br>GGCGGGGTCGATCTCCGCCCCCTCTTCGAGTTTTTCCTCCTC<br>GAGGGGACTTTCTCGAATGACATCATCTTTGACCCCTTTGAT<br>CTTCGAAGGAAGTTCGATCACGTCTCGATTTGGCGCTCGAG<br>GGGACTTTCTCGAAGATCAAAGGGTCGAGTAGAGTCTAGACT<br>CTACATTTTGACACCCCAT                                                                                                                                                                                 |
| 47 | S1-4  | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGATTTC<br>CAAGAAATCGAGGAAAGTCCCCTCGACCCTTTGATCTTCGAGG<br>AGGAAAACTCGAATGAGTCACATCGACCCTTTGATCTTCGAA<br>GAGGGGGCGGAGATCGATCTCCGCCCCCTCTTCGAGGAAAGT<br>CCCCTCGACCCTTTGATCTTCGACCCCGCCCCCTCGAGGAAA<br>GTCCCCTCGAGTAGAGTCTAGACTCTACATTTTGACACCCCCA<br>T                                                                                                                                                 |
| 48 | S1-1  | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGATCTC<br>CGCCCCCTCTTCGAAGATCAAAGGGTCGAGGGGACTTTCTC<br>GATTTCTTGAAATCGAATGACATCATCTTTGATTTGGCGCTC<br>GAATGACATCATCTTTGACCCCTTTGATCTTCGATGTGACTCAT<br>TCGAGGGGACTTTCTCGAAGATCAAAGGGTCGAGGGGACTT<br>TCCTCGAGTAGAGTCTAGACTCTACATTTTGACACCCCAT                                                                                                                                                          |
| 49 | S1-3  | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAGGG<br>GACTTTCTCGAGGGGGCGGGGTCGATTTGGCGCTCGATCTC<br>CGCCCCCTCTTCGAATGAGTCACATCGAGGAAAGTCCCCTCG<br>AGGAGGAAAACTCGATGTGACTCATTGATTTCCAAGAAATC<br>GAGTAGAGTCTAGACTCTACATTTTGACACCCCAT                                                                                                                                                                                                                |
| 50 | S1-42 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGATTTC<br>CAAGAAATCGATCTCCGCCCCCTCTTCGAATGACATCATCTTT<br>CGAATGACATCATCTTTGAGGAGGAAAACTCGACCCCGCCC<br>CCTCGATTTCTTGAAATCGAGGAAAGTCCCCTCGAGCGCCAA<br>ATCGAAGGAAGTTTGAATGACATCATCTTTGAATGAGTCACA<br>TCGAGGAAAGTCCCCTCGAGGAGGAAAACTCGAGTAGAGTC<br>TAGACTCTACATTTTGACACCCCAT                                                                                                                            |
| 51 | S1-61 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAATGA<br>GTCACATCGATCTCCGCCCCCTCTTCGAGGGGGCGGGGTCGA<br>GGAGGAAAACTCGAATGAGTCACATCGACCCTTTGATCTTCG<br>AGGGGACTTTCCGGGGTGGAGCAAGCGTGACAAAGTCCACGTA<br>TGACCCGACCGACGATATCGAAGCCTACGCGCTGAACGCCAG<br>CCCCGATCGACCCCGCCCCCTCGATTTCCAAGAAATCGAATGA<br>CATCATCTTTGAATGACATCATCTTTGAGGGGACTTTCTCTCG<br>AACTTCCTTCGAGGGGACTTTCTCGAGGGGACTTTCTCTCGAG<br>GAGGAAAACTCGAGTAGAGTCTAGACTCTACATTTTGACACC<br>CCCAT |

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| 52 | S1-62 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGATTTC<br>CAAGAAATCGAGTTTTTCCTCCTCGAGGGGGCGGGTTCGAGG<br>GGACTTTCCTCGACCCTTTGATCTTCGAGGAAAGTCCCCTCGA<br>GCGCCAAATCGATCTCCGCCCCCTCTTCGAGTAGAGTCTAGAC<br>TCTACATTTTGACACCCCCAT                                                                                                                                                                                                                                  |
| 53 | S1-15 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAAGAT<br>CAAAGGGTCGATTTCTTGAAATCGATGTGACTCATTTCGATCA<br>CGTCCTCGAGGAGGAAAACTCGAGGAAAGTCCCCTCGAACT<br>TCCTTCGAGGGGGCGGGGTGGAATGAGTCACATCGAGGAAAG<br>TCCCCTCGAGGGGACTTTCCTCGATTTCTTGAAATCGAAGAG<br>GGGGCGGAGATCGAGTTTTTCCTCCTCGAGGAAAGTCCCCTC<br>GATCGACTCTACATTTTGACACCCCCAT                                                                                                                                 |
| 54 | S1-2  | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAATGA<br>GTCACATCGAAGATCAAAGGGTCGACCCTTTGATCTTCGATTT<br>GGCGCTCGATGTGACTCATTTCGACCCCGCCCCCTCGAGGAAA<br>GTCCCCTCGAGGAAAGTCCCCTCGAGGAAAGTCCCCTCGAGG<br>AAAGTCCCCTCGAGTTTTTCCTCCTCGAAGAGGGGGCGGAGA<br>TCGATTTGGCGCTCGAGGACGTGATCGAGTAGAGTCTAGACT<br>CTACATTTTGACACCCCCAT                                                                                                                                       |
| 55 | S1-27 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGATTTC<br>CAAGAAATCGAGTTTTTCCTCCTCGATTTCCAAGAAATCGAGG<br>GGACTTTCCTCGAGGGGACTTTCCTCGAATGACATCATCTTTC<br>GATGTGACTCATTTCGAAGATCAAAGGGTCGAAGGAAGTTCGAA<br>TGAGTCACATCGAATGAGTCACATCGACCCTTGATCTTCGAGG<br>AGGAAAACTCGATTTGGCGCTCGATTTGGCGCTCGAGTAGA<br>GTCTAGACTCTACATTTTGACACCCCCAT                                                                                                                            |
| 56 | S1-8  | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGATTTC<br>TTGAAATCGATTTGGCGCTCGAAGGAAGTTCGAGGGGGCGG<br>GGTCGATTTCCAAGAAATCGAGTTTTTCCTCCTCGAGCGCCAA<br>ATCGACCCTTTGATCTTCGATCACGTCCTCGAGCGCCAAATCG<br>AGGACGTGATCGAATGAGTCACATCGAATGAGTCACATCGAAT<br>GAGTCACATCGATTTGGCGCTCGATCGGGGGGGCGGGTTCGA<br>GGAAAGTCCCCTCGAGGAAAGTCCCCTCGATTTCCAAGAAATC<br>GATTTGGCGCTCGATCTCCGCCCCCTCTTCGATCTCCGCCCC<br>CTCTTCGAGTTTTTCCTCCTCGAGTAGAGTCTAGACTCTACATT<br>TTGACACCCCCAT |
| 57 | S1-30 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAATGA<br>CATCATCTTTCGAGGAAAGTCCCCTCGACCCTTTGATCTTCGA<br>CCCCGCCCCCTCGAGTTTTTCCTCCTCGAGGAAAGTCCCCTC<br>GACCCTTTGATCTTCGAGGGGACTTTCCTCGAGGGGGCGGGG<br>TCGAGGACGTGATCGAGGAAAGTCCCCTCGAGTAGAGTCTAG<br>ACTCTACATTTTGACACCCCCAT                                                                                                                                                                                   |
| 58 | S1-33 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAAGAT<br>CAAAGGGTCGAGGAGGAAAACTCGAGGGGACTTTCCTCGAC<br>CCTTTGATCTTCGAATGAGTCACATCGATTTCTTGAAATCGAA<br>GGAAGTTCGAGGGGACTTTCCTCGAGTTTTTCCTCCTCGAACT<br>TCCTTCGAGGAAAGTCCCCTCGAAGATCAAAGGGTCAAGGA<br>AGTTCGAAAGATGATGTCATTTCGATTTCTTGAAATCGAAGATC<br>AAAGGGTCGAAGAGGGGGCGGAGATCGATTTCCAAGAAATCG<br>AGTAGAGTCTAGACTCTACATTTTGACACCCCCAT                                                                          |



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| 59 | S1-41 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAATGA<br>GTCACATCGAGCGCCAAATCGAGGGGACTTTCTCGAGGGGG<br>CGGGGTCGAGTTTTTCTCCTCGATTTCCAAGAAATCGAGGGG<br>ACTTTCCTCGAGGGGACTTTCTCGAGGGGACTTTCTCGAGT<br>AGAGCTAGACTCTACATTTTGACACCCCCAT                                                                                                                                                                          |
| 60 | S1-59 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAGTTT<br>TTCTCCTCGAGGGGACTTTCTCGAAAGATGATGTCATTGCA<br>TCTCCGCCCCCTCTTCGAGGGGACTTTCTCGAGGAAAGTCC<br>CCTCGAGGAAAGTCCCCTCGAAGATCAAAGGGTCAATGAGT<br>CACATCGACCCTTTGATCTTCGACCCTTTGATCTTCGATTTCCA<br>AGAAATCGAGGAAAGTCCCCTCGAGGAAAGTCCCCTCGAGTA<br>GAGTCTAGACTCTACATTTTGACACCCCCAT                                                                             |
| 61 | S1-66 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAGGG<br>GACTTTCTCGAGGAAAGTCCCCTCGATTTCTTGAAATCGAT<br>TTGGCGCTCGAGGAGGAAAACTCGATGTGACTCATTGACCC<br>CTTTGATCTTCGAGGGGACTTTCTCGAGGGGACTTTCTCGA<br>GCGCCAAATCGAGGAGGAAAACTCGAGGGGACTTTCTCGA<br>TTTCTTGAAATCGAAAGATGATGTCATTGACCCCTTTGATCTT<br>CGAGTAGAGTCTAGACTCTACATTTTGACACCCCCAT                                                                           |
| 62 | S1-71 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAGGA<br>AAGTCCCCTCGAGGAAAGTCCCCTCGATTTCTTGAAATCGAA<br>TGACATCATCTTTGATCACGTCCTCGAGGAAAGTCCCCTCGA<br>GTTTTCTCCTCGAGGGGACTTTCTCGATTTCCAAGAAATC<br>GATTTCTTGAAATCGACCCTTTGATCTTCGAGGAGGAAAAAC<br>TCGAGTAGAGTCTAGACTCTACATTTTGACACCCCCAT                                                                                                                      |
| 63 | S1-56 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGATGTG<br>ACTCATTGAGGAAAGTCCCCTCGATTTGGCGCTCGATGTGAC<br>TCATTGATTTGGCGCTCGAGGGGACTTTCTCGATTTCCAAG<br>AAATCGAGGGGACTTTCTCGAGGAAAGTCCCCTCGAGTTTTT<br>CCTCCTCGAGTAGAGTCTAGACTCTACATTTTGACACCCCCAT                                                                                                                                                             |
| 64 | S1-6  | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGATGTG<br>ACTCATTGAGCGCCAAATCGATTTCCAAGAAATCGAATGAGT<br>CACATCGAATGACATCATCTTTGATGTGACTCATTGAGGAA<br>AGTCCCCTCGATTTCTTGAAATCGAGGAAAGTCCCCTCGAGT<br>TTTTCTCCTCGAAGAGGGGGCGGAGATCGAATGACATCATCT<br>TTCGAGTAGAGTCTAGACTCTACATTTTGACACCCCCAT                                                                                                                   |
| 65 | S1-60 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAGGG<br>GACTTTCTCGATCTCCGCCCCCTCTTCGAGGAAAGTCCCCTC<br>GAAGGAAGTTCGAATCTCCTTCGAGGGGACTTTCTCGAAGGA<br>AGTTCGAGCGCCAAATCGAATGAGTCACATCGATTTCCAAGAA<br>ATCGATCTCCGCCCCCTCTTCGAGGAGGAAAACTCGAGGGG<br>ACTTTCCTCGATTTCCAAGAAATCGAGGGGACTTTCTCGATC<br>ACGTCCTCGAGGACGTGATCGAATGAGTCACATCGATGTGACT<br>CATTCGACCCTTTGATCTTCGAGTAGAGTCTAGACTCTACATTT<br>TGACACCCCCAT |
| 66 | S1-86 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGATTTT<br>CAAGAAATCGAGGGGGCGGGTTCGAGGGGACTTTCTCGAG<br>GGGACTTTCTCGAGGAAAGTCCCCTCGATCTCCGCCCCCTC<br>TTCGAGGAAAGTCCCCTCGAATCTCCTTCGAGGAAAGTCCCCT<br>CGAATGAGTCACATCGATTTGGCGCTCGAGGAAAGTCCCCTC                                                                                                                                                               |

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|    |       | GAGGGGACTTTCTCGAGGAAAGTCCCCTCGAGGGGGCGGG<br>GTCGAACCTCCTTCGAGTAGAGTCTAGACTCTACATTTTGACAC<br>CCCCAT                                                                                                                                                                                                                                                                           |
| 67 | S1-32 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAATGA<br>GTCACATCGATCTCCGCCCCCTCTTCGATTTGGCGCTCGAGG<br>GGACTTTCTCGACCCCTTTGATCTTCGAGGGGACTTTCTCGA<br>GGAAAGTCCCCTCGAGGAAAGTCCCCTCGAGTAGAGTCTAGA<br>CTCTACATTTTGACACCCCCAT                                                                                                                                                              |
| 68 | S1-10 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGATTTG<br>GCGCTCGATGTGACTCATTTCGAGGAAAGTCCCCTCGAGGGGA<br>CTTTCCTCGAAGATCAAAGGGTCGAAAGATGATGTCATTTCGAG<br>GAAAGTCCCCTCGACCCCGCCCCCTCGATTTCTTGAAATCGA<br>ATGAGTCACATCGATTTCTTGAAATCGATGTGACTCATTTCGA<br>GTAGAGTCTAGACTCTACATTTTGACACCCCCAT                                                                                                |
| 69 | S1-18 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAGGG<br>GACTTTCTCGATGTGACTCATTTCGATTTCTTGAAATCGATTT<br>CCAAGAAATCGACCCCGCCCCCTCGAGGAGGAAAACTCGAT<br>TTCCAAGAAATCGAATGACATCATCTTTCGAGGGGACTTTCT<br>CGAAGATCAAAGGGTCGAAGATCAAAGGGTCGAGGAGGAAAA<br>ACTCGATTTCCAAGAAATCGAATGAGTCACATCGAGTTTTCT<br>CCTCGATTTCTTGAAATCGAAGAGGGGGCGGAGATCGATTT<br>GGCGCTCGAGTAGAGTCTAGACTCTACATTTTGACACCCCCAT |
| 70 | S1-14 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAAAGA<br>TGATGTCATTTCGAATGAGTCACATCGATTTGGCGCTCGAGGGG<br>GCGGGGTCGATTTGGCGCTCGAAAGATGATGTCATTTCGAGTTT<br>TTCCTCCTCGACCCCTTTGATCTTCGAAGAGGGGGCGGAGATC<br>GAAAGATGATGTCATTTCGAATGAGTCACATCGATTTCCAAGAA<br>ATCGATTTCCAAGAAATCGAGTAGAGTCTAGACTCTACATTTTG<br>ACACCCCCAT                                                                     |
| 71 | S1-16 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAGGA<br>GAAAAAACTCGATTTCTTGAAATCGAGGGGACTTTCTTCGAA<br>AGATGATGTCATTTCGAAGATCAAAGGGTCGATGTGACTCATT<br>GAGGGGACTTTCTTCGAGGGGGCGGGGTCGAATGACATCATC<br>TTTCGAGGGGACTTTCTTCGAGGGGACTTTCTTCGAATGACAT<br>CATCTTTCGATTTCTTGAAATCGAGTAGAGTCTAGACTCTACA<br>TTTTGACACCCCCAT                                                                       |
| 72 | S1-19 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGATCTC<br>CGCCCCCTCTTCGAGTTTTTCTCCTCGATTTCTTGAAATCGA<br>AGATCAAAGGGTCGATTTCTTGAAATCGAGGGGACTTTCTC<br>GAGGAAAGTCCCCTCGAGGGGACTTTCTTCGAGGAGGAAAAA<br>CTCGATTTGGCGCTCGAGTAGAGTCTAGACTCTACATTTTGAC<br>ACCCCCAT                                                                                                                              |
| 73 | S1-26 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAAAGA<br>TGATGTCATTTCGAGTTTTTCTCCTCGAAGGAAGTTCGAGGAC<br>GTGATCGAAGAGGGGGCGGAGATCGAATGAGTCACATCGAAG<br>GAAGTTCGAGGAAAGTCCCCTCGAGGAAAGTCCCCTCGAAGA<br>TCAAAGGGTCGAGTAGAGTCTAGACTCTACATTTTGACACCCC<br>CAT                                                                                                                                 |
| 74 | S1-65 | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGACCC<br>CGCCCCCTCGAGCGCCAAATCGAGGGGACTTTCTTCGAGGGG                                                                                                                                                                                                                                                                                     |

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|    |        | ACTTTCCTCGAGGGGACTTTCCTCGAGTAGAGTCTAGACTCTA<br>CATTTTGACACCCCCAT                                                                                                                                                                                                                                                                                                                                                                                                                   |
| 75 | S2-n1  | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAGGG<br>GGCGGGTCAATGACATCATCTTTCGAGGAAAGTCCCCTCG<br>AGGAAAGTCCCCTCGAGGAAAGTCCCCTCGATTTCCAAGAAAT<br>CGACCCTTTGATCTTCGAAGATCAAAGGGTCGATGTGACTCAT<br>TCGAAGATCAAAGGGTCGATTTGGCGCTCGAGTAGAGTCTAGA<br>CTCTACATTTTGACACCCCCAT                                                                                                                                                                                                                      |
| 76 | S4-n1  | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGAGGA<br>AAGTCCCCTCGAGTTTTTCCTCCTCGAAGAGGGGGCGGAGAT<br>CGATTTCCAAAAAATCGAATGACATCATCTTTCGAAGATCAAG<br>GGGTCGAAGATCAAAGGGTCGATTTCCAAGAAATCGATTTCTT<br>GGAAATCGAGGAAAGTCCCCTCGACCCTTTGATCTTCGATCTC<br>CGCCCCCTCTTCGAAGATCAAAGGGTCGAAGAGGGGGCGGA<br>GATCGAGTAGAGTCTAGACTCTACATTTTGACACCCCCAT                                                                                                                                                     |
| 77 | S6-n1  | CTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATCGATTTT<br>CAAGAAATCGATTTCTTGAAATCGAATGACATCATCTTTCGAG<br>TTTTTCTCCTCGAAGAGGGGGCGGAGATCGAGGAAAGTCCC<br>CTCGATTTCCAAGAAATCGAACTTCCTTCGAAGGAAGTTCGAG<br>GAGGAAAAACTCGAGGGGGCGGGGTGAGTTTTTCTCCTCG<br>AGGGGACTTTCTCGACCCCGCCCCCTCGACCCTTTGATCTTC<br>GATTTCCAAGAAATCGAGTAGAGTCTAGACTCTACATTTTGACA<br>CCCCCAT                                                                                                                                        |
| 78 | S1-325 | TCGAAGATCAAAGGGTCGATTTCCAAGAAATCGATGTGACTCA<br>TTCGATTTGGCGCTCGACCCCGCCCCCTCGAAGAGGGGGCG<br>GAGATCGAATGAGTCACATCGAGGAAAGTCCCCTCGATTTCTT<br>GGAAATCGAGGAAAGTCCCCTCGAGGGGACTTTCTCGAGGA<br>GGAAAAACTCGAATGACATCATCTTTCGAATGAGTCACATCGA<br>TTTCTTGAAATCGAGGGGACTTTCTCGACCCCGCCCCCTCG<br>AGGTGACTTTCTCGAGGGGACTTTCTCGATGTGACTCATTC<br>GAGGGGACTTTCTCGAGGGGACTTTCTCGACCCTTTGATCT<br>TCGATTTGGCGCTCGAGGGGACTTTCTCGAGGGGACTTTCC<br>TCGAGGGGACTTTCTCGAGTAGAGTCTAGACTCTACATTTTG<br>ACACCCCCA |
| 79 | S1-60  | TCGAATGAGTCACATCGATCTCCGCCCCCTCTTCGAGGGGGC<br>GGGGTCGAGGAGGAAAAACTCGAATGAGTCACATCGACCCTT<br>TGATCTTCGAGGGGACTTTCCGGGGTGGAGCAAGCGTGACAA<br>GTCCACGTATGACCCGACCGACGATATCGAAGCCTACGCGCT<br>GAACGCCAGCCCCGATCGACCCCGCCCCCTCGATTTCCAAGA<br>AATCGAATGACATCATCTTTCGAATGACATCATCTTTCGAGGG<br>GACTTTCTCGAACTTCCTTCGAGGGGACTTTCTCGAGGGGA<br>CTTTCCTCGAGGAGGAAAAACTCGAGTAGAGTCTAGACTCTAC<br>ATTTTGACACCCCCA                                                                                   |
| 80 | S2-274 | TCGAATGAGTCACATCGATCTCCGCCCCCTCTTCGAGGGGGC<br>GGGGTCGAGGAGGAAAAACTCGAATGAGTCACATCGACCCTT<br>TGATCTTCGAGGGGACTTTCCGGGGTGGAGCAAGCGTGACAA<br>GTCCACGTATGACCCGACCGACGATATCGAAGCCTACGCGCT<br>GAACGCCAGCCCCGATCGACCCCGCCCCCTCGATTTCCAAGA<br>AATCGAATGACATCATCTTTCGAATGACATCATCTTTCGAGGG<br>GACTTTCTCGAACTTCCTTCGAGGGGACTTTCTCGAGGGGA                                                                                                                                                     |

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|    |                      | CTTTCCTCGAGGGGACTTTTCCTCGAGGAGGAAAACTCGAAAG<br>ATGATGTCATTTCGAGTTTTTCTCCTCGAGGAGGAAAACTCG<br>AGTAGAGTCTAGACTCTACATTTTGACACCCCCA                                                                                                                                                                                                                                                                                                                                    |
| 81 | S2-310               | TGGAATGAGTCACATCGATCTCCGCCCCCTCTTCGAGGGGGC<br>GGGGTCGAGGAGGAAAACTCGAATGAGTCACATCGACCCCTT<br>TGATCTTCGAGGGGACTTTCCGGGGTGGAGCAAGCGTGACAA<br>GTCCACGTATGACCCGACCGACGATATCGAAGCCTACGCGCT<br>GAACGCCAGCCCCGATCGACCCCGCCCCCTCGATTTCCAAGA<br>AATCGAATGACATCATCTTTCGAATGACATCATCTTTCGAGGG<br>GACTTTCTCGAACTTCCTTCGAGGGGACTTTCTCGAGGGGA<br>CTTTCCTCGAGGGGACTTTCTCGAGGAGGAAAACTCGAGTA<br>GAGTCTAGACTCTACATTTTGACACCCCCA                                                      |
| 82 | S1-367               | TCGATTTCCAAGAAATCGACCCCGCCCCCTCGAACTTCCTTCG<br>ATTTCTTGGAATCGAGGAAAGTCCCCTCGATTTCTTGAAATC<br>GATTTCTTGGAATCGAATGACATCATCTTTCGATTTCTTGAA<br>ATCGACCCCTTTGATCTTCGAGGAGGAAAACTCGAATGACATC<br>ATCTTTCGATCACGTCCTCGAAGATCAAAGGGTCGAGTTTTTC<br>CTCCTCGAGGAAAGTCCCCTCGATGTGACTCATTTCGATTTCTT<br>GGAAATCGAGGGGACTTTCTCGAGGGGACTTTCTCGAGTTT<br>TTCCTCCTCGAGGAGGAAAACTCGATTTCCAAGAAATCGAGG<br>GGACTTTCCTCGACCCCTTTGATCTTCGAGCGCCAAATCGAGTA<br>GAGTCTAGACTCTACATTTTGACACCCCCA |
| 83 | S1-7                 | TCGATTTCTTGGAATCGATTTGGCGCTCGAAGGAAGTTCGAG<br>GGGGCGGGGTCGATTTCCAAGAAATCGAGTTTTTCTCCTCGA<br>GCGCCAAATCGACCCCTTTGATCTTCGATCACGTCCTCGAGCGC<br>CAAATCGAGGACGTGATCGAATGAGTCACATCGAATGAGTCAC<br>ATCGAATGAGTCACATCGATTTGGCGCTCGATCGGGGGGCGG<br>GGTCGAGGAAAGTCCCCTCGAGGAAAGTCCCCTCGATTTCCA<br>AGAAATCGATTTGGCGCTCGATCTCCGCCCCCTCTTCGATCTC<br>CGCCCCCTCTTCGAGTTTTTCTCCTCGAGTAGAGTCTAGACT<br>CTACATTTTGACACCCCCA                                                            |
| 84 | Minimal CD4<br>hinge | SNIKVLPTWSTPVQP                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| 85 | CD3ε                 | DGNEEMGGITQTPYKVSISGTTVILTCPQYPGSEILWQHNDKNIG<br>GDEDDKNIGSDEDLHSLKEFSELEQSGYYVCYPRGSKPEDANFY<br>LYLRARVCENCMEMDVMSVATIVIVDICITGLLLLVYYWSKNRKA<br>KAKPVTRGAGAGGRQRGQNKERPPVPNPDIPIRKGQRDLYS<br>GLNQRR                                                                                                                                                                                                                                                              |
| 86 | FRB*                 | WHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQT<br>LKETSFNQAYGRDLMEAEWCRKYMKSGNVKDLLQAWDLYYHV<br>FRRISK                                                                                                                                                                                                                                                                                                                                                                 |
| 87 | FRB                  | WHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQT<br>LKETSFNQAYGRDLMEAEWCRKYMKSGNVKDLTQAWDLYYHV<br>FRRISK                                                                                                                                                                                                                                                                                                                                                                 |
| 88 | FKBP12               | GVQVETISPGDGRTFPKRGQTCVVHYTGMLDGGKKFDSSRDRN<br>KPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDIYAYGATGH<br>PGIIPPHATLVFDVELLKE                                                                                                                                                                                                                                                                                                                                                |
| 89 | FKBP12<br>F36V       | GVQVETISPGDGRTFPKRGQTCVVHYTGMLDGGKKVDSSRDRN<br>KPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDIYAYGATGH<br>PGIIPPHATLVFDVELLKE                                                                                                                                                                                                                                                                                                                                                |

|     |                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|-----|--------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 90  | CD4 transmembrane                                      | MALIVLGGVAGLLLLFIGLGIFF                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| 91  | Truncated intracellular CD4 – v1                       | CVRC                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| 92  | Truncated intracellular CD4 – v2                       | CVRCRHRRRQ                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| 93  | anti-CD33_VHH_1 CDR1                                   | RSSGIDVMG                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| 94  | anti-CD33_VHH_1 CDR2                                   | EISGVGDTN                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| 95  | anti-CD33_VHH_1 CDR3                                   | HSFLDLVGA                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| 96  | anti-CD33_VHH_1                                        | EVQLVESGGGEVQPGGSLRLSCAASRSSGIDVMGWYRQAPGKE<br>RELVAEISGVGDTNYAASLADRFTVSRDNAKNTVYLMQSSSLRAED<br>TAVYYCNAHSFLDLVGAWGQGTLVTVKP                                                                                                                                                                                                                                                                                                                               |
| 97  | anti-CLL1_VHH_1 CDR1                                   | GFLFSIYDMN                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| 98  | anti-CLL1_VHH_1 CDR2                                   | GITNNGYSTA                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| 99  | anti-CLL1_VHH_1 CDR3                                   | DLTKAYDVEYA                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| 100 | anti-CLL1_VHH_1                                        | EVQLVESGGGEVQPGGSLRLSCAASGFLFSIYDMNWYRQAPGK<br>EREWAGITNNGYSTAYAESVKGRFTISRDNANKNTIYLMQSSSLRA<br>EDTAVYYCHADLTAKAYDVEYAWGQGTLVTVKP                                                                                                                                                                                                                                                                                                                          |
| 101 | CD8 signal sequence                                    | ALPVTALLPLALLLHAARP                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| 102 | IgK signal sequence                                    | ETPAQLFLLLLWLPDTTG                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| 103 | FRBstar.3xG4S.CD3e                                     | WHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQT<br>LKETSFNQAYGRDLMEAEWCRKYMKSGNVKDLLQAWDLYYHV<br>FRRISKGGGGSGGGGSGGGGSDGNEEMGGITQTPYKVSISGTT<br>VILTCPQYPGSEILWQHNDKNIGGDEDDKNIGSDEDHLSLKEFSEL<br>EQSGYYVCYPRGSKPEDANFYLYLRARVCENCMEMDMVMSVATIV<br>IVDICITGLLLLVYYWSKNRKAKAKPVTRGAGAGGRQGRQNKER<br>PPPVPNPDPYPIRKGGQRDLYSGLNQRR                                                                                                                                  |
| 104 | antiCLL1.G4S.antiCD33.G4S.FKBP.minCD4hinge.CD4tm.CD4ic | EVQLVESGGGEVQPGGSLRLSCAASGFLFSIYDMNWYRQAPGK<br>EREWAGITNNGYSTAYAESVKGRFTISRDNANKNTIYLMQSSSLRA<br>EDTAVYYCHADLTAKAYDVEYAWGQGTLVTVKPGGGGSEVQLVE<br>SGGGEVQPGGSLRLSCAASRSSGIDVMGWYRQAPGKERELVAE<br>ISGVGDTNYAASLADRFTVSRDNANKNTVYLMQSSSLRAEDTAVYY<br>CNAHSFLDLVGAWGQGTLVTVKPGGGGSGVQVETISPGDGRTF<br>PKRGQTCVHYTGMLDGKKFDSSRDNRNPKFKFMLGKQEVIRG<br>WEEGVAAQMSVGVQRAKLTISPDIYAYGATGHPGIIPPHATLVFDVEL<br>LKLESNIKVLPTWSTPVQPMALIVLGGVAGLLLLFIGLGIFFCVRCRH<br>RRRQ |

|     |                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|-----|---------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 105 | CD8ss.FRBs<br>tar.CD3e.P2<br>A.IgKss.anti<br>CLL1.<br>antiCD33.FK<br>BP.minCD4h<br>inge.CD4tm.<br>CD4ic | ALPVTALLPLALLLHAARPGSILWHEMWHEGLEEASRLYFGERN<br>VKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEQWCR<br>KYMKSGNVKDILLQAWDLYYHVFRISKGGGGSGGGGSGGGGS<br>DGNEEMGGITQTPYKVSISGTTVILTCPQYPGSEILWQHNDKNIG<br>GDEDDKNIGSDEDHLSLKEFSELEQSGYYVCYPRGSKPEDANFY<br>LYLRARVCENCMEMDVM SVATIVIVDITGGLLLL VYYWSKNRKA<br>KAKPVTRGAGAGGRQRGQNKERPPVPNPDYEPKRGQRDLYS<br>GLNQRRISGSGATNFSLLKQAGDVEENPGPSMETPAQLLFLLLW<br>LPDTTGEVQLVESGGGEVQPGGSLRLS CAASGFLFSIYDMNWYR<br>QAPGKEREWVAGITNNGYSTAYAESVKGRFTISRDNANTYILQM<br>SSLRAEDTAVYYCHADLT KAYDVEYAWGQGT LVTVPKPGGGGSE<br>VQLVESGGGEVQPGGSLRLS CAASRSGIDVMGWYRQAPGKER<br>ELVAEISGVGDTNYAASLADRFTVSRDNANTVYLQMSSLRAEDT<br>AVYYCNAHSFLDLVGAWGQGT LVTVPKPGGGGSGVQVETISPGD<br>GRTFPKRGQTCVVHYTGMLEDGKKFDSSRDNRNPKFKMLGKQE<br>VIRGWEEGVAQMSVGQRAKL TISPDYAYGATGHPGIIPPHATLVF<br>DVELLKLESNIKVLPTWSTPVQPMALIVLGGVAGLLFFIGLGIFFCV<br>RCRHRRRQ |
| 106 | Kozak                                                                                                   | (GCC)RCCATGG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| 107 | IL2 minimal<br>promoter                                                                                 | ACATTTTGACACCCCATATAATATTTTCCAGAATTAACAGTATAA<br>ATTGCATCTCTTGTTCAGAGTTCCTATCACTCTCTTTAATCA<br>CTACTCACAGTAACCTCAACTCCTG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| 108 | CD28<br>transmembrane<br>domain                                                                         | MFWWLVVVGGVLACYSLLVTVAFIIFW                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| 109 | CD8 $\alpha$<br>transmembrane<br>domain                                                                 | IWAPLAGTCGVLLLSLVITLYC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| 110 | CD3z<br>signaling<br>domain                                                                             | RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP<br>EMGGKPRRKPNQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH<br>DGLYQGLSTATKDTYDALHMQALPPR                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| 111 | 4-1BB<br>signaling<br>domain                                                                            | VKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |

J. Exemplary Embodiments.

1. An artificial expression construct including a sequence encoding a mutated interleukin 15 (IL-15), wherein the sequence encoding the mutated IL-15 is under the regulatory control of a promoter including i) a minimal promoter operably linked to a sequence having at least 95% sequence identity to the sequence as set forth in any of SEQ ID Nos: 45-83 or ii) a sequence having at least 95% sequence identity to the sequence as set forth in SEQ ID NO: 15.
2. The artificial expression construct of embodiment 1, wherein the promoter has at least 98% sequence identity to the sequence as set forth in SEQ ID NO: 15.
3. The artificial expression construct of embodiments 1 or 2, wherein the promoter has at least 99% sequence identity to the sequence as set forth in SEQ ID NO: 15.
4. The artificial expression construct of any of embodiments 1-3, wherein the promoter has the sequence as set forth in SEQ ID NO: 15.
5. The artificial expression construct of embodiment 1, wherein promoter includes the minimal promoter operably linked to a sequence having at least 98% or at least 99% sequence identity

to the sequence as set forth in any of SEQ ID Nos: 45-83.

6. The artificial expression construct of any of embodiments 1-5, wherein promoter includes the minimal promoter operably linked to the sequence as set forth in any of SEQ ID Nos: 45-83.
7. The artificial expression construct of any of embodiments 1-6, wherein the minimal promoter is an IL2 minimal promoter.
8. The artificial expression construct of embodiment 7, wherein the IL2 minimal promoter includes the sequence as set forth in SEQ ID NO: 107 or a sequence having at least 95% sequence identity to the sequence as set forth in SEQ ID NO: 107.
9. The artificial expression construct of any of embodiments 1-9, wherein the mutated IL-15 binds to an IL15 receptor complex including IL-15R $\alpha$ .
10. The artificial expression construct of claim 1, wherein the mutated IL-15 has lower affinity to complexes including IL2R $\beta$  and common gamma receptor without IL-15R $\alpha$ , as compared to IL2R $\beta$  and common gamma receptor with IL-15R $\alpha$ .
11. The artificial expression construct of claim 1, wherein the mutated IL-15 has lower affinity to the common gamma receptor and/or atypical binding to IL2R $\beta$  as compared to a wild-type IL-15.
12. The artificial expression construct of claim 1, wherein the mutated IL-15 is unable to bind the common gamma receptor.
13. The artificial expression construct of any of embodiments 1-12, wherein the mutated IL-15 includes a D to S mutation at position 8 compared to a wild-type IL-15.
14. The artificial expression construct of embodiment 13, wherein the wild-type IL-15 includes the sequence as set forth in SEQ ID NO: 8 or SEQ ID NO: 41.
15. The artificial expression construct of embodiments 13 or 14, wherein the wild-type IL-15 is encoded by the sequence as set forth in SEQ ID NO: 10 or SEQ ID NO: 43.
16. The artificial expression construct of any of embodiments 1-15, wherein the mutated IL-15 includes the sequence as set forth in SEQ ID NO: 9 or SEQ ID NO: 42; or a sequence having at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to the sequence set forth SEQ ID NO: 9 or SEQ ID NO: 42.
17. The artificial expression construct of any of embodiments 1-16, wherein the mutated IL-15 is encoded by the sequence as set forth in SEQ ID NO: 11 or SEQ ID NO: 44; or a sequence having at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to the sequence set forth in SEQ ID NO: 11 or SEQ ID NO: 44.
18. The artificial expression construct of any of embodiments 1-17, wherein the artificial expression construct includes a sequence having at least 95% sequence identity to the

sequence as set forth in SEQ ID NO: 19 (iSynPro.IL15.D8S.Fwd).

19. The artificial expression construct of any of embodiments 1-18, wherein the artificial expression construct includes a sequence having at least 98% sequence identity to the sequence as set forth in SEQ ID NO: 19 (iSynPro.IL15.D8S.Fwd).
20. The artificial expression construct of any of embodiments 1-19, wherein the artificial expression construct includes a sequence having at least 99% sequence identity to the sequence as set forth in SEQ ID NO: 19 (iSynPro.IL15.D8S.Fwd).
21. The artificial expression construct of any of embodiments 1-20, wherein the artificial expression construct includes the sequence as set forth in SEQ ID NO: 19 (iSynPro.IL15.D8S.Fwd).
22. The artificial expression construct of any of embodiments 1-21, further including a sequence encoding a recombinant receptor or exogenous lymphocyte receptor, wherein the recombinant receptor or exogenous lymphocyte receptor include a binding domain that binds an antigen expressed on a surface of targeted cells.
23. The artificial expression construct of embodiment 22, wherein the recombinant receptor includes a chimeric antigen receptor (CAR), an engineered TCR (eTCR), Dimerizing Agent-Regulated Immunoreceptor Complex (DARIC), or a hybrid thereof.
24. The artificial expression construct of claim 22, wherein the exogenous lymphocyte receptor comprises a T cell receptor (TCR) or a B cell receptor (BCR).
25. The artificial expression construct of embodiments 22 or 23, wherein the sequence encoding the recombinant receptor or exogenous lymphocyte receptor is operably linked to a second promoter.
26. The artificial expression construct of embodiment 24, wherein the second promoter is selected from the group including a cytomegalovirus immediate early gene promoter (CMV); an elongation factor 1 alpha promoter (EF1- $\alpha$ ); a phosphoglycerate kinase-1 promoter (PGK); a ubiquitin-C promoter (UBQ-C); a cytomegalovirus enhancer/chicken beta-actin promoter (CAG); polyoma enhancer/herpes simplex thymidine kinase promoter (MC1); a beta actin promoter ( $\beta$ -ACT); a simian virus 40 promoter (SV40); a myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer-binding site substituted (MND) U3 promoter; a mouse mammary tumor virus (MMTV) promoter; a human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter; a MoMuLV promoter; an avian leukemia virus promoter; an Epstein-Barr virus immediate early promoter; a Rous sarcoma virus promoter; an actin promoter; a myosin promoter; a hemoglobin promoter; or a creatine kinase promoter.
27. The artificial expression construct of embodiments 24 or 25, wherein the second promoter



- includes an MNDU3 promoter or an EF1- $\alpha$  promoter.
28. The artificial expression construct of embodiments 25 or 26, wherein the EF1- $\alpha$  promoter includes the first intron of a human EF1- $\alpha$  gene.
  29. The artificial expression construct of embodiments 25 or 26, wherein the EF1- $\alpha$  promoter lacks the first intron of a human EF1- $\alpha$  gene.
  30. The artificial expression construct of any of embodiments 22-29, wherein the binding domain is part of an extracellular component.
  31. The artificial expression construct of any of embodiments 22-30, wherein the binding domain comprises an anti-CD33 binding domain or an anti-CLL1 binding domain.
  32. The artificial expression construct of any of embodiments 22-31, wherein the targeted cells include cancer cells or cells infected with bacteria, virus, fungi, parasites, or arthropods.
  33. The artificial expression construct of any of embodiments 22-32, wherein the recombinant receptor further includes an intracellular component.
  34. The artificial expression construct of embodiment 33, wherein the intracellular component includes a CD3 $\zeta$  signaling domain and/or a 4-1BB signaling domain.
  35. The artificial expression construct of embodiment 33 or 34, wherein the intracellular component is linked to the extracellular component through a transmembrane domain.
  36. The artificial expression construct of embodiment 35, wherein the transmembrane domain includes a CD8 $\alpha$  transmembrane domain, a CD4 transmembrane domain, or a CD28 transmembrane domain.
  37. The artificial expression construct of any of embodiments 22-36, wherein the recombinant receptor further includes a multimerization domain.
  38. The artificial expression construct of any of embodiments 22-37, wherein the recombinant receptor multimerizes upon administration of a drug.
  39. The artificial expression construct of embodiment 38, wherein the drug includes rapamycin or a rapalog thereof.
  40. The artificial expression construct of embodiment 38 or embodiment 39, wherein the drug includes AP1903, AP20187, AP21967 (also known as C16-(S)-7-methylindolerapamycin), everolimus, novolimus, pimecrolimus, ridaforolimus, sirolimus, tacrolimus, temsirolimus, umirolimus, zotarolimus, or BPC015.
  41. The artificial expression construct of any of embodiments 37-40, wherein the multimerization domain includes an FK506 binding protein (FKBP) multimerization domain or a variant thereof, and an FKBP-rapamycin binding (FRB) multimerization domain or a variant thereof.
  42. The artificial expression construct of any one of embodiments 23-41, wherein the DARIC

includes a signaling component including an amino acid sequence as set forth in SEQ ID NOs: 1 or 2; or a sequence having at least 95%, at least 98%, or at least 99% sequence identity to the sequence as set forth in SEQ ID NOs: 1 or 2.

43. The artificial expression construct of any one of embodiments 23-42, wherein the DARIC includes a targeting component including an amino acid sequence as set forth in SEQ ID NOs: 3 or 4; or a sequence having at least 95%, at least 98%, or at least 99% sequence identity to the sequence as set forth in SEQ ID NOs: 3 or 4.
44. The artificial expression construct of any one of embodiments 23-43, wherein the DARIC includes an amino acid sequence as set forth in SEQ ID NO: 5 ; or a sequence having at least 95%, at least 98%, or at least 99% sequence identity to the sequence as set forth in SEQ ID NO: 5.
45. The artificial expression construct of any one of embodiments 23-44, wherein the recombinant receptor includes:
- (a) a signaling component including (i) a first multimerization domain, (ii) a first linker, and (iii) an intracellular component; and
  - (b) a targeting component including (i) a binding domain, (ii) a second linker, (iii) a second multimerization domain, and (iv) a transmembrane domain.
46. The artificial expression construct of any one of embodiments 23-45, wherein the recombinant receptor includes:
- (a) a signaling component including (i) a first multimerization domain including an FRB polypeptide or a FKBP polypeptide, (ii) a first linker, and (iii) a CD3 $\epsilon$  ; and
  - (b) a targeting component including (i) an anti-CLL1 binding domain, (ii) an anti-CD33 binding domain, (iii) a second linker, (iv) a second multimerization domain including an FRB polypeptide or a FKBP polypeptide, (v) a spacer, and (vi) a transmembrane domain.
47. The artificial expression construct of any one of embodiments 23-46, wherein the recombinant receptor includes:
- (a) a signaling component including (i) a first multimerization domain including an FRB polypeptide or a FKBP polypeptide, (ii) a first linker, and (iii) a CD3 $\epsilon$ ; and
  - (b) a targeting component including (i) an anti-CLL1 binding domain, (ii) an anti-CD33 binding domain, (iii) a second linker, (iv) a second multimerization domain including an FRB

polypeptide or a FKBP polypeptide, (v) a spacer including a CD4 hinge region, (vi) a CD4 transmembrane domain, and (vii) a truncated CD4 intracellular polypeptide.

48. The artificial expression construct of any of embodiments 45 - 47, wherein the targeting component does not include a functional intracellular domain or costimulatory domain having signaling capabilities.
49. The artificial expression construct of embodiment 47 or embodiment 48, wherein the CD4 hinge region includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 84.
50. The artificial expression construct of any one of embodiments 48-49, wherein the CD4 hinge region includes an amino acid sequence as set forth in SEQ ID NO: 84.
51. The artificial expression construct of any one of embodiments 47-50, wherein the CD3 $\epsilon$  includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 85.
52. The artificial expression construct of any one of embodiments 47-51, wherein the CD3 $\epsilon$  includes an amino acid sequence as set forth in SEQ ID NO: 85.
53. The artificial expression construct of any one of embodiments 47-52, wherein the CD3 $\epsilon$  includes both extracellular and intracellular portions of CD3 $\epsilon$ .
54. The artificial expression construct of any one of embodiments 47-53, wherein the FRB polypeptide and FKBP polypeptide localize extracellularly when the signaling and targeting components are expressed.
55. The artificial expression construct of any one of embodiments 46-54, wherein the first multimerization domain and second multimerization domain are different.
56. The artificial expression construct of any one of embodiments 46-55, wherein the first multimerization domain includes an FRB polypeptide, and the second multimerization domain includes an FKBP polypeptide.
57. The artificial expression construct of any one of embodiments 46-55, wherein the first multimerization domain includes an FKBP polypeptide, and the second multimerization domain includes an FRB polypeptide.
58. The artificial expression construct of any one of embodiments 47-57, wherein the FRB polypeptide includes an FRB T2098L variant.

59. The artificial expression construct of any one of embodiments 47-58, wherein the FRB polypeptide includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, 99%, sequence identity to, or including a sequence as set forth in SEQ ID NO: 86.
60. The artificial expression construct of any one of embodiments 47-59, wherein the FRB polypeptide includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, 99%, sequence identity to, or including a sequence as set forth in SEQ ID NO: 87.
61. The artificial expression construct of any one of embodiments 47-60, wherein the FKBP polypeptide includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, 99%, sequence identity to, or including a sequence as set forth in SEQ ID NO: 88.
62. The artificial expression construct of any one of embodiments 47-61, wherein the FKBP polypeptide includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, 99%, sequence identity to, or including a sequence as set forth in SEQ ID NO: 89.
63. The artificial expression construct of any one of embodiments 46-62, wherein the first multimerization domain and the second multimerization domain associate with a drug.
64. The artificial expression construct of embodiment 63, wherein the drug includes a rapamycin or a rapalog thereof.
65. The artificial expression construct of embodiment 63 or embodiment 64, wherein the drug is AP1903, AP20187, AP21967 (also known as C16-(S)-7-methylindolerapamycin), everolimus, novolimus, pimecrolimus, ridaforolimus, sirolimus, tacrolimus, temsirolimus, umirolimus, zotarolimus, or BPC015.
66. The artificial expression construct of any one of embodiments 46-65, wherein the first linker is a linker of 2 to 40 amino acids in length.
67. The artificial expression construct of embodiment 66, wherein the first linker is selected from the group including: GG, GS, SG, SS, GSS, SSG, GSG, SGS, SGG, G4S, 2xG4S, 3xG4S, 4xG4S, 5xG4S, and any combination thereof.
68. The artificial expression construct of embodiments 66 or 67, wherein the first linker is a 3xG4S linker.
69. The artificial expression construct of any one of embodiments 46-68, wherein the second linker is a linker of 2 to 40 amino acids in length.

70. The artificial expression construct of embodiment 69, wherein the second linker is selected from the group including: GG, GS, SG, SS, GSS, SSG, GSG, SGS, SGG, G4S, 2xG4S, 3xG4S, 4xG4S, and any combination thereof.
71. The artificial expression construct of embodiments 69 or 70, wherein the second linker is a G4S linker.
72. The artificial expression construct of any one of embodiments 48-71, wherein the CD4 transmembrane domain includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 90.
73. The artificial expression construct of any one of embodiments 48-72, wherein the CD4 transmembrane domain includes an amino acid sequence as set forth in SEQ ID NO: 90.
74. The artificial expression construct of any one of embodiments 48-73, wherein the truncated intracellular CD4 polypeptide includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 91 or SEQ ID NO: 92.
75. The artificial expression construct of any one of embodiments 48-74, wherein the truncated intracellular CD4 polypeptide includes an amino acid sequence as set forth in SEQ ID NO: 91 or SEQ ID NO: 92.
76. The artificial expression construct of any one of embodiments 47-75, wherein the anti-CLL1 binding domain includes a single domain antibody (sdAb) or single chain variable fragment (scFv).
77. The artificial expression construct of embodiment 76, wherein the sdAb is a VHH or heavy chain-only antibody (HcAb).
78. The artificial expression construct of embodiments 76 or 77, wherein the sdAb is a camelid VHH.
79. The artificial expression construct of any one of embodiments 76-78, wherein the scFv or sdAb is human or humanized.
80. The artificial expression construct of any one of embodiments 47-79, wherein the anti-CLL1 binding domain includes a complementarity determining region (CDR)1 including the sequence as set forth in SEQ ID NO: 97, a CDR2 including the sequence as set forth in SEQ ID NO: 98, and a CDR3 including the sequence as set forth in SEQ ID NO: 99.

81. The artificial expression construct of any one of embodiments 47-80, wherein the anti-CLL1 binding domain includes a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 100.
82. The artificial expression construct of any one of embodiments 47-81, wherein the anti-CLL1 binding domain includes the sequence as set forth in SEQ ID NO: 100.
83. The artificial expression construct of embodiment 47-82, wherein the anti-CD33 binding domain includes an sdAb or scFv.
84. The artificial expression construct of embodiment 83, wherein the sdAb is a VHH or heavy chain-only antibody (HcAb).
85. The artificial expression construct of embodiments 83 or 84, wherein the sdAb is a camelid VHH.
86. The artificial expression construct of any one of embodiments 83-85, wherein the scFv or sdAb is human or humanized.
87. The artificial expression construct of any one of embodiments 47-86, wherein the anti-CD33 binding domain includes a CDR1 including the sequence as set forth in SEQ ID NO: 93, a CDR2 including the sequence as set forth in SEQ ID NO: 94, and a CDR3 including the sequence as set forth in SEQ ID NO: 95.
88. The artificial expression construct of any one of embodiments 47-87, wherein the anti-CD33 binding domain includes a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 96.
89. The artificial expression construct of any one of embodiments 47-88, wherein the anti-CD33 binding domain includes the sequence as set forth SEQ ID NO:96.
90. The artificial expression construct of any one of embodiments 47-89, wherein the signaling component further includes a signal sequence.
91. The artificial expression construct of embodiment 90, wherein the signal sequence is a CD8 signal sequence.
92. The artificial expression construct of embodiment 91, wherein the CD8 signal sequence includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 101.

93. The artificial expression construct of embodiment 91 or embodiment 92, wherein the CD8 signal sequence includes the amino acid sequence as set forth in SEQ ID NO: 101.
94. The artificial expression construct of any one of embodiments 47-93, wherein the targeting component further includes a signal sequence.
95. The artificial expression construct of embodiment 94, wherein the signal sequence is an IgK signal sequence.
96. The artificial expression construct of embodiment 95, wherein the IgK signal sequence includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 102.
97. The artificial expression construct of embodiment 95 or embodiment 96, wherein the IgK signal sequence includes an amino acid sequence as set forth in SEQ ID NO: 102.
98. The artificial expression construct of any one of embodiments 46-97, wherein the signaling component includes a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 103.
99. The artificial expression construct of any one of embodiments 46-98, wherein the signaling component includes the sequence as set forth in SEQ ID NO: 103.
100. The artificial expression construct of any one of embodiments 46-99, wherein the targeting component includes a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 104.
101. The artificial expression construct of any one of embodiments 46-100, wherein the targeting component includes the sequence as set forth in SEQ ID NO: 104.
102. The artificial expression construct of any one of embodiments 22-101, wherein the recombinant receptor includes a fusion polypeptide which includes a targeting component and a signaling component of an eTCR.
103. The artificial expression construct of embodiment 102, wherein the fusion polypeptide includes a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 105.
104. The artificial expression construct of embodiment 102 or embodiment 103, wherein the fusion polypeptide includes the sequence as set forth in SEQ ID NO: 105.
105. The artificial expression construct of any of embodiments 1-104, further including a first control feature.

106. The artificial expression construct of embodiment 105, wherein the first control feature includes or encodes a transduction marker, a selection cassette, or a suicide gene.
107. The artificial expression construct of embodiment 106, wherein the transduction marker includes a truncated HER2 protein (Her2tG), epidermal growth factor receptor (EGFRt), or truncated CD19 (tCD19).
108. The artificial expression construct of embodiment 106, wherein the selection cassette includes a dihydrofolate reductase double mutant (DHFRdm).
109. The artificial expression construct of any of embodiments 1-108, further including a first skip sequence.
110. The artificial expression construct of embodiment 109, wherein the first skip sequence is between the sequence encoding the mutated IL-15 and the first control feature.
111. The artificial expression construct of embodiment 109 or embodiment 110, wherein the first skip sequence encodes a 2A self-cleaving polypeptide.
112. The artificial expression construct of embodiment 111, wherein the 2A self-cleaving polypeptide includes T2A, P2A, E2A, or F2A.
113. The artificial expression construct of any of embodiments 105-112, further including a second control feature.
114. The artificial expression construct of any of embodiments 109-113, further including a second skip sequence.
115. The artificial expression construct of embodiment 114, wherein the second skip sequence is between the mutated IL-15 and the recombinant receptor.
116. The artificial expression construct of embodiment 114 or embodiment 115, wherein the second skip sequence encodes a 2A self-cleaving polypeptide.
117. The artificial expression construct of embodiment 116, wherein the 2A skip self-cleaving polypeptide includes T2A, P2A, E2A, or F2A.
118. A nanoparticle encapsulating the artificial expression construct of any of embodiments 1-117.
119. A method of improving persistence (or function) of an adoptive cell therapy (ACT) including: transducing an immune effector cell or population of immune effector cells with a polynucleotide encoding an exogenous mutant IL-15 polypeptide, wherein the exogenous mutant IL-15 polypeptide binds to an IL15 receptor complex including IL-15R $\alpha$ , compared to an IL15 receptor complex without IL-15R $\alpha$ .



120. The method of claim 119, wherein the exogenous mutant IL-15 polypeptide has lower affinity to complexes including IL2R $\beta$  and common gamma receptor without IL-15R $\alpha$  as compared to IL2R $\beta$  and common gamma receptor with IL-15R $\alpha$ .
121. The artificial expression construct of claim 119, wherein the exogenous mutant IL-15 polypeptide has lower affinity to the common gamma receptor and/or atypical binding to IL2R $\beta$  as compared to a wild-type IL-15.
122. The method of claim 119, wherein the mutated IL-15 is unable to bind the common gamma receptor.
123. The method of embodiment 119, wherein the mutant IL-15 polypeptide includes a D to S mutation at position 8 compared to a wild-type IL-15.
124. The method of any one of embodiments 120-123, wherein the wild-type IL-15 includes the sequence as set forth in SEQ ID NO: 8 or SEQ ID NO: 41.
125. The method of any one of embodiments 120-124, wherein the wild-type IL-15 is encoded by the sequence as set forth in SEQ ID NO: 10 or SEQ ID NO: 43.
126. The method of any of embodiments 119-125, wherein the mutant IL-15 polypeptide includes an amino acid sequence as set forth in SEQ ID NO: 9 or SEQ ID NO: 42; or a sequence having at least 90%, 95%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 9 or SEQ ID NO: 42.
127. The method of any of embodiments 119-126, wherein the mutant IL-15 is encoded by the sequence as set forth in SEQ ID NO: 11 or SEQ ID NO: 44; or a sequence having at least 90%, 95%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 11 or SEQ ID NO: 44.
128. The method of any of embodiments 119-127, wherein the method results in increased persistence and improved function.
129. The method of embodiment 128, wherein the persistence is increased compared to an immune effector cell or population of immune effector cells transduced with an exogenous wild-type IL-15 polypeptide.
130. The method of embodiments 128 or 129, wherein the improved function includes decreased antigen-independent IFN $\gamma$  release compared to an immune effector cell or population of immune effector cells including a polynucleotide encoding an exogenous wildtype IL-15 polypeptide.
131. The method of any of embodiments 128-130, wherein the improved function includes increased immune cell proliferation compared to an immune effector cell including a polynucleotide encoding an exogenous wildtype IL-15 polypeptide.

132. The method of any of embodiments 128-131, wherein the immune effector cell or population of immune effector cells include a recombinant receptor or exogenous lymphocyte receptor.
133. The method of embodiment 132, wherein the recombinant receptor includes a chimeric antigen receptor (CAR), an engineered T cell receptor (eTCR), a dimerizing agent-regulated immunoreceptor complex (DARIC), or a hybrid thereof.
134. The method of claim 132, wherein the exogenous lymphocyte receptor comprises a T cell receptor (TCR) or a B cell receptor (BCR).
135. The method of embodiments 132 or 133, wherein the recombinant receptor binds to a target antigen.
136. The method of embodiment 135, wherein the target antigen includes CD33, CLL1, CD19, CD20, CD22, EGFR, EphA2, Her2, IL13Ra2, ROR1, CD133, mesothelin, CD123, or L1-CAM.
137. The method of any one of embodiments 133-136, wherein the DARIC includes a signaling component including an amino acid sequence as set forth in SEQ ID NO: 1 or SEQ ID NO: 2; or a sequence having at least 95%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 1 or SEQ ID NO: 2.
138. The method of any one of embodiments 133-137, wherein the DARIC includes a targeting component including an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 4; or a sequence having at least 95%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO:3 or SEQ ID NO: 4.
139. The method of any one of embodiments 133-138, wherein the DARIC includes an amino acid sequence as set forth in SEQ ID NO: 5; or a sequence having at least 95%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 5.
140. The method of any one of embodiments 133-139, wherein the recombinant receptor includes:
- (a) a signaling component including (i) a first multimerization domain, (ii) a first linker, and (iii) an intracellular component; and
  - (b) a targeting component including (i) a binding domain, (ii) a second linker, (iii) a second multimerization domain, and (iv) a transmembrane domain.
141. The method of any one of embodiments 133-140, wherein the recombinant receptor includes:
- (a) a signaling component including (i) a first multimerization domain including an FRB polypeptide or a FKBP polypeptide, (ii) a first linker, and (iii) a CD3ε ; and

(b) a targeting component including (i) an anti-CLL1 binding domain, (ii) an anti-CD33 binding domain, (iii) a second linker, (iv) a second multimerization domain including an FRB polypeptide or a FKBP, (v) a spacer, and (vi) a transmembrane domain.

142. The method of any one of embodiments 133-141, wherein the recombinant receptor includes:

(a) a signaling component including (i) a first multimerization domain including an FRB polypeptide or a FKBP polypeptide, (ii) a first linker, and (iii) a CD3 $\epsilon$ ; and

(b) a targeting component including (i) an anti-CLL1 binding domain, (ii) an anti-CD33 binding domain, (iii) a second linker, (iv) a second multimerization domain including an FRB polypeptide or a FKBP polypeptide, (v) a CD4 hinge region, (vi) a CD4 transmembrane domain, and (vii) a truncated CD4 intracellular polypeptide.

143. The method of any of embodiments 140-142, wherein the targeting component does not include a functional intracellular domain or costimulatory domain having signaling capabilities.

144. The method of embodiment 142 or embodiment 143, wherein the CD4 hinge region includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 84.

145. The method of any one of embodiments 142-144, wherein the CD4 hinge region includes the amino acid sequence as set forth in SEQ ID NO: 84.

146. The method of any one of embodiments 141-145, wherein the CD3 $\epsilon$  includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 85.

147. The method of any one of embodiments 141-146, wherein the CD3 $\epsilon$  includes the amino acid sequence as set forth in SEQ ID NO: 85.

148. The method of any one of embodiments 141-147, wherein the CD3 $\epsilon$  includes both extracellular and intracellular portions of the CD3 $\epsilon$ .

149. The method of any one of embodiments 141-148, wherein the FRB polypeptide and FKBP polypeptide localize extracellularly when the signaling and targeting components are expressed.

150. The method of any one of embodiments 140-149, wherein the first multimerization domain and the second multimerization domain are different.

151. The method of any one of embodiments 140-150, wherein the first multimerization domain includes an FRB polypeptide, and the second multimerization domain includes an FKBP polypeptide.
152. The method of any one of embodiments 140-151, wherein the first multimerization domain includes an FKBP polypeptide, and the second multimerization domain includes an FRB polypeptide.
153. The method of any one of embodiments 141-152, wherein the FRB polypeptide includes an FRB T2098L variant.
154. The method of any one of embodiments 141-153, wherein the FRB polypeptide includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, 99%, sequence identity to, or including a sequence as set forth in SEQ ID NO: 86.
155. The method of any one of embodiments 141-154, wherein the FRB polypeptide includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, 99%, sequence identity to, or including a sequence as set forth in SEQ ID NO: 87.
156. The method of any one of embodiments 141-155, wherein the FKBP polypeptide includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, 99%, sequence identity to, or including a sequence as set forth in SEQ ID NO: 88.
157. The method of any one of embodiments 141-156, wherein the FKBP polypeptide includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, 99%, sequence identity to, or including a sequence as set forth in SEQ ID NO: 89.
158. The method of any one of embodiments 140-157, wherein the first multimerization domain and second multimerization domain associate with a drug.
159. The method of embodiment 158, wherein the drug includes a rapamycin or a rapalog thereof.
160. The method of embodiment 158 or embodiment 159, wherein the drug is AP1903, AP20187, AP21967 (also known as C16-(S)-7-methylindolerapamycin), everolimus, novolimus, pimecrolimus, ridaforolimus, sirolimus, tacrolimus, temsirolimus, umirolimus, zotarolimus, or BPC015.
161. The method of any one of embodiments 140-160, wherein the first linker is a linker of 2 to 40 amino acids in length.

162. The method of embodiment 161, wherein the first linker is selected from the group including: GG, GS, SG, SS, GSS, SSG, GSG, SGS, SGG, G4S, 2xG4S, 3xG4S, 4xG4S, 5xG4S, and any combination thereof.
163. The method of embodiment 162, wherein the first linker is a 3xG4S linker.
164. The method of any one of embodiments 140-163, wherein the second linker is a linker of 2 to 40 amino acids in length.
165. The method of embodiment 164, wherein the second linker is selected from the group including: GG, GS, SG, SS, GSS, SSG, GSG, SGS, SGG, G4S, 2x G4S, 3xG4S, 4xG4S, and any combination thereof.
166. The method of embodiments 164 or 165, wherein the second linker is a G4S linker.
167. The method of any one of embodiments 142-166, wherein the CD4 transmembrane domain includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 90.
168. The method of any one of embodiments 142-167, wherein the CD4 transmembrane domain includes the amino acid sequence as set forth in SEQ ID NO: 90.
169. The method of any one of embodiments 142-168, wherein the truncated intracellular CD4 polypeptide includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 91 or SEQ ID NO: 92.
170. The method of any one of embodiments 142-169, wherein the truncated intracellular CD4 polypeptide includes the amino acid sequence as set forth in SEQ ID NO: 91 or SEQ ID NO: 92.
171. The method of any one of embodiments 141-170, wherein the anti-CLL1 binding domain includes a single domain antibody (sdAb) or a single chain variable fragment (scFv).
172. The method of embodiment 171, wherein the sdAb is a VHH or heavy chain-only antibody (HcAb).
173. The method of embodiments 171 or 172, wherein the sdAb is a camelid VHH.
174. The method of any one of embodiments 171-173, wherein the scFv or sdAb is human or humanized.
175. The method of any one of embodiments 171-174, wherein the anti-CLL1 binding domain includes a complementarity determining region (CDR)<sub>1</sub> including the sequence as set forth in

SEQ ID NO: 97, a CDR2 including the sequence as set forth in SEQ ID NO: 98, and a CDR3 including the sequence as set forth in SEQ ID NO: 99.

176. The method of any one of embodiments 171-175, wherein the anti-CLL1 binding domain includes a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 100.
177. The method of any one of embodiments 171-176, wherein the anti-CLL1 binding domain includes the sequence as set forth in SEQ ID NO: 100.
178. The method of any one of embodiments 141-177, wherein the anti-CD33 binding domain includes an sdAb or scFv.
179. The method of embodiment 178 wherein the sdAb is a VHH or heavy chain-only antibody (HcAb).
180. The method of embodiments 178 or 179, wherein the sdAb is a camelid VHH.
181. The method of any one of embodiments 178-180, wherein the scFv or sdAb is human or humanized.
182. The method of any one of embodiments 178-181, wherein the anti-CD33 binding domain includes a CDR1 including the sequence as set forth in SEQ ID NO: 93, a CDR2 including the sequence as set forth in SEQ ID NO: 94, and a CDR3 including the sequence as set forth in SEQ ID NO: 95.
183. The method of any one of embodiments 178-182, wherein the anti-CD33 binding domain includes a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 96.
184. The method of any one of embodiments 178-183, wherein the anti-CD33 binding domain includes the sequence as set forth in SEQ ID NO: 96.
185. The method of any one of embodiments 140-184, wherein the signaling component further includes a signal sequence.
186. The method of embodiment 185, wherein the signal sequence is a CD8 signal sequence.
187. The method of embodiment 186, wherein the CD8 signal sequence includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 101.

188. The method of embodiment 186 or embodiment 187, wherein the CD8 signal sequence includes the amino acid sequence as set forth in SEQ ID NO: 101.
189. The method of any one of embodiments 140-188, wherein the targeting component further includes a signal sequence.
190. The method of embodiment 189, wherein the signal sequence is an IgK signal sequence.
191. The method of embodiment 190, wherein the IgK signal sequence includes an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 102.
192. The method of embodiment 190 or embodiment 191, wherein the IgK signal sequence includes the amino acid sequence as set forth in SEQ ID NO: 102.
193. The method of any one of embodiments 140-192, wherein the signaling component includes a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 103.
194. The method of any one of embodiments 140-193, wherein the signaling component includes the sequence as set forth in SEQ ID NO: 103.
195. The method of any one of embodiments 140-194, wherein the targeting component includes a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 104.
196. The method of any one of embodiments 140-195, wherein the targeting component includes the sequence as set forth in SEQ ID NO: 104.
197. The method of any one of embodiments 140-196, wherein the recombinant receptor includes a fusion polypeptide which includes the targeting component and the signaling component of the recombinant receptor.
198. The method of embodiment 197, wherein the fusion polypeptide includes a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 105.
199. The method of embodiment 197 or embodiment 198, wherein the fusion polypeptide includes the sequence as set forth in SEQ ID NO: 105.
200. The method of any of embodiments 119-199, wherein the immune effector cell or population of immune effector cells includes:

- a) a T cell, an  $\alpha\beta$  T cell, or a  $\gamma\delta$  T cell;
  - b) a CD3+, CD4+, and/or CD8+ cell;
  - c) a cytotoxic T lymphocyte (CTL), a tumor infiltrating lymphocyte (TIL), or a helper T cell; or
  - d) a natural killer (NK) cell or natural killer T (NKT) cell.
201. The method of any of embodiments 119-200, wherein the polynucleotide includes an exogenous promoter operably linked to the polynucleotide encoding the exogenous mutant IL-15 polypeptide.
202. The method of embodiment 201, wherein the exogenous promoter is a constitutive promoter.
203. The method of embodiment 202, wherein the constitutive promoter is selected from the group including: a cytomegalovirus immediate early gene promoter (CMV); an elongation factor 1 alpha promoter (EF1- $\alpha$ ); a phosphoglycerate kinase-1 promoter (PGK); a ubiquitin-C promoter (UBQ-C); a cytomegalovirus enhancer/chicken beta-actin promoter (CAG); polyoma enhancer/herpes simplex thymidine kinase promoter (MC1); a beta actin promoter ( $\beta$ -ACT); a simian virus 40 promoter (SV40); a myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer-binding site substituted (MND) U3 promoter; a mouse mammary tumor virus (MMTV) promoter; a human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter; a MoMuLV promoter; an avian leukemia virus promoter; an Epstein-Barr virus immediate early promoter; a Rous sarcoma virus promoter; an actin promoter; a myosin promoter; a hemoglobin promoter; or a creatine kinase promoter.
204. The method of embodiments 202 or 203, wherein the constitutive promoter includes an MNDU3 promoter or a EF1- $\alpha$  promoter.
205. The method of embodiment 204, wherein the EF1- $\alpha$  promoter includes the first intron of a human EF1- $\alpha$  gene.
206. The method of embodiment 204, wherein the EF1- $\alpha$  promoter lacks the first intron of a human EF1- $\alpha$  gene.
207. The method of any of embodiments 202-206, wherein the constitutive promoter includes the nucleotide sequence as set forth in any of SEQ ID NOs: 12, 13, or 14.
208. The method of embodiment 201, wherein the exogenous promoter is an inducible promoter.
209. The method of embodiment 208, wherein the inducible promoter is an iSynPro promoter.



210. The method of embodiment 209, wherein the iSynPro promoter includes the sequence as set forth in SEQ ID NO: 15 or a sequence having at least 95% sequence identity to the sequence as set forth in SEQ ID NO: 15.
211. The method of embodiments 209 or 210, wherein the iSynPro promoter includes a minimal promoter operably linked to a sequence having at least 95% sequence identity to the sequence as set forth in any of SEQ ID Nos: 45-83.
212. The method of any one of embodiment 209 -211, wherein the iSynPro promoter includes a minimal promoter operably linked to a sequence as set forth in any of SEQ ID Nos: 45-83.
213. The method of any one of embodiments 119-212, wherein the cell includes the artificial expression construct of any one of embodiments 1-117.
214. A non-natural cell or population thereof including the artificial expression construct of any of embodiments 1-117.
215. The non-natural cell or population thereof of embodiment 214, wherein the cell or population thereof include an autologous cell or an allogeneic cell in reference to a subject.
216. The non-natural cell or population thereof of embodiments 214 or 215, including an *in vivo* or *ex vivo* cell or population thereof.
217. The non-natural cell or population thereof of any of embodiments 214-216, wherein the cell or population thereof include an immune cell.
218. The non-natural cell or population thereof of embodiment 217, wherein the immune cell is a lymphocyte.
219. The non-natural cell or population thereof of embodiment 218, wherein the lymphocyte includes a T cell, B cell, natural killer (NK) cell, or NK-T cell.
220. The non-natural cell or population thereof of any of embodiments 214-219, wherein the cell or population thereof include a T cell selected from a CD3+ T cell, a CD4+ T cell, a CD8+ T cell, a central memory T cell, an effector memory T cell, and/or a naïve T cell.
221. The non-natural cell or population thereof of any of embodiments 214-220, wherein the cell or population thereof include a CD8+ T cell.
222. The non-natural cell or population thereof of any of embodiments 214-221, wherein the cell or population thereof include a CD4+ T cell.
223. A composition including the non-natural cell or population thereof of any of embodiments 214-222 and a pharmaceutically acceptable carrier.
224. A method of treating a subject in need thereof including administering a therapeutically effective amount of the artificial expression construct of any of embodiments 1-117, the nanoparticle of embodiment 118, a non-natural cell or population thereof of any of

embodiments 214-222, or the composition of embodiment 223 to the subject thereby treating the subject in need thereof.

225. The method of embodiment 224, wherein the subject in need thereof has cancer or an infection.
226. The method of embodiment 225, wherein the cancer includes a hematological malignancy.
227. The method of embodiment 226, wherein the hematological malignancy includes leukemia, lymphoma, or multiple myeloma.
228. The method of embodiment 227, wherein the leukemia includes acute myeloid leukemia (AML).
229. The method of embodiment 225, wherein the cancer includes a solid cancer.
230. The method of embodiment 229, wherein the solid cancer includes lung cancer, squamous cell carcinoma, colorectal cancer, pancreatic cancer, breast cancer, thyroid cancer, bladder cancer, cervical cancer, esophageal cancer, ovarian cancer, gastric cancer, endometrial cancer, or brain cancer.
231. The method of method of embodiment 230, wherein the lung cancer is a non-small cell lung carcinoma.
232. The method of method of embodiment 230, wherein the brain cancer includes gliomas, glioblastomas, or oligodendrogliomas.
233. The method of any of embodiments 224-232, wherein the administering a therapeutically effective amount includes administering intravesically, intravenously, intradermally, intraarterially, intraparenterally, intranodally, intralymphatically, intraperitoneally, intralesionally, intraprostatically, intravaginally, intrarectally, topically, intrathecally, intratumorally, intramuscularly, or subcutaneously.

**[0268]** Although the foregoing embodiments have been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings contemplated herein that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims. The following examples are provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of noncritical parameters that could be changed or modified to yield essentially similar results.

K. Experimental Examples.

**[0269]** Example 1. Generation of Cytokine Expressing Engineered T Cells and Characterization Thereof. Lentiviral vectors including constructs that include a polynucleotide encoding at least a recombinant receptor and/or an IL15 polypeptide were designed, constructed, and verified. In

certain lentiviral vectors the recombinant receptor is a anti-CD33 dimerizing agent-regulated immunoreceptor complex (DARIC33) including an anti-CD33 VHH and signaling components. The anti-CD33 VHH DARIC (DARIC33) containing lentiviral vectors were constructed including a constitutive MNDU3 promoter operably linked to a polynucleotide encoding: a DARIC signaling component (CD8 $\alpha$ -signal peptide, an FRB variant (T82L), a CD8 $\alpha$  transmembrane domain, an intracellular 4-1BB costimulatory domain, and a CD3 zeta signaling domain); a P2A sequence; and a DARIC targeting component (an Igk-signal peptide, a CD33-specific VHH binding domain (camelid or humanized), a G4S linker, an FKBP12 domain, and a CD4 derived transmembrane domain with a truncated intracellular domain. See, e.g., SEQ ID NOs: 1-5. Some lentiviral vectors also included a polynucleotide encoding an IL15 polypeptide operably linked to the polynucleotide encoding the recombinant receptor via a polynucleotide(s) encoding one or more viral 2A self-cleaving polypeptides (e.g., P2A). See FIG. 1.

**[0270]** T cells from 3 donors were transduced with lentiviral vector (LVV) encoding either DARIC33 alone or DARIC33 with various IL15 variants. The transduced T cells were expanded for 10 days with similar growth kinetics between T cells transduced with control or IL15 containing lentiviruses (FIG. 2). Expression of both DARIC33 targeting component (FIGs. 3A and 3B) and DARIC33 signaling component (FIGs. 4A and 4B) was similar between all LVVs, with the exception of T cells transduced with soluble wild-type (WT) IL15, which had reduced expression of DARIC33 components. The viral copy number was within an acceptable range for all DARIC33 variants (FIG. 5). Inclusion of either soluble or membrane bound IL15 had minimal impact on CD4:CD8 ratio (FIG. 6). T cells transduced with IL15 containing vectors had a slight reduction in the central memory T cell compartment, as determined by CD62L staining (FIG. 7). The expression of CD54 is a marker of tonic signaling in LVV transduced T cells and the inclusion of WT, but not sIL-15 variant (D8S mutation) resulted in increased CD54 expression compared to control DARIC33 cells (FIG. 8).

**[0271]** Example 2. IL15 Expressing DARIC33 T Cells Are Functionally Active. Transduced T cells were co-cultured either with media alone or with endogenous CD33+ tumor cell line MV4-11 and A549 cell line engineered to over-express CD33. Cytokine secretion was quantified by Meso Scale Discovery (MSD®, Merck Sharp & Dohme Corp., Rahway, NJ) analysis of cultured supernatants. Minimal IFN $\gamma$  production was observed when untransduced or anti-CD33 dimerizing agent-regulated immunoreceptor complex (DARIC33) T cells were cultured in the absence of tumor. Expression of DARIC33 with soluble wildtype (WT) IL15 resulted in significant IFN $\gamma$  production in the absence of tumor. Neither the D8S sIL15 mutant (IL-15 with D8S mutation) nor the membrane-tethered IL15 variants showed high levels of IFN $\gamma$  production in the absence

of tumor. Dimerization of the DARIC33 signaling complex with rapamycin or the non-immunosuppressive rapalog AP21967 had no impact on IL15 secretion from any of the constructs (FIG. 9).

**[0272]** To analyze functional capacity of IL15 expressing T cells, the DARIC33 T cells were cultured with CD33+ MV4-11 tumor cells at an E:T ratio of 1:1 in the presence or absence of rapamycin or AP21967 rapalog for 24 hours. All constructs had inducible IFN $\gamma$  production in response to tumor co-culture, with similar levels of IFN $\gamma$  secretion for all DARIC33 variants (FIG. 10). Minimal cytokine production was detected in untransduced controls. Similarly, DARIC33 targeting T cells secreted equivalent amounts of IFN $\gamma$  to an A549 cell line engineered to express CD33 (FIG. 11). However, when co-cultured with a control A549 cell line, engineered to over-express B-cell maturation antigen (BCMA), the T cells engineered to express DARIC33 in combination with WT soluble IL15 secreted high levels of IFN $\gamma$  (FIG. 12).

**[0273]** To evaluate DARIC33 cytotoxicity, parental and IL15 expressing DARIC33 T cells were co-cultured with A549 cells engineered to express a fluorescent reporter gene (NLR) as well as CD33 or BCMA as a negative control. Co-culture of DARIC33 cells with A549-CD33 spheroids in the absence of dimerization drug resulted in some cytotoxicity, particularly for T cells expressing the soluble WT IL-15 variant (FIG. 13). Addition of AP21967 produced rapid and similar tumor cell killing for all DARIC33 variants (FIG. 14). Similar to the findings above, co-culture of DARIC33 variants with control A549-BCMA tumor cell line resulted in minimal cell killing in the presence or absence of AP21967 (FIGs. 15 and 16). T cells expressing WT soluble IL15 exhibited antigen-independent cytotoxicity.

**[0274]** Example 3. Generation Of Lentiviral Vectors And Characterization Thereof. Lentiviral vectors including constructs that include a polynucleotide encoding at least a recombinant receptor (e.g., DARIC33) and/or a polynucleotide including an inducible/regulatable promoter (e.g., iSynPro) operably linked to a polynucleotide encoding an sIL15 or sIL15.D8S polypeptide were designed, constructed, and verified. As shown in FIG. 17, the iSynPro-IL15 polypeptides were constructed in either forward or reverse orientations relative to the polynucleotide encoding the recombinant receptor.

**[0275]** T cells were activated, transduced and expanded as described in Example 1. Following a 10-day expansion protocol, transduced T cells were cultured with NLR+ A549 engineered to over-express either B-cell maturation antigen (BCMA) or CD33, in the presence or absence of rapamycin. Activation of iSynPro promoter and secretion of IL-15 was analyzed by enzyme-linked immunosorbent assay (ELISA). In the absence of target, or in the presence of a non-specific BCMA, T cells transduced with constitutively-expressing sIL-15 LVVs secreted IL-15, and IL-15

secretion was not impacted by addition of rapamycin (FIG. 18A and 18B). However, co-culture of DARIC33.iSynPro T cells with CD33+ tumor cells resulted in IL-15 secretion, only in the presence of rapamycin (FIG. 18C). Both Forward and Reverse iSynPro orientations induced IL-15 productions following tumor and rapamycin co-culture.

**[0276]** The functionality of engineered T cells was analyzed by quantifying IFN $\gamma$  secretion in culture supernatant. The engineered T cells were co-cultured with engineered A549 cells and IFN $\gamma$  production was analyzed by MSD. T cells transduced with constitutively expressing soluble WT IL-15 LVV secreted large quantities of IFN $\gamma$  when cultured with control A549-BCMA cell line (FIG. 19A). In contrast, engineered T cells had robust IFN $\gamma$  production when co-cultured with A549-CD33 cell line, in the presence of rapamycin (FIG. 19B).

**[0277]** The T cells engineered to express DARIC33 or DARIC33.iSynPro.IL15/IL-15.D8S were also cultured with AML-derived tumor cell lines and cytokine production analyzed via MSD. Engineered T cells had high IFN $\gamma$  production when co-cultured with HL60 tumor cells in the presence of rapamycin. However, inclusion of either WT or D8S IL-15 mutant led to higher levels of secretion when T cells were co-cultured with CD33<sup>low</sup> OCI-AML tumor line (FIG. 20A). Inclusion of either IL-15 or IL-15(D8S) also led to higher IL-2 secretion when T cells were stimulated with either OCI-AML or HL-60 tumor line (FIG. 20B). Similar to previous observations, T cells cultured without antigen did not produce any IL15. However, DARIC33.iSynPro T cells cultured with HL60 in the presence of rapamycin showed inducible IL15 production (FIG. 20C).

**[0278]** Example 4. Proliferation of Engineered Cells In Vitro. T cells were activated, transduced and expanded as described in Examples 1-3. Following a 10-day expansion protocol, transduced T cells were cultured with CD33+ MV4-11 in the presence of rapamycin in different media. The T cells were counted and media exchanged at 3, 7, 10, and 15 days following activation. While the addition of exogenous IL-2 and IL-15 to the culture led to persistent T cell expansion (FIG. 21A), expression of IL-15 or IL-15(D8S) transgene resulted in higher levels of peak T cell expansion, but similar kinetics of T cell contraction compared to control samples (FIGs. 21B and 21C).

**[0279]** Example 5. Characterization Of Engineered Cells In Vivo. The in vivo activity of IL15 secreting T cells was analyzed using a xenograft tumor model. T cells were activated, transduced and expanded as described in Example 1. Immunodeficient NSG mice were engrafted with CD33+ MV4-11 tumor cells expressing firefly luciferase for in vivo tracking. Similar tumor growth was observed in all animals receiving DARIC33 T cells in the absence of rapamycin (FIG. 22). At the high  $10 \times 10^6$  T cell dose, addition of rapamycin (0.1mg/kg, mwf (Monday, Wednesday, Friday)) resulted in equivalent tumor control in all DARIC33 T cells (FIG. 23). At the low  $3 \times 10^6$  T cell dose,

inclusion of the D8S IL15 transgene resulted in improved tumor control compared to DARIC33 T cells (FIG. 24).

**[0280]** Example 6. Construction of Rapamycin-Inducible Engineered T Cell Receptors (eTCRs). Lentiviral vectors including constructs that encode at least a multimerization domain (e.g., a rapamycin-inducible dimerization domain), a CD3 subunit (e.g., CD3 $\epsilon$ ), and at least one extracellular antigen targeting domain were designed, cloned, and sequence verified. The constructs include or encode various combinations of the following units: a signal sequence (e.g., a CD8 $\alpha$  or IgK derived signal sequence), one or more multimerization domains (e.g., an FK506-binding protein (FKBP12 or FKBP) and an FKBP-rapamycin binding protein (FRB or FRB\*)), a CD3 $\epsilon$  subunit, one or more viral self-cleaving peptides (e.g., P2A or T2A self-cleaving peptides) one or more extracellular antigen targeting domains (e.g., an antibody derived targeting domain or a natural ligand derived targeting domain, one hinge and transmembrane domain (e.g., those derived from CD4) and one secreted cytokine molecule, either expressed behind a traditional promoter (e.g., MND) or a T-cell activation inducible promoter (see FIGs. 25A and 25B).

**[0281]** Example 7. Evaluation of Rapamycin-Inducible eTCR T Cells with IL-15 D8S In Vivo. T cells expressing rapamycin-inducible T cell receptors (TEA-T cells) were generated using a 7-day transduction and expansion process, then evaluated for expression and biological activity against specific target antigens. Briefly, enriched CD4<sup>+</sup> and CD8<sup>+</sup> T cells were cultured in an IL-2 containing media and activated with a formulation of human CD3 and human CD28. Lentiviral vectors encoding rapamycin-inducible eTCRs with CD4 hinge and transmembrane domains anchoring a FKBP12 multimerization domain and two antigen targeting domains with or without IL-15 with the D8S mutation driven by an inducible promoter (TEA<sup>+</sup>/IL-15 D8S) were used to transduce the enriched T cells one day after culture initiation, then cells were transferred to a 1L G-REX® (Wilson Wolf Corporation, St. Paul MN) culture system 24 hours later. After a total of 7 days in culture, TEA-T cells were evaluated for rapamycin-dependent, antigen-dependent activity, as well as rapamycin-independent, antigen-independent background activity.

**[0282]** Female NSG-MHCI/MHCII knockout mice were dosed intravenously with a CD33+CLL1+ MV-411 xenograft tumor cells expressing firefly luciferase. After 8 days of tumor growth, 12x10<sup>6</sup> untransduced or 6x10<sup>6</sup> TEA<sup>+</sup>/IL-15 D8S T cells were administered intravenously without rapamycin or with rapamycin dosed three times per week (FIGs. 26A-26D). Survival was monitored for 70 days (FIG. 27). TEA+IL-15 D8S outperformed TEA (no IL15-D8S) in both overall tumor growth and in percentage of mice surviving to study end.

L. Closing Paragraphs.

**[0283]** The nucleic acid and amino acid sequences provided herein are shown using letter abbreviations for nucleotide bases and amino acid residues, as defined in 37 C.F.R. §1.831-1.835 and set forth in WIPO Standard ST.26 (implemented on July 1, 2022). Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included in embodiments where it would be appropriate.

**[0284]** Variants of the sequences disclosed and referenced herein are also included. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological activity can be found using computer programs well known in the art, such as DNASTAR™ (Madison, Wisconsin) software. Preferably, amino acid changes in the protein variants disclosed herein are conservative amino acid changes, i.e., substitutions of similarly charged or uncharged amino acids. A conservative amino acid change involves substitution of one of a family of amino acids which are related in their side chains.

**[0285]** In a peptide or protein, suitable conservative substitutions of amino acids are known to those of skill in this art and generally can be made without altering a biological activity of a resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson et al. *Molecular Biology of the Gene*, 4th Edition, 1987, The Benjamin/Cummings Pub. Co., p. 224). Naturally occurring amino acids are generally divided into conservative substitution families as follows: Group 1: Alanine (Ala), Glycine (Gly), Serine (Ser), and Threonine (Thr); Group 2: (acidic): Aspartic acid (Asp), and Glutamic acid (Glu); Group 3: (acidic; also classified as polar, negatively charged residues and their amides): Asparagine (Asn), Glutamine (Gln), Asp, and Glu; Group 4: Gln and Asn; Group 5: (basic; also classified as polar, positively charged residues): Arginine (Arg), Lysine (Lys), and Histidine (His); Group 6 (large aliphatic, nonpolar residues): Isoleucine (Ile), Leucine (Leu), Methionine (Met), Valine (Val) and Cysteine (Cys); Group 7 (uncharged polar): Tyrosine (Tyr), Gly, Asn, Gln, Cys, Ser, and Thr; Group 8 (large aromatic residues): Phenylalanine (Phe), Tryptophan (Trp), and Tyr; Group 9 (non-polar): Proline (Pro), Ala, Val, Leu, Ile, Phe, Met, and Trp; Group 11 (aliphatic): Gly, Ala, Val, Leu, and Ile; Group 10 (small aliphatic, nonpolar or slightly polar residues): Ala, Ser, Thr, Pro, and Gly; and Group 12 (sulfur-containing): Met and Cys. Additional information can be found in Creighton (1984) *Proteins*, W.H. Freeman and Company.

**[0286]** In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982, *J. Mol. Biol.* 157(1), 105-32). Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and

charge characteristics (Kyte and Doolittle, 1982). These values are: Ile (+4.5); Val (+4.2); Leu (+3.8); Phe (+2.8); Cys (+2.5); Met (+1.9); Ala (+1.8); Gly (−0.4); Thr (−0.7); Ser (−0.8); Trp (−0.9); Tyr (−1.3); Pro (−1.6); His (−3.2); Glutamate (−3.5); Gln (−3.5); aspartate (−3.5); Asn (−3.5); Lys (−3.9); and Arg (−4.5).

**[0287]** It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, i.e., still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within  $\pm 2$  is preferred, those within  $\pm 1$  are particularly preferred, and those within  $\pm 0.5$  are even more particularly preferred. It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity.

**[0288]** As detailed in US 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: Arg (+3.0); Lys (+3.0); aspartate (+3.0 $\pm$ 1); glutamate (+3.0 $\pm$ 1); Ser (+0.3); Asn (+0.2); Gln (+0.2); Gly (0); Thr (−0.4); Pro (−0.5 $\pm$ 1); Ala (−0.5); His (−0.5); Cys (−1.0); Met (−1.3); Val (−1.5); Leu (−1.8); Ile (−1.8); Tyr (−2.3); Phe (−2.5); Trp (−3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within  $\pm 2$  is preferred, those within  $\pm 1$  are particularly preferred, and those within  $\pm 0.5$  are even more particularly preferred.

**[0289]** As outlined above, amino acid substitutions may be based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. As indicated elsewhere, variants of gene sequences can include codon optimized variants, sequence polymorphisms, splice variants, and/or mutations that do not affect the function of an encoded product to a statistically-significant degree.

**[0290]** Variants of the protein, nucleic acid, and gene sequences disclosed herein also include sequences with at least 70% sequence identity, 80% sequence identity, 85% sequence identity, 90% sequence identity, 95% sequence identity, 96% sequence identity, 97% sequence identity, 98% sequence identity, or 99% sequence identity to the protein, nucleic acid, or gene sequences disclosed herein.

**[0291]** "% sequence identity" refers to a relationship between two or more sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between protein, nucleic acid, or gene sequences as determined by the match between strings of such sequences. "Identity" (often referred to as "similarity") can be readily



calculated by known methods, including those described in: Computational Molecular Biology (Lesk, A. M., ed.) Oxford University Press, NY (1988); Biocomputing: Informatics and Genome Projects (Smith, D. W., ed.) Academic Press, NY (1994); Computer Analysis of Sequence Data, Part I (Griffin, A. M., and Griffin, H. G., eds.) Humana Press, NJ (1994); Sequence Analysis in Molecular Biology (Von Heijne, G., ed.) Academic Press (1987); and Sequence Analysis Primer (Gribkov, M. and Devereux, J., eds.) Oxford University Press, NY (1992). Methods to determine identity are designed to give the best match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Sequence alignments and percent identity calculations may be performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR, Inc., Madison, Wisconsin). Multiple alignment of the sequences can also be performed using the Clustal method of alignment (Higgins and Sharp CABIOS, 5, 151-153 (1989) with default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Relevant programs also include the GCG suite of programs (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wisconsin); BLASTP, BLASTN, BLASTX (Altschul, et al., J. Mol. Biol. 215:403-410 (1990); DNASTAR (DNASTAR, Inc., Madison, Wisconsin); and the FASTA program incorporating the Smith-Waterman algorithm (Pearson, Comput. Methods Genome Res., [Proc. Int. Symp.] (1994), Meeting Date 1992, 111-20. Editor(s): Suhai, Sandor. Publisher: Plenum, New York, N.Y.). Within the context of this disclosure it will be understood that where sequence analysis software is used for analysis, the results of the analysis are based on the "default values" of the program referenced. "default values" mean any set of values or parameters, which originally load with the software when first initialized.

**[0292]** Variants also include nucleic acid molecules that hybridize under stringent hybridization conditions to a sequence disclosed herein and provide the same function as the reference sequence. Exemplary stringent hybridization conditions include an overnight incubation at 42 °C in a solution including 50% formamide, 5XSSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5XDenhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1XSSC at 50 °C. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, moderately high stringency conditions include an overnight incubation at 37°C in a solution including 6XSSPE (20XSSPE=3M NaCl; 0.2M NaH<sub>2</sub>PO<sub>4</sub>; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 µg/ml salmon sperm blocking DNA; followed by washes at 50 °C with 1XSSPE, 0.1% SDS. In addition, to achieve even

lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5XSSC). Variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

**[0293]** "Bind" refers to an association of a binding domain (of, for example, a CAR binding domain) to its cognate binding molecule. "Preferentially binds" refers to an association of a binding domain (of, for example, a recombinant receptor binding domain) to its cognate binding molecule with an affinity or  $K_a$  (i.e., an equilibrium association constant of a particular binding interaction with units of  $1/M$ ) equal to or greater than  $10^5 M^{-1}$ , while not significantly associating with any other molecules or components in a relevant environment sample. Binding domains may be classified as "high affinity" or "low affinity". In particular embodiments, "high affinity" binding domains refer to those binding domains with a  $K_a$  of at least  $10^7 M^{-1}$ , at least  $10^8 M^{-1}$ , at least  $10^9 M^{-1}$ , at least  $10^{10} M^{-1}$ , at least  $10^{11} M^{-1}$ , at least  $10^{12} M^{-1}$ , or at least  $10^{13} M^{-1}$ . In particular embodiments, "low affinity" binding domains refer to those binding domains with a  $K_a$  of up to  $10^7 M^{-1}$ , up to  $10^6 M^{-1}$ , up to  $10^5 M^{-1}$ . Alternatively, affinity may be defined as an equilibrium dissociation constant ( $K_d$ ) of a particular binding interaction with units of  $M$  (e.g.,  $10^{-5} M$  to  $10^{-13} M$ ). In certain embodiments, a binding domain may have "enhanced affinity," which refers to a selected or engineered binding domains with stronger binding to a cognate binding molecule than a wild type (or parent) binding domain. For example, enhanced affinity may be due to a  $K_a$  (equilibrium association constant) for the cognate binding molecule that is higher than the reference binding domain or due to a  $K_d$  (dissociation constant) for the cognate binding molecule that is less than that of the reference binding domain, or due to an off-rate ( $K_{off}$ ) for the cognate binding molecule that is less than that of the reference binding domain. A variety of assays are known for detecting binding domains that preferentially bind a particular cognate binding molecule as well as determining binding affinities, such as Western blot, ELISA, and BIACORE® analysis (see also, e.g., Scatchard, et al., 1949, Ann. N.Y. Acad. Sci. 51:660; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent).

**[0294]** Unless otherwise indicated, the practice of the present disclosure can employ conventional techniques of immunology, molecular biology, microbiology, cell biology and recombinant DNA. These methods are described in the following publications. See, e.g., Sambrook, et al. Molecular Cloning: A Laboratory Manual, 2nd Edition (1989); F. M. Ausubel, et al. eds., Current Protocols in Molecular Biology, (1987); the series Methods IN Enzymology (Academic Press, Inc.); M.

MacPherson, et al., PCR: A Practical Approach, IRL Press at Oxford University Press (1991); MacPherson et al., eds. PCR 2: Practical Approach, (1995); Harlow and Lane, eds. Antibodies, A Laboratory Manual, (1988); and R. I. Freshney, ed. Animal Cell Culture (1987).

**[0295]** As will be understood by one of ordinary skill in the art, each embodiment disclosed herein can comprise, consist essentially of or consist of its particular stated element, step, ingredient or component. "Include" or "including" should be interpreted to recite: "comprise, consist of, or consist essentially of." The transition term "comprise" or "comprises" means has, but is not limited to, and allows for the inclusion of unspecified elements, steps, ingredients, or components, even in major amounts. The transitional phrase "consisting of" excludes any element, step, ingredient or component not specified. The transition phrase "consisting essentially of" limits the scope of the embodiment to the specified elements, steps, ingredients or components and to those that do not materially affect the embodiment. A material effect would cause a statistically significant decrease in immune cell function, as described herein.

**[0296]** Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. When further clarity is required, "about" has the meaning reasonably ascribed to it by a person skilled in the art when used in conjunction with a stated numerical value or range, i.e. denoting somewhat more or somewhat less than the stated value or range, to within a range of  $\pm 20\%$  of the stated value;  $\pm 19\%$  of the stated value;  $\pm 18\%$  of the stated value;  $\pm 17\%$  of the stated value;  $\pm 16\%$  of the stated value;  $\pm 15\%$  of the stated value;  $\pm 14\%$  of the stated value;  $\pm 13\%$  of the stated value;  $\pm 12\%$  of the stated value;  $\pm 11\%$  of the stated value;  $\pm 10\%$  of the stated value;  $\pm 9\%$  of the stated value;  $\pm 8\%$  of the stated value;  $\pm 7\%$  of the stated value;  $\pm 6\%$  of the stated value;  $\pm 5\%$  of the stated value;  $\pm 4\%$  of the stated value;  $\pm 3\%$  of the stated value;  $\pm 2\%$  of the stated value; or  $\pm 1\%$  of the stated value.

**[0297]** Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain

errors necessarily resulting from the standard deviation found in their respective testing measurements.

**[0298]** Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other members of the group or other elements found herein. It is anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

**[0299]** Certain embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Of course, variations on these described embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventor expects skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

**[0300]** Furthermore, numerous references have been made to patents, printed publications, journal articles and other written text throughout this specification (referenced materials herein). Each of the referenced materials are individually incorporated herein by reference in their entirety for their referenced teaching.

**[0301]** In closing, it is to be understood that the embodiments of the invention disclosed herein are illustrative of the principles of the present invention. Other modifications that may be employed are within the scope of the invention. Thus, by way of example, but not of limitation, alternative configurations of the present invention may be utilized in accordance with the teachings herein. Accordingly, the present invention is not limited to that precisely as shown and described.

**[0302]** The particulars shown herein are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of various embodiments of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for the fundamental understanding of the invention, the description taken with the drawings and/or

examples making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

**[0303]** Definitions and explanations used in the present disclosure are meant and intended to be controlling in any future construction unless clearly and unambiguously modified in the examples or when application of the meaning renders any construction meaningless or essentially meaningless. In cases where the construction of the term would render it meaningless or essentially meaningless, the definition should be taken from Webster's Dictionary, 3rd Edition or a dictionary known to those of ordinary skill in the art, such as the Oxford Dictionary of Biochemistry and Molecular Biology (Eds. Attwood T et al., Oxford University Press, Oxford, 2006).

**[0304]** In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

## CLAIMS

What is claimed is:

1. A method of improving function of an adoptive cell therapy (ACT) comprising: transducing an immune effector cell or population of immune effector cells with a polynucleotide encoding a mutated interleukin 15 (IL-15) and a recombinant receptor, wherein the sequence encoding the mutated IL-15 is under the regulatory control of a promoter having the sequence as set forth in SEQ ID NO: 15.
2. A method of improving persistence (or function) of an adoptive cell therapy (ACT) comprising: transducing an immune effector cell or population of immune effector cells with a polynucleotide encoding an exogenous mutant IL-15 polypeptide, wherein the exogenous mutant IL-15 polypeptide binds to an IL15 receptor complex comprising IL-15R $\alpha$ , compared to an IL15 receptor complex without IL-15R $\alpha$ .
3. The method of claim 2, wherein the exogenous mutant IL-15 polypeptide has lower affinity to complexes including IL2R $\beta$  and common gamma receptor without IL-15R $\alpha$  as compared to IL2R $\beta$  and common gamma receptor with IL-15R $\alpha$ .
4. The artificial expression construct of claim 2, wherein the exogenous mutant IL-15 polypeptide has lower affinity to the common gamma receptor and/or atypical binding to IL2R $\beta$  as compared to wild type IL-15 as compared to wild-type IL-15.
5. The method of claim 2, wherein the mutated IL-15 is unable to bind the common gamma receptor.
6. The method of claim 2, wherein the mutant IL-15 polypeptide comprises a D to S mutation at position 8 compared to a wild-type IL-15.
7. The method of claim 6, wherein the wild-type IL-15 comprises the sequence as set forth in SEQ ID NO: 8 or SEQ ID NO: 41.
8. The method of claim 6, wherein the wild-type IL-15 is encoded by the sequence as set forth in SEQ ID NO: 10 or SEQ ID NO: 43.
9. The method of claim 2, wherein the mutant IL-15 polypeptide comprises an amino acid sequence as set forth in SEQ ID NO: 9 or SEQ ID NO: 42; or a sequence having at least 90%, 95%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 9 or SEQ ID NO: 42.
10. The method of claim 2, wherein the mutant IL-15 polypeptide is encoded by the sequence as set forth in SEQ ID NO: 11 or SEQ ID NO: 44; or a sequence having at least 90%, 95%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 11 or SEQ ID NO:

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11. The method of claim 2, wherein the method results in increased persistence and improved function.
12. The method of claim 11, wherein the persistence is increased compared to an immune effector cell or population of immune effector cells transduced with an exogenous wild-type IL-15 polypeptide.
13. The method of claim 11, wherein the improved function comprises decreased antigen-independent IFN $\gamma$  release compared to an immune effector cell or population of immune effector cells comprising a polynucleotide encoding an exogenous wildtype IL-15 polypeptide.
14. The method of claim 11, wherein the improved function comprises increased immune cell proliferation compared to an immune effector cell comprising a polynucleotide encoding an exogenous wildtype IL-15 polypeptide.
15. The method of claim 11, wherein the immune effector cell or population of immune effector cells comprise a recombinant receptor or exogenous lymphocyte receptor.
16. The method of claim 15, wherein the recombinant receptor comprises a chimeric antigen receptor (CAR), an engineered T cell receptor (eTCR), a dimerizing agent-regulated immunoreceptor complex (DARIC), or a hybrid thereof.
17. The method of claim 15, wherein the exogenous lymphocyte receptor comprises a T cell receptor (TCR) or a B cell receptor (BCR).
18. The method of claim 15, wherein the recombinant receptor binds to a target antigen.
19. The method of claim 18, wherein the target antigen comprises CD33, CLL1, CD19, CD20, CD22, EGFR, EphA2, Her2, IL13Ra2, ROR1, CD133, mesothelin, CD123, or L1-CAM.
20. The method of claim 16, wherein the DARIC comprises a signaling component comprising an amino acid sequence as set forth in SEQ ID NO: 1 or SEQ ID NO: 2; or a sequence having at least 95%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 1 or SEQ ID NO: 2.
21. The method of claim 16, wherein the DARIC comprises a targeting component comprising an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 4; or a sequence having at least 95%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 4.
22. The method of claim 16, wherein the DARIC comprises an amino acid sequence as set forth in SEQ ID NO: 5; or a sequence having at least 95%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 5.
23. The method of claim 16, wherein the recombinant receptor comprises:

(a) a signaling component comprising (i) a first multimerization domain, (ii) a first linker, and (iii) an intracellular component; and

(b) a targeting component comprising (i) a binding domain, (ii) a second linker, (iii) a second multimerization domain, and (iv) a transmembrane domain.

24. The method of claim 16, wherein the recombinant receptor comprises:

(a) a signaling component comprising (i) a first multimerization domain comprising an FRB polypeptide or a FKBP polypeptide, (ii) a first linker, and (iii) a CD3 $\epsilon$ ; and

(b) a targeting component comprising (i) an anti-CLL1 binding domain, (ii) an anti-CD33 binding domain, (iii) a second linker, (iv) a second multimerization domain comprising an FRB polypeptide or a FKBP, (v) a spacer, and (vi) a transmembrane domain.

25. The method of claim 16, wherein the recombinant receptor comprises:

(a) a signaling component comprising (i) a first multimerization domain comprising an FRB polypeptide or a FKBP polypeptide, (ii) a first linker, and (iii) a CD3 $\epsilon$ ; and

(b) a targeting component comprising (i) an anti-CLL1 binding domain, (ii) an anti-CD33 binding domain, (iii) a second linker, (iv) a second multimerization domain comprising an FRB polypeptide or a FKBP polypeptide, (v) a CD4 hinge region, (vi) a CD4 transmembrane domain, and (vii) a truncated CD4 intracellular polypeptide.

26. The method of claim 23, wherein the targeting component does not comprise a functional intracellular domain or costimulatory domain having signaling capabilities.

27. The method of claim 25, wherein the CD4 hinge region comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 84.

28. The method of claim 25, wherein the CD4 hinge region comprises the amino acid sequence as set forth in SEQ ID NO: 84.

29. The method of claim 24, wherein the CD3 $\epsilon$  comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 85.

30. The method of claim 24, wherein the CD3 $\epsilon$  comprises the amino acid sequence as set forth in SEQ ID NO: 85.



31. The method of claim 24, wherein the CD3 $\epsilon$  comprises both extracellular and intracellular portions of the CD3 $\epsilon$ .
32. The method of claim 24, wherein the FRB polypeptide and FKBP polypeptide localize extracellularly when the signaling and targeting components are expressed.
33. The method of claim 23, wherein the first multimerization domain and the second multimerization domain are different.
34. The method of claim 23, wherein the first multimerization domain comprises an FRB polypeptide, and the second multimerization domain comprises an FKBP polypeptide.
35. The method of claim 23, wherein the first multimerization domain comprises an FKBP polypeptide, and the second multimerization domain comprises an FRB polypeptide.
36. The method of claim 24, wherein the FRB polypeptide comprises an FRB T2098L variant.
37. The method of claim 24, wherein the FRB polypeptide comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, 99%, sequence identity to, or comprising a sequence as set forth in SEQ ID NO: 86.
38. The method of claim 24, wherein the FRB polypeptide comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, 99%, sequence identity to, or comprising a sequence as set forth in SEQ ID NO: 87.
39. The method of claim 24, wherein the FKBP polypeptide comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, 99%, sequence identity to, or comprising a sequence as set forth in SEQ ID NO: 88.
40. The method of claim 24, wherein the FKBP polypeptide comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, 99%, sequence identity to, or comprising a sequence as set forth in SEQ ID NO: 89.
41. The method of claim 23, wherein the first multimerization domain and second multimerization domain associate with a drug.
42. The method of claim 41, wherein the drug comprises a rapamycin or a rapalog thereof.
43. The method of claim 41, wherein the drug is AP1903, AP20187, AP21967 (also known as C16-(S)-7-methylindolerapamycin), everolimus, novolimus, pimecrolimus, ridaforolimus, sirolimus, tacrolimus, temsirolimus, umirolimus, zotarolimus, or BPC015.
44. The method of claim 23, wherein the first linker is a linker of 2 to 40 amino acids in length.

45. The method of claim 44, wherein the first linker is selected from the group consisting of: GG, GS, SG, SS, GSS, SSG, GSG, SGS, SGG, G4S, 2xG4S, 3xG4S, 4xG4S, 5xG4S, and any combination thereof.
46. The method of claim 44, wherein the first linker is a 3xG4S linker.
47. The method of claim 23, wherein the second linker is a linker of 2 to 40 amino acids in length.
48. The method of claim 47, wherein the second linker is selected from the group consisting of: GG, GS, SG, SS, GSS, SSG, GSG, SGS, SGG, G4S, 2x G4S, 3xG4S, 4xG4S, and any combination thereof.
49. The method of claim 47, wherein the second linker is a G4S linker.
50. The method of claim 25, wherein the CD4 transmembrane domain comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 90.
51. The method of claim 25, wherein the CD4 transmembrane domain comprises the amino acid sequence as set forth in SEQ ID NO: 90.
52. The method of claim 25, wherein the truncated intracellular CD4 polypeptide comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 91 or SEQ ID NO: 92.
53. The method of claim 25, wherein the truncated intracellular CD4 polypeptide comprises the amino acid sequence as set forth in SEQ ID NO: 91 or SEQ ID NO: 92.
54. The method of claim 24, wherein the anti-CLL1 binding domain comprises a single domain antibody (sdAb) or a single chain variable fragment (scFv).
55. The method of claim 54, wherein the sdAb is a VHH or heavy chain-only antibody (HcAb).
56. The method of claim 54, wherein the sdAb is a camelid VHH.
57. The method of claim 54, wherein the scFv or sdAb is human or humanized.
58. The method of claim 54, wherein the anti-CLL1 binding domain comprises a complementarity determining region (CDR)1 comprising the sequence as set forth in SEQ ID NO: 97, a CDR2 comprising the sequence as set forth in SEQ ID NO: 98, and a CDR3 comprising the sequence as set forth in SEQ ID NO: 99.

59. The method of claim 54, wherein the anti-CLL1 binding domain comprises a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 100.
60. The method of claim 54, wherein the anti-CLL1 binding domain comprises the sequence as set forth in SEQ ID NO: 100.
61. The method of claim 24, wherein the anti-CD33 binding domain comprises an sdAb or scFv.
62. The method of claim 61, wherein the sdAb is a VHH or heavy chain-only antibody (HcAb).
63. The method of claim 61, wherein the sdAb is a camelid VHH.
64. The method of claim 61, wherein the scFv or sdAb is human or humanized.
65. The method of claim 61, wherein the anti-CD33 binding domain comprises a CDR1 comprising the sequence as set forth in SEQ ID NO: 93, a CDR2 comprising the sequence as set forth in SEQ ID NO: 94, and a CDR3 comprising the sequence as set forth in SEQ ID NO: 95.
66. The method of claim 61, wherein the anti-CD33 binding domain comprises a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 96.
67. The method of claim 61, wherein the anti-CD33 binding domain comprises the sequence as set forth in SEQ ID NO: 96.
68. The method of claim 23, wherein the signaling component further comprises a signal sequence.
69. The method of claim 68, wherein the signal sequence is a CD8 signal sequence.
70. The method of claim 69, wherein the CD8 signal sequence comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 101.
71. The method of claim 69, wherein the CD8 signal sequence comprises the amino acid sequence as set forth in SEQ ID NO: 101.
72. The method of claim 23, wherein the targeting component further comprises a signal sequence.
73. The method of claim 72, wherein the signal sequence is an IgK signal sequence.

74. The method of claim 73, wherein the IgK signal sequence comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 102.
75. The method of claim 73, wherein the IgK signal sequence comprises the amino acid sequence as set forth in SEQ ID NO: 102.
76. The method of claim 23, wherein the signaling component comprises a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 103.
77. The method of claim 23, wherein the signaling component comprises the sequence as set forth in SEQ ID NO: 103.
78. The method of claim 23, wherein the targeting component comprises a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 104.
79. The method of claim 23, wherein the targeting component comprises the sequence as set forth in SEQ ID NO: 104.
80. The method of claim 23, wherein the recombinant receptor comprises a fusion polypeptide which comprises the targeting component and the signaling component of the recombinant receptor.
81. The method of claim 80, wherein the fusion polypeptide comprises a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 105.
82. The method of claim 80, wherein the fusion polypeptide comprises the sequence as set forth in SEQ ID NO: 105.
83. The method of claim 2, wherein the immune effector cell or population of immune effector cells comprises:
- a) a T cell, an  $\alpha\beta$  T cell, or a  $\gamma\delta$  T cell;
  - b) a CD3+, CD4+, and/or CD8+ cell;
  - c) a cytotoxic T lymphocyte (CTL), a tumor infiltrating lymphocyte (TIL), or a helper T cell; or
  - d) a natural killer (NK) cell or natural killer T (NKT) cell.

84. The method of claim 2, wherein the polynucleotide comprises an exogenous promoter operably linked to the polynucleotide encoding the exogenous mutant IL-15 polypeptide.
85. The method of claim 84, wherein the exogenous promoter is a constitutive promoter.
86. The method of claim 85, wherein the constitutive promoter is selected from the group consisting of: a cytomegalovirus immediate early gene promoter (CMV); an elongation factor 1 alpha promoter (EF1- $\alpha$ ); a phosphoglycerate kinase-1 promoter (PGK); a ubiquitin-C promoter (UBQ-C); a cytomegalovirus enhancer/chicken beta-actin promoter (CAG); polyoma enhancer/herpes simplex thymidine kinase promoter (MC1); a beta actin promoter ( $\beta$ -ACT); a simian virus 40 promoter (SV40); a myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer-binding site substituted (MND) U3 promoter; a mouse mammary tumor virus (MMTV) promoter; a human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter; a MoMuLV promoter; an avian leukemia virus promoter; an Epstein-Barr virus immediate early promoter; a Rous sarcoma virus promoter; an actin promoter; a myosin promoter; a hemoglobin promoter; and a creatine kinase promoter.
87. The method of claim 85, wherein the constitutive promoter comprises an MNDU3 promoter or a EF1- $\alpha$  promoter.
88. The method of claim 87, wherein the EF1- $\alpha$  promoter comprises the first intron of a human EF1- $\alpha$  gene.
89. The method of claim 87, wherein the EF1- $\alpha$  promoter lacks the first intron of a human EF1- $\alpha$  gene.
90. The method of claim 85, wherein the constitutive promoter comprises the nucleotide sequence as set forth in any of SEQ ID NOs: 12, 13, or 14.
91. The method of claim 84, wherein the exogenous promoter is an inducible promoter.
92. The method of claim 91, wherein the inducible promoter is an iSynPro promoter.
93. The method of claim 92, wherein the iSynPro promoter comprises the sequence as set forth in SEQ ID NO: 15 or a sequence having at least 95% sequence identity to the sequence as set forth in SEQ ID NO: 15.
94. The method of claim 92, wherein the iSynPro promoter comprises a minimal promoter operably linked to a sequence having at least 95% sequence identity to the sequence as set forth in any of SEQ ID Nos: 45-83.
95. The method of claims 92, wherein the iSynPro promoter comprises a minimal promoter operably linked to a sequence as set forth in any of SEQ ID Nos: 45-83.
96. An artificial expression construct comprising a sequence encoding a mutated interleukin 15 (IL-15), wherein the sequence encoding the mutated IL-15 is under the regulatory control of a

- promoter comprising i) a minimal promoter operably linked to a sequence having at least 95% sequence identity to the sequence as set forth in any of SEQ ID Nos: 45-83 or ii) a sequence having at least 95% sequence identity to the sequence as set forth in SEQ ID NO: 15.
97. The artificial expression construct of claim 96, wherein the promoter has at least 98% sequence identity to the sequence as set forth in SEQ ID NO: 15.
98. The artificial expression construct of claim 96, wherein the promoter has at least 99% sequence identity to the sequence as set forth in SEQ ID NO: 15.
99. The artificial expression construct of claim 96, wherein the promoter has the sequence as set forth in SEQ ID NO: 15.
100. The artificial expression construct of claim 96, wherein promoter comprises the minimal promoter operably linked to a sequence having at least 98% or at least 99% sequence identity to the sequence as set forth in any of SEQ ID Nos: 45-83.
101. The artificial expression construct of claim 96, wherein promoter comprises the minimal promoter operably linked to the sequence as set forth in any of SEQ ID Nos: 45-83.
102. The artificial expression construct of claim 96, wherein the minimal promoter is an IL2 minimal promoter.
103. The artificial expression construct of claim 102, wherein the IL2 minimal promoter comprises the sequence as set forth in SEQ ID NO: 107 or a sequence having at least 95% sequence identity to the sequence as set forth in SEQ ID NO: 107.
104. The artificial expression construct of claim 96, wherein the mutated IL-15 binds to an IL15 receptor complex comprising IL-15R $\alpha$ .
105. The artificial expression construct of claim 96, wherein the mutated IL-15 lower affinity to complexes including IL2R $\beta$  and common gamma receptor without IL-15R $\alpha$  as compared to IL2R $\beta$  and common gamma receptor with IL-15R $\alpha$ .
106. The artificial expression construct of claim 96, lower affinity to the common gamma receptor and/or atypical binding to IL2R $\beta$  as compared to wild-type IL-15.
107. The artificial expression construct of claim 96, wherein the mutated IL-15 is unable to bind the common gamma receptor.
108. The artificial expression construct of claim 96, wherein the mutated IL-15 comprises a D to S mutation at position 8 compared to a wild-type IL-15.
109. The artificial expression construct of claim 108, wherein the wild-type IL-15 comprises the sequence as set forth in SEQ ID NO: 8 or SEQ ID NO: 41.
110. The artificial expression construct of claim 108, wherein the wild-type IL-15 is encoded by the sequence as set forth in SEQ ID NO: 10 or SEQ ID NO: 43.

111. The artificial expression construct of claim 96, wherein the mutated IL-15 comprises the sequence as set forth in SEQ ID NO: 9 or SEQ ID NO: 42; or a sequence having at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to the sequence set forth SEQ ID NO: 9 or SEQ ID NO: 42.
112. The artificial expression construct of claim 96, wherein the mutated IL-15 is encoded by the sequence as set forth in SEQ ID NO: 11 or SEQ ID NO: 44; or a sequence having at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to the sequence set forth in SEQ ID NO: 11 or SEQ ID NO: 44.
113. The artificial expression construct of claim 96, wherein the artificial expression construct comprises a sequence having at least 95% sequence identity to the sequence as set forth in SEQ ID NO: 19 (iSynPro.IL15.D8S.Fwd).
114. The artificial expression construct of claim 96, wherein the artificial expression construct comprises a sequence having at least 98% sequence identity to the sequence as set forth in SEQ ID NO: 19 (iSynPro.IL15.D8S.Fwd).
115. The artificial expression construct of claim 96, wherein the artificial expression construct comprises a sequence having at least 99% sequence identity to the sequence as set forth in SEQ ID NO: 19 (iSynPro.IL15.D8S.Fwd).
116. The artificial expression construct of claim 96, wherein the artificial expression construct comprises the sequence as set forth in SEQ ID NO: 19 (iSynPro.IL15.D8S.Fwd).
117. The artificial expression construct of claim 96, further comprising a sequence encoding a recombinant receptor or exogenous lymphocyte receptor, wherein the recombinant receptor or exogenous lymphocyte receptor comprise a binding domain that binds an antigen expressed on a surface of targeted cells.
118. The artificial expression construct of claim 117, wherein the recombinant receptor comprises a chimeric antigen receptor (CAR), an engineered TCR (eTCR), Dimerizing Agent-Regulated Immunoreceptor Complex (DARIC), or a hybrid thereof.
119. The artificial expression construct of claim 117, wherein the exogenous lymphocyte receptor comprises a T cell receptor (TCR) or a B cell receptor (BCR).
120. The artificial expression construct of claim 117, wherein the sequence encoding the recombinant receptor or exogenous lymphocyte receptor is operably linked to a second promoter.
121. The artificial expression construct of claim 120, wherein the second promoter is selected from the group consisting of: a cytomegalovirus immediate early gene promoter (CMV); an elongation factor 1 alpha promoter (EF1- $\alpha$ ); a phosphoglycerate kinase-1 promoter (PGK); a

ubiquitin-C promoter (UBQ-C); a cytomegalovirus enhancer/chicken beta-actin promoter (CAG); polyoma enhancer/herpes simplex thymidine kinase promoter (MC1); a beta actin promoter ( $\beta$ -ACT); a simian virus 40 promoter (SV40); a myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer-binding site substituted (MND) U3 promoter; a mouse mammary tumor virus (MMTV) promoter; a human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter; a MoMuLV promoter; an avian leukemia virus promoter; an Epstein-Barr virus immediate early promoter; a Rous sarcoma virus promoter; an actin promoter; a myosin promoter; a hemoglobin promoter; and a creatine kinase promoter.

122. The artificial expression construct of claim 120, wherein the second promoter comprises an MNDU3 promoter or an EF1- $\alpha$  promoter.
123. The artificial expression construct of claim 121, wherein the EF1- $\alpha$  promoter comprises the first intron of a human EF1- $\alpha$  gene.
124. The artificial expression construct of claim 121, wherein the EF1- $\alpha$  promoter lacks the first intron of a human EF1- $\alpha$  gene.
125. The artificial expression construct of claim 117, wherein the binding domain is part of an extracellular component.
126. The artificial expression construct of claim 117, wherein the binding domain comprises an anti-CD33 binding domain or an anti-CLL1 binding domain.
127. The artificial expression construct of claim 117, wherein the targeted cells comprise cancer cells or cells infected with bacteria, virus, fungi, parasites, or arthropods.
128. The artificial expression construct of claim 117, wherein the recombinant receptor further comprises an intracellular component.
129. The artificial expression construct of claim 128, wherein the intracellular component comprises a CD3 $\zeta$  signaling domain and/or a 4-1BB signaling domain.
130. The artificial expression construct of claim 128, wherein the intracellular component is linked to the extracellular component through a transmembrane domain.
131. The artificial expression construct of claim 130, wherein the transmembrane domain comprises a CD8 $\alpha$  transmembrane domain, a CD4 transmembrane domain, or a CD28 transmembrane domain.
132. The artificial expression construct of claim 117, wherein the recombinant receptor further comprises a multimerization domain.
133. The artificial expression construct of claim 117, wherein the recombinant receptor multimerizes upon administration of a drug.



134. The artificial expression construct of claim 133, wherein the drug comprises rapamycin or a rapalog thereof.
135. The artificial expression construct of claim 133, wherein the drug comprises AP1903, AP20187, AP21967 (also known as C16-(S)-7-methylindolerapamycin), everolimus, novolimus, pimecrolimus, ridaforolimus, sirolimus, tacrolimus, temsirolimus, umirolimus, zotarolimus, or BPC015.
136. The artificial expression construct of claim 132, wherein the multimerization domain comprises an FK506 binding protein (FKBP) multimerization domain or a variant thereof, and an FKBP-rapamycin binding (FRB) multimerization domain or a variant thereof.
137. The artificial expression construct of claim 118, wherein the DARIC comprises a signaling component comprising an amino acid sequence as set forth in SEQ ID NOs: 1 or 2; or a sequence having at least 95%, at least 98%, or at least 99% sequence identity to the sequence as set forth in SEQ ID NOs: 1 or 2.
138. The artificial expression construct of claim 118, wherein the DARIC comprises a targeting component comprising an amino acid sequence as set forth in SEQ ID NOs: 3 or 4; or a sequence having at least 95%, at least 98%, or at least 99% sequence identity to the sequence as set forth in SEQ ID NOs: 3 or 4.
139. The artificial expression construct of claim 118, wherein the DARIC comprises an amino acid sequence as set forth in SEQ ID NO: 5; or a sequence having at least 95%, at least 98%, or at least 99% sequence identity to the sequence as set forth in SEQ ID NO: 5.
140. The artificial expression construct of claim 118, wherein the recombinant receptor comprises:
- (a) a signaling component comprising (i) a first multimerization domain, (ii) a first linker, and (iii) an intracellular component; and
  - (b) a targeting component comprising (i) a binding domain, (ii) a second linker, (iii) a second multimerization domain, and (iv) a transmembrane domain.
141. The artificial expression construct of claim 118, wherein the recombinant receptor comprises:
- (a) a signaling component comprising (i) a first multimerization domain comprising an FRB polypeptide or a FKBP polypeptide, (ii) a first linker, and (iii) a CD3 $\epsilon$ ; and

(b) a targeting component comprising (i) an anti-CLL1 binding domain, (ii) an anti-CD33 binding domain, (iii) a second linker, (iv) a second multimerization domain comprising an FRB polypeptide or a FKBP polypeptide, (v) a spacer, and (vi) a transmembrane domain.

142. The artificial expression construct of claim 118, wherein the recombinant receptor comprises:

(a) a signaling component comprising (i) a first multimerization domain comprising an FRB polypeptide or a FKBP polypeptide, (ii) a first linker, and (iii) a CD3 $\epsilon$ ; and

(b) a targeting component comprising (i) an anti-CLL1 binding domain, (ii) an anti-CD33 binding domain, (iii) a second linker, (iv) a second multimerization domain comprising an FRB polypeptide or a FKBP polypeptide, (v) a spacer comprising a CD4 hinge region, (vi) a CD4 transmembrane domain, and (vii) a truncated CD4 intracellular polypeptide.

143. The artificial expression construct of claim 140, wherein the targeting component does not comprise a functional intracellular domain or costimulatory domain having signaling capabilities.

144. The artificial expression construct of claim 142, wherein the CD4 hinge region comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 84.

145. The artificial expression construct of claim 142, wherein the CD4 hinge region comprises an amino acid sequence as set forth in SEQ ID NO: 84.

146. The artificial expression construct of claim 141, wherein the CD3 $\epsilon$  comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 85.

147. The artificial expression construct of claim 141, wherein the CD3 $\epsilon$  comprises an amino acid sequence as set forth in SEQ ID NO: 85.

148. The artificial expression construct of claim 141, wherein the CD3 $\epsilon$  comprises both extracellular and intracellular portions of CD3 $\epsilon$ .

149. The artificial expression construct of claim 141, wherein the FRB polypeptide and FKBP polypeptide localize extracellularly when the signaling and targeting components are expressed.

150. The artificial expression construct of claim 140, wherein the first multimerization domain and second multimerization domain are different.
151. The artificial expression construct of claim 140, wherein the first multimerization domain comprises an FRB polypeptide, and the second multimerization domain comprises an FKBP polypeptide.
152. The artificial expression construct of claim 140, wherein the first multimerization domain comprises an FKBP polypeptide, and the second multimerization domain comprises an FRB polypeptide.
153. The artificial expression construct of claim 141, wherein the FRB polypeptide comprises an FRB T2098L variant.
154. The artificial expression construct of claim 141, wherein the FRB polypeptide comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, 99%, sequence identity to, or comprising a sequence as set forth in SEQ ID NO: 86.
155. The artificial expression construct of claim 141, wherein the FRB polypeptide comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, 99%, sequence identity to, or comprising a sequence as set forth in SEQ ID NO: 87.
156. The artificial expression construct of claim 141, wherein the FKBP polypeptide comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, 99%, sequence identity to, or comprising a sequence as set forth in SEQ ID NO: 88.
157. The artificial expression construct of claim 141, wherein the FKBP polypeptide comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, 99%, sequence identity to, or comprising a sequence as set forth in SEQ ID NO: 89.
158. The artificial expression construct of claim 140, wherein the first multimerization domain and the second multimerization domain associate with a drug.
159. The artificial expression construct of claim 158, wherein the drug comprises a rapamycin or a rapalog thereof.
160. The artificial expression construct of claim 158, wherein the drug is AP1903, AP20187, AP21967 (also known as C16-(S)-7-methylindolerapamycin), everolimus, novolimus, pimecrolimus, ridaforolimus, sirolimus, tacrolimus, temsirolimus, umirolimus, zotarolimus, or BPC015.

161. The artificial expression construct of claim 140, wherein the first linker is a linker of 2 to 40 amino acids in length.
162. The artificial expression construct of claim 161, wherein the first linker is selected from the group consisting: GG, GS, SG, SS, GSS, SSG, GSG, SGS, SGG, G4S, 2xG4S, 3xG4S, 4xG4S, 5xG4S, and any combination thereof.
163. The artificial expression construct of claim 161, wherein the first linker is a 3xG4S linker.
164. The artificial expression construct of claim 140, wherein the second linker is a linker of 2 to 40 amino acids in length.
165. The artificial expression construct of claim 164, wherein the second linker is selected from the group consisting of: GG, GS, SG, SS, GSS, SSG, GSG, SGS, SGG, G4S, 2xG4S, 3xG4S, 4xG4S, and any combination thereof.
166. The artificial expression construct of claim 164, wherein the second linker is a G4S linker.
167. The artificial expression construct of claim 142, wherein the CD4 transmembrane domain comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 90.
168. The artificial expression construct of claim 142, wherein the CD4 transmembrane domain comprises an amino acid sequence as set forth in SEQ ID NO: 90.
169. The artificial expression construct of claim 142, wherein the truncated intracellular CD4 polypeptide comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 91 or SEQ ID NO: 92.
170. The artificial expression construct of claim 142, wherein the truncated intracellular CD4 polypeptide comprises an amino acid sequence as set forth in SEQ ID NO: 91 or SEQ ID NO: 92.
171. The artificial expression construct of claim 141, wherein the anti-CLL1 binding domain comprises a single domain antibody (sdAb) or single chain variable fragment (scFv).
172. The artificial expression construct of claim 171, wherein the sdAb is a VHH or heavy chain-only antibody (HcAb).
173. The artificial expression construct of claim 171, wherein the sdAb is a camelid VHH.
174. The artificial expression construct of claim 171, wherein the scFv or sdAb is human or humanized.

175. The artificial expression construct of claim 141, wherein the anti-CLL1 binding domain comprises a complementarity determining region (CDR)1 comprising the sequence as set forth in SEQ ID NO: 97, a CDR2 comprising the sequence as set forth in SEQ ID NO: 98, and a CDR3 comprising the sequence as set forth in SEQ ID NO: 99.
176. The artificial expression construct of claim 141, wherein the anti-CLL1 binding domain comprises a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 100.
177. The artificial expression construct of claim 141, wherein the anti-CLL1 binding domain comprises the sequence as set forth in SEQ ID NO: 100.
178. The artificial expression construct of claim 141, wherein the anti-CD33 binding domain comprises an sdAb or scFv.
179. The artificial expression construct of claim 178, wherein the sdAb is a VHH or heavy chain-only antibody (HcAb).
180. The artificial expression construct of claim 178, wherein the sdAb is a camelid VHH.
181. The artificial expression construct of claim 178, wherein the scFv or sdAb is human or humanized.
182. The artificial expression construct of claim 141, wherein the anti-CD33 binding domain comprises a CDR1 comprising the sequence as set forth in SEQ ID NO: 93, a CDR2 comprising the sequence as set forth in SEQ ID NO: 94, and a CDR3 comprising the sequence as set forth in SEQ ID NO: 95.
183. The artificial expression construct of claim 141, wherein the anti-CD33 binding domain comprises a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 96.
184. The artificial expression construct of claim 141, wherein the anti-CD33 binding domain comprises the sequence as set forth SEQ ID NO:96.
185. The artificial expression construct of claim 140, wherein the signaling component further comprises a signal sequence.
186. The artificial expression construct of claim 185, wherein the signal sequence is a CD8 signal sequence.

187. The artificial expression construct of claim 186, wherein the CD8 signal sequence comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 101.
188. The artificial expression construct of claim 186, wherein the CD8 signal sequence comprises the amino acid sequence as set forth in SEQ ID NO: 101.
189. The artificial expression construct of claim 140, wherein the targeting component further comprises a signal sequence.
190. The artificial expression construct of claim 189, wherein the signal sequence is an IgK signal sequence.
191. The artificial expression construct of claim 190, wherein the IgK signal sequence comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 102.
192. The artificial expression construct of claim 190, wherein the IgK signal sequence comprises an amino acid sequence as set forth in SEQ ID NO: 102.
193. The artificial expression construct of claim 140, wherein the signaling component comprises a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 103.
194. The artificial expression construct of claim 140, wherein the signaling component comprises the sequence as set forth in SEQ ID NO: 103.
195. The artificial expression construct of claim 140, wherein the targeting component comprises a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 104.
196. The artificial expression construct of claim 140, wherein the targeting component comprises the sequence as set forth in SEQ ID NO: 104.
197. The artificial expression construct of claim 117, wherein the recombinant receptor comprises a fusion polypeptide which comprises a targeting component and a signaling component of an eTCR.
198. The artificial expression construct of claim 197, wherein the fusion polypeptide comprises a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence as set forth in SEQ ID NO: 105.

199. The artificial expression construct of claim 197, wherein the fusion polypeptide comprises the sequence as set forth in SEQ ID NO: 105.
200. The artificial expression construct of claim 96, further comprising a first control feature.
201. The artificial expression construct of claim 200, wherein the first control feature comprises or encodes a transduction marker, a selection cassette, or a suicide gene.
202. The artificial expression construct of claim 201, wherein the transduction marker comprises a truncated HER2 protein (Her2tG), epidermal growth factor receptor (EGFRt), or truncated CD19 (tCD19).
203. The artificial expression construct of claim 201, wherein the selection cassette comprises a dihydrofolate reductase double mutant (DHFRdm).
204. The artificial expression construct of claim 96, further comprising a first skip sequence.
205. The artificial expression construct of claim 204, wherein the first skip sequence is between the sequence encoding the mutated IL-15 and the first control feature.
206. The artificial expression construct of claim 204, wherein the first skip sequence encodes a 2A self-cleaving polypeptide.
207. The artificial expression construct of claim 206, wherein the 2A self-cleaving polypeptide comprises T2A, P2A, E2A, or F2A.
208. The artificial expression construct of claim 200, further comprising a second control feature.
209. The artificial expression construct of claim 204, further comprising a second skip sequence.
210. The artificial expression construct of claim 209, wherein the second skip sequence is between the mutated IL-15 and the recombinant receptor.
211. The artificial expression construct of claim 209, wherein the second skip sequence encodes a 2A self-cleaving polypeptide.
212. The artificial expression construct of claim 211, wherein the 2A skip self-cleaving polypeptide comprises T2A, P2A, E2A, or F2A.
213. A method of comprising: transducing an immune effector cell or population of immune effector cells with the artificial expression construct of claim 96.
214. A nanoparticle encapsulating the artificial expression construct of claim 96.
215. A non-natural cell or population thereof comprising the artificial expression construct of claim 96.
216. The non-natural cell or population thereof of claim 215, wherein the cell or population thereof comprise an autologous cell or an allogeneic cell in reference to a subject.

217. The non-natural cell or population thereof of claim 215, comprising an *in vivo* or *ex vivo* cell or population thereof.
218. The non-natural cell or population thereof of claim 215, wherein the cell or population thereof comprise an immune cell.
219. The non-natural cell or population thereof of claim 218, wherein the immune cell is a lymphocyte.
220. The non-natural cell or population thereof of claim 219, wherein the lymphocyte comprises a T cell, B cell, natural killer (NK) cell, or NK-T cell.
221. The non-natural cell or population thereof of claim 215, wherein the cell or population thereof comprise a T cell selected from a CD3+ T cell, a CD4+ T cell, a CD8+ T cell, a central memory T cell, an effector memory T cell, and/or a naïve T cell.
222. The non-natural cell or population thereof of claim 215, wherein the cell or population thereof comprise a CD8+ T cell.
223. The non-natural cell or population thereof of claim 215, wherein the cell or population thereof comprise a CD4+ T cell.
224. A composition comprising the non-natural cell or population thereof of claim 215 and a pharmaceutically acceptable carrier.
225. A method of treating a subject in need thereof comprising administering a therapeutically effective amount of the artificial expression construct of claim 96, the nanoparticle of claim 214, a non-natural cell or population thereof of claim 215, or the composition of claim 224 to the subject thereby treating the subject in need thereof.
226. The method of claim 225, wherein the subject in need thereof has cancer or an infection.
227. The method of claim 226, wherein the cancer comprises a hematological malignancy.
228. The method of claim 227, wherein the hematological malignancy comprises leukemia, lymphoma, or multiple myeloma.
229. The method of claim 228, wherein the leukemia comprises acute myeloid leukemia (AML).
230. The method of claim 226, wherein the cancer comprises a solid cancer.
231. The method of claim 230, wherein the solid cancer comprises lung cancer, squamous cell carcinoma, colorectal cancer, pancreatic cancer, breast cancer, thyroid cancer, bladder cancer, cervical cancer, esophageal cancer, ovarian cancer, gastric cancer, endometrial cancer, or brain cancer.
232. The method of method of claim 231, wherein the lung cancer is a non-small cell lung carcinoma.



233. The method of method of claim 217, wherein the brain cancer comprises gliomas, glioblastomas, or oligodendrogliomas.
234. The method of claim 211, wherein the administering a therapeutically effective amount comprises administering intravesically, intravenously, intradermally, intraarterially, intraparenterally, intranodally, intralymphatically, intraperitoneally, intralesionally, intraprostatically, intravaginally, intrarectally, topically, intrathecally, intratumorally, intramuscularly, or subcutaneously.