(12) STANDARD PATENT (11) Application No. **AU 2018367452 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
IL-36 secreting immunoresponsive cells and uses thereof

(51) International Patent Classification(s)
C07K 14/54 (2006.01) ***A61P 35/00*** (2006.01)
A61K 35/17 (2015.01) ***C07K 14/725*** (2006.01)
A61K 38/20 (2006.01) ***C07K 16/28*** (2006.01)

(21) Application No: **2018367452** (22) Date of Filing: **2018.11.14**

(87) WIPO No: **WO19/099483**

(30) Priority Data

(31) Number	(32) Date	(33) Country
62/585,879	**2017.11.14**	**US**

(43) Publication Date: **2019.05.23**
(44) Accepted Journal Date: **2024.06.13**

(71) Applicant(s)
Memorial Sloan-Kettering Cancer Center

(72) Inventor(s)
DANIYAN, Anthony;BRENTJENS, Renier J.

(74) Agent / Attorney
Spruson & Ferguson, GPO Box 3898, Sydney, NSW, 2001, AU

(56) Related Art
WO 2017/075537 A1

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 May 2019 (23.05.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/099483 A1

(51) International Patent Classification:
A61K 35/17 (2015.01) *C07K 14/54* (2006.01)
A61K 38/20 (2006.01) *C07K 14/725* (2006.01)
A61P 35/00 (2006.01) *C07K 16/28* (2006.01)

(21) International Application Number:
PCT/US2018/061003

(22) International Filing Date:
14 November 2018 (14.11.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/585,879 14 November 2017 (14.11.2017) US

(71) Applicant: **MEMORIAL SLOAN-KETTERING CANCER CENTER** [US/US]; 1275 York Avenue, New York, NY 10065 (US).

(72) Inventors: **DANIYAN, Anthony**; 303 East 60th Street, Apt. 12C, New York, NY 10022 (US). **BRENTJENS, Renier, J.**; 1275 York Avenue, New York, NY 10065 (US).

(74) Agent: **LENDARIS, Steven, P.** et al.; Baker Botts LLP, 30 Rockefeller Plaza, New York, NY 10112-4498 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

(54) Title: IL-36 SECRETING IMMUNORESPONSIVE CELLS AND USES THEREOF

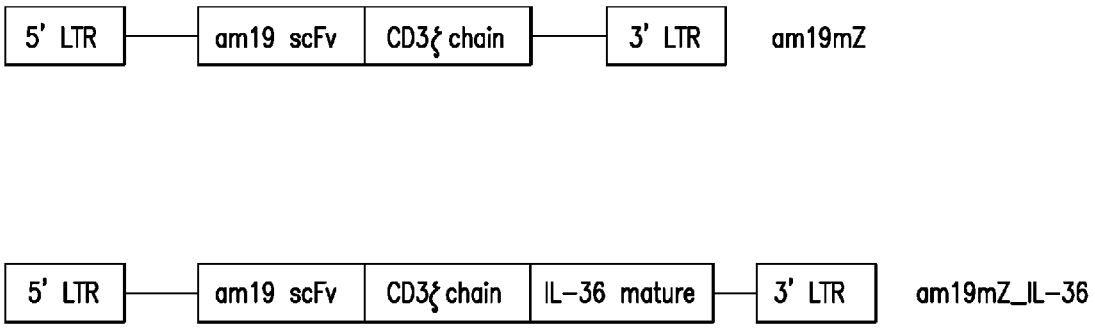


FIG. 1A

(57) Abstract: The present disclosure provides methods and compositions for enhancing the immune response toward cancers and pathogens. It relates to an immunoresponsive cell comprising an antigen-recognizing receptor (e.g., a chimeric antigen receptor (CAR) or a T cell receptor (TCR)), and expressing increased level of IL-36. In certain embodiments, the engineered immunoresponsive cells are antigen-directed and have enhanced immune- activating properties.

WO 2019/099483 A1

IL-36 SECRETING IMMUNORESPONSIVE CELLS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Application No.: 62/585,879 filed on November 14, 2017, the content of which is hereby incorporated by reference in its entirety, and to which priority is claimed.

INTRODUCTION

The presently disclosed subject matter provides methods and compositions for enhancing the immune response toward cancers and pathogens. It relates to immunoresponsive cells comprising antigen-recognizing receptors (e.g., chimeric antigen receptors (CARs) or T cell receptors (TCRs)) that are engineered to express an IL-36 polypeptide. These engineered immunoresponsive cells are antigen-directed, promote recruitment of other cytokines and exhibit enhanced anti-target efficacy.

BACKGROUND OF THE INVENTION

The majority of adult B-cell malignancies, including acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, and non-Hodgkin's lymphoma, are incurable despite currently available therapies. Adoptive therapy with genetically engineered autologous T cells has shown evidence of therapeutic efficacy in melanoma and indolent B cell malignancies. T cells may be modified to target tumor-associated antigens through the introduction of genes encoding artificial T-cell receptors, termed chimeric antigen receptors (CAR), specific to such antigens. Immunotherapy is a targeted therapy that has the potential to provide for the treatment of cancer.

However, malignant cells adapt to generate an immunosuppressive microenvironment to protect themselves from immune recognition and elimination. This "hostile" tumor microenvironment poses a challenge to methods of treatment involving stimulation of an immune response, such as targeted T cell therapies. Various modifications have been made toward improving the antitumor effect of CAR- or TCR-engineered T cells. For example, Pegram et al. describes a murine model of CAR-engineered T cells that constitutively secrete interleukin 12 (IL-12) and showed increased cytotoxicity towards $CD19^+$ tumor cells (Pegram et al., BLOOD, Vol. 119, No. 18, 2012). However, the secretion of IL-12 led to suppression of interleukin 2 (IL-2), an important cytokine that promotes the proliferation and anti-tumor effect of T and B lymphocytes. Dotti et al. discloses CAR-engineered T cells that constitutively secrete

interleukin 15 (IL-15) and an inducible caspase-9 based suicide gene (iC9), which showed increase cytotoxicity towards $CD19^+$ tumor cells (US 20130071414 A1). This modified CAR-T cell demonstrated unchanged levels of IL-2 expression both *in vivo* and *in vitro*. Accordingly, novel therapeutic strategies for treating neoplasia are urgently required.

SUMMARY OF THE INVENTION

The presently disclosed subject matter provides immunoresponsive cells (e.g., T cells, Tumor Infiltrating Lymphocytes, Natural Killer (NK) cells, cytotoxic T lymphocytes (CTLs), Natural Killer T (NK-T) cells or regulatory T cells) that (a) express an antigen-recognizing receptor (e.g., CAR or TCR) directed toward a target antigen of interest, and (b) express (and secrete) an interleukin 36 ("IL36") polypeptide (e.g., IL-36 alpha, IL-36 beta and/or IL-36 gamma). In certain non-limiting embodiments, the immunoresponsive cell comprises a nucleotide acid encoding an IL-36 polypeptide (e.g., IL-36 polypeptide-encoding nucleic acid), in expressible form.

In a first aspect, the present invention provides an isolated immunoresponsive cell comprising:

an antigen-recognizing receptor comprising an extracellular antigen-binding domain that binds to an antigen and an intracellular signaling domain that is capable of inducing cytotoxicity of the cell upon the binding of the extracellular antigen-binding domain to the antigen; and

(a) an exogenous IL-36 polypeptide or a fragment thereof that is constitutively expressed; or

(b) a modified promoter at an endogenous *IL-36* gene locus, wherein the modified promoter enhances gene expression of the endogenous *IL-36* gene, thereby the endogenous IL-36 gene is constitutively expressed.

In a second aspect, the present invention provides a pharmaceutical composition comprising an effective amount of an immunoresponsive cell of the first aspect and a pharmaceutically acceptable excipient.

In a third aspect, the present invention provides a method of reducing tumor burden, reducing number of tumor cells, reducing tumor size, and/or eradicating a tumor in a subject, treating and/or preventing a neoplasm, lengthening survival of a subject having a neoplasm, or increasing immune-activating cytokine production in response to a tumor antigen or a pathogen antigen in a subject, the method comprising administering to

the subject an effective amount of immunoresponsive cells of the first aspect or a pharmaceutical composition of the second aspect.

In a fourth aspect, the present invention provides a method of treating blood cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of an immunoresponsive cell of the first aspect, wherein the immunoresponsive cell is a T cell, and wherein the antigen is CD19, and/or wherein the blood cancer is selected from the group consisting of B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, and non-Hodgkin's lymphoma.

In a fifth aspect, the present invention provides a nucleic acid composition comprising (a) a first nucleic acid sequence encoding an antigen-recognizing receptor comprising an extracellular antigen-binding domain that that binds to an antigen and an intracellular signaling domain that is capable of inducing cytotoxicity of the cell upon the binding of the extracellular antigen-binding domain to the antigen; and (b) a second nucleic acid sequence encoding an exogenous IL-36 polypeptide or a fragment thereof that is constitutively expressed, each optionally operably linked to a promoter element.

In a sixth aspect, the present invention provides a vector comprising the nucleic acid composition of the fifth aspect.

In a seventh aspect, the present invention provides use of an immunoresponsive cell of the first aspect in the manufacture of a medicament for reducing tumor burden, treating and/or preventing a neoplasm, lengthening survival of a subject having a neoplasm, and/or increasing immune-activating cytokine production in response to a tumor antigen or a pathogen antigen in a subject.

In an eighth aspect, the present invention provides a method for producing an antigen-specific immunoresponsive cell, the method comprising introducing into an immunoresponsive cell the nucleic acid composition of the fifth aspect or the vector of the sixth aspect.

The presently disclosed subject matter also provides an immunoresponsive cell comprising (a) an antigen-recognizing receptor (e.g., CAR or TCR) directed toward a target antigen of interest, and (b) a modified promoter at an endogenous (native) *IL-36* gene locus, wherein the modified promoter enhances the gene expression of the endogenous *IL-36* gene locus. In certain non-limiting embodiments, the modification comprises replacement of an endogenous promoter with a constitutive promoter or an inducible promoter, or insertion of a constitutive promoter or inducible promoter to the

promoter region of the endogenous *IL-36* gene locus. In certain non-limiting embodiments, the constitutive promoter is selected from the group consisting of a CMV promoter, an EF1a promoter, a SV40 promoter, a PGK1 promoter, a Ubc promoter, a beta-actin promoter, and a CAG promoter. In certain non-limiting embodiments, the inducible promoter is selected from the group consisting of a tetracycline response element (TRE) promoter and an estrogen response element (ERE) promoter.

In certain embodiments, the immunoresponsive cell constitutively expresses the IL-36 polypeptide (mature or non-mature form of IL-36 protein). In certain embodiments, the IL-36 polypeptide is secreted. The antigen-recognizing receptor can be a TCR or a CAR. In certain embodiments, the antigen-recognizing receptor is a CAR. In certain embodiments, the immunoresponsive cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NK-T) cell, a human embryonic stem cell, and a pluripotent stem

cell from which lymphoid cells may be differentiated. In certain embodiments, the immunoresponsive cell is autologous.

Furthermore, the presently disclosed subject matter provides methods of using such immunoresponsive cells for inducing and/or enhancing an immune response, and/or for treating and/or preventing a neoplasm (e.g., cancer), infectious disease, and other diseases/disorders that would benefit from an augmented immune response.

In certain non-limiting embodiments, the presently disclosed subject matter provides an isolated immunoresponsive cell (a) comprising an antigen-recognizing receptor that binds to an antigen, and (b) expressing or secreting an IL-36 polypeptide. In certain embodiments, the immunoresponsive cell comprises an exogenous IL-36 polypeptide. In certain embodiments, the immunoresponsive cell comprises a nucleic acid encoding an IL-36 polypeptide. In certain embodiments, binding of the antigen-recognizing receptor to the antigen is capable of activating the immunoresponsive cell. In certain embodiments, the antigen-recognizing receptor is a CAR.

The presently disclosed subject matter further provides a composition comprising the immunoresponsive cells disclosed herein. In certain embodiments, the composition is a pharmaceutical composition that comprises a pharmaceutically acceptable excipient. In certain embodiments, the pharmaceutical composition is for treating and/or preventing a neoplasm (e.g., cancer), wherein the antigen to which the antigen-recognizing receptor binds is a tumor antigen.

The presently disclosed subject matter provides the immunoresponsive cells disclosed herein or the composition disclosed herein for use in a therapy, e.g., for use in reducing tumor burden, treating and/or preventing a neoplasm, lengthening survival of a subject having a neoplasm, and/or increasing immune-activating cytokine production in response to a tumor antigen or a pathogen antigen in a subject.

The presently disclosed subject matter also provides a method of treating and/or preventing a neoplasm in a subject. In certain embodiments, the method comprises administering to the subject an effective amount of the immunoresponsive cells or the pharmaceutical composition disclosed herein. The presently disclosed subject matter also provides a method of reducing tumor burden in a subject. In certain embodiments, the method comprises administering to the subject an effective amount of the immunoresponsive cells or the pharmaceutical composition disclosed herein. The presently disclosed subject matter further provides a method of lengthening survival of a subject having neoplasm (e.g., cancer). In certain embodiments, the method comprises

administering to the subject an effective amount of the immunoresponsive cells or the pharmaceutical composition disclosed herein.

The presently disclosed subject matter also provides a method of enhancing or increasing an immune response to a target antigen in a subject. In certain embodiments, the method comprises administering to the subject an effective amount of the immunoresponsive cells or the pharmaceutical composition disclosed herein. The cell can express and secrete the IL-36 polypeptide that enhances the subject's immune response toward the target antigen.

The presently disclosed subject matter further provides a method of increasing immune-activating cytokine production in response to a tumor antigen or a pathogen antigen in a subject. In certain embodiments, the method comprises administering to the subject an effective amount of the immunoresponsive cells or the pharmaceutical composition disclosed herein. In certain non-limiting embodiments, the immune-activating cytokine is selected from the group consisting of IL-10, GM-SCF and IFN-γ.

The presently disclosed subject matter further provides a method of treating blood cancer in a subject in need thereof. In certain embodiments, the method comprises administering to the subject a therapeutically effective amount of the immunoresponsive cells or the pharmaceutical composition disclosed herein. In certain embodiments, the cells are T cells. In certain embodiments, the antigen to which the antigen-recognizing receptor binds is CD19.

The presently disclosed subject matter further provides a method for producing an immunoresponsive cell disclosed herein. In certain embodiments, the method comprises introducing into an immunoresponsive cell (a) a first nucleic acid sequence that encodes an antigen-recognizing receptor that binds to an antigen, and (b) a second nucleic acid sequence that encodes an IL-36 polypeptide.

The presently disclosed subject matter further provides a nucleic acid composition comprising (a) a first nucleic acid sequence encoding an antigen-recognizing receptor (e.g., a CAR or TCR) that binds to an antigen and (b) a second nucleic acid sequence encoding an IL-36 polypeptide (mature or non-mature form of IL-36).

In certain non-limiting embodiments, the first or the second nucleic acid sequence is operably linked to a promoter element constitutively or inducibly expressed in the immunoresponsive cell. The promoter for the first nucleic acid sequence may be the same or different from the promoter for the second nucleic acid sequence. In certain

non-limiting embodiments, each of the first and second nucleic acid sequences is operably linked to a promoter element constitutively or inducibly expressed in the immunoresponsive cell. One or both of the first and second nucleic acid sequences may be comprised in a vector, which may be the same vector (bicistronic) or separate vectors. In certain non-limiting embodiments, the vector is a virus vector, e.g., a retroviral vector.

In certain embodiments, the nucleic acid composition is comprised in a vector. In certain non-limiting embodiments, the vector is a virus vector, e.g., a retroviral vector. The presently disclosed subject matter also provides a vector comprising the nucleic acid composition disclosed herein.

The presently disclosed subject matter provides a kit for inducing and/or enhancing an immune response and/or treating and/or preventing a neoplasm (e.g., cancer) or, pathogen infection. In certain embodiments, the kit comprises the immunoresponsive cells disclosed herein, the pharmaceutical composition disclosed herein, the nucleic acid composition disclosed herein, or the vector disclosed herein. In certain embodiments, the kit further comprises written instructions for inducing and/or enhancing an immune response and/or treating and/or preventing a neoplasm or a pathogen infection.

In various non-limiting embodiments, the immunoresponsive cell is autologous to its intended recipient subject.

In various embodiments of any of the aspects delineated herein, the antigen-recognizing receptor is a TCR or a CAR. In various embodiments of any of the aspects delineated herein, the antigen-recognizing receptor is exogenous or endogenous. In various embodiments of any of the aspects delineated herein, the antigen-recognizing receptor is recombinantly expressed. In various embodiments of any of the aspects delineated herein, the antigen-recognizing receptor is expressed from a vector. In various embodiments of any of the aspects delineated herein, the antigen-recognizing receptor is a CAR. In certain embodiments, the CAR comprises an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain. In certain embodiments, wherein the CAR does not comprise a co-stimulatory signaling domain. In certain embodiments, the CAR is 19z.

In various embodiments of any of the aspects delineated herein, the antigen-recognizing receptor is a TCR. In certain embodiments, the TCR is a recombinant TCR. In certain embodiments, the TCR is a non-naturally occurring TCR. In certain embodiments, the TCR differs from any naturally occurring TCR by at least one amino

acid residue. In certain embodiments, the TCR is modified from a naturally occurring TCR by at least one amino acid residue.

In various embodiments of any of the aspects delineated herein, the antigen to which the antigen-recognizing receptor binds is a tumor antigen or a pathogen antigen. In certain embodiments, the antigen is a tumor antigen. In various embodiments of any of the aspects delineated herein, the tumor antigen is selected from the group consisting of CD19, MUC16, MUC1, CAlX, CEA, CD8, CD7, CD10, CD20, CD22, CD30, CD33, CLL1 CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD133, CD138, a cytomegalovirus (CMV) infected cell antigen, EGP-2, EGP-40, EpCAM, erb-B2,3,4, FBP, Fetal acetylcholine receptor, folate receptor-α, GD2, GD3, HER-2, hTERT, IL-13R-a2, κ-light chain, KDR, LeY, L1 cell adhesion molecule, MAGE-A1, Mesothelin, ERBB2, MAGEA3, p53, MART1,GP100, Proteinase3 (PR1), Tyrosinase, Survivin, hTERT, EphA2, NKG2D ligands, NY-ES0-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, WT-1, BCMA, CD123, CD44V6, NKCS1, EGF1R, EGFR-VIII, CD99, CD70, ADGRE2, CCR1, LILRB2, PRAME, and ERBB. In certain embodiments, the antigen is CD19. Amino acid sequences that specifically bind to said antigens are known in the art or may be prepared using methods known in the art; examples include immunoglobulins, variable regions of immunoglobulins (e.g. variable fragment ("Fv") or bivalent variable fragment ("Fab")), single chain antibodies, etc. In certain embodiments, the antigen is a pathogen antigen.

In various non-limiting embodiments of any of the aspects delineated herein, the exogenous IL-36 polypeptide is secreted. In various non-limiting embodiments of any of the aspects delineated herein, the IL-36 polypeptide is comprised (and expressed) from a vector. In various non-limiting embodiments of any of the aspects delineated herein, the IL-36 polypeptide comprises a heterologous signal sequence at the amino-terminus (e.g., a signal sequence that is not naturally associated with IL-36). In various embodiments of any of the aspects delineated herein, the heterologous signal sequence is selected from the group consisting of IL-2 signal sequence, the kappa leader sequence, the CD8 leader sequence, and combinations and/or synthetic variations thereof which retain the capacity to promote secretion of IL-36 polypeptide (either mature or non-mature). In certain embodiments, the IL-36 peptide is a mature form of IL-36 alpha, IL-36 beta, IL-36 gamma, or a functional fragment thereof. In certain embodiments, the IL-36 peptide comprises an amino acid sequence that is at least about 80% homologous to the sequence

set forth in SEQ ID NO: 4, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 30, SEQ ID NO: 31, or SEQ ID NO: 32. In certain embodiments, wherein the IL-36 peptide comprises the amino acid sequence set forth in SEQ ID NO: 4, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 30, SEQ ID NO: 31, or SEQ ID NO: 32. In various embodiments of any of the aspects delineated herein, the IL-36 polypeptide enhances an immune response of the immunoresponsive cell. In certain embodiments, the exogenous IL-36 polypeptide increases anti-tumor cytokine production. In certain embodiments, the anti-tumor cytokine is selected from the group consisting of IL-10, GM-CSF and IFN-γ.

In various embodiments of any of the aspects delineated herein, the method reduces the number of tumor cells, reduces tumor size, eradicates the tumor in the subject, reduces the tumor burden in the subject, eradicates the tumor burden in the subject, increases the period of time to relapse/recurrence, and/or increases the period of survival.

Illustrative neoplasms for which the presently disclosed subject matter can be used include, but are not limited to leukemias (e.g., acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia a, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia), polycythemia vera, lymphoma (Hodgkin's disease, non-Hodgkin's disease), Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors such as sarcomas and carcinomas (e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, nile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodenroglioma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma).

In various non-limiting embodiments of any of the aspects delineated herein, the neoplasm is one or more of blood cancer, B cell leukemia, multiple myeloma, lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, non-Hodgkin's lymphoma, and ovarian cancer. In certain embodiments, the blood cancer is one or more of B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, and non-Hodgkin's lymphoma. In certain embodiments, the antigen is CD19. In certain embodiments, the neoplasm is ovarian cancer, and the antigen is MUC16.

BRIEF DESCRIPTION OF THE FIGURES

The following Detailed Description, given by way of example, but not intended to limit the presently disclosed subject matter to specific embodiments described, may be understood in conjunction with the accompanying drawings.

Figures 1A-1D depict representations of various CAR constructs. A) Schematic representation of am19mZ (a first generation CAR comprising a rat anti-mouse CD19 scFv and an intracellular signaling domain that comprises a mouse CD3ζ polypeptide. The amino acid sequence and corresponding nucleotide sequence for am19mZ are set forth in SEQ ID NOS: 5 and 65, respectively); and ah19mZ_IL-36 (a first generation CAR (am19mZ) secreting murine IL-36. Both CARs utilized a CD28 proximal extracellular and transmembrane domain as a hinge. In all murine CAR constructs, the cytokine was separated from the CAR by a self-cleaving P2A element. B) first-generation anti-mouse CD 19 myc-tag CAR incorporating constitutively-secreted murine IL36-alpha. C) first-generation anti-mouse CD 19 myc-tag CAR incorporating constitutively-secreted murine IL36-beta. D) first-generation anti-mouse CD 19 myc-tag CAR incorporating constitutively-secreted murine IL36-gamma. All vectors comprised SFG backbone.

Figure 2 depicts flow cytometry analyses of cell surface expression of various CAR constructs. Surface expressions of anti-CD19 myc-tag first generation CAR secreting IL-36 beta (d5M19ZTSB) and gamma (d5M19ZTSG) and control constructs including M19del (non-functional CAR), M19Z (first generation CAR) were confirmed. d5B6emp served as a negative control.

Figure 3 depicts murine granulocyte macrophage colony-stimulating factor (GM-CSF) secretion in T cells transduced with various CAR constructs. Mean differences between indicated samples, 95% confidence interval thereof and adjusted P values are shown in the table below.

Figure 4 depicts murine Interferon gamma (mINF-gamma) secretion in T cells transduced with various CAR constructs. Mean differences between indicated samples, 95% confidence interval thereof and adjusted P values are shown in the table below.

Figure 5 depicts murine Interleukin 10 (IL-10) secretion in T cells transduced with various CAR constructs. Mean differences between indicated samples, 95% confidence interval thereof and adjusted P values are shown in the table below.

Figures 6A-6C depict survival curves of tumor-bearing mice treated with various modified T cells. A) Survival curves of all subjects. Untreated (untreated control group); am19MTmZ (a first generation CAR comprising a rat anti-mouse CD19 scFv and an intracellular signaling domain that comprises a mouse CD3ζ polypeptide); am19MTmZpIL36B (a first generation CAR (am19mZ) secreting murine IL-36 beta), and am19MTmZpIL36G (a first generation CAR (am19mZ) secreting murine IL-36 gamma). Median survival numbers are shown in the table below. B) Survival curves of am19MTmZ (M19Z) and am19MTmZpIL36B (M19Z_36B). Median survival numbers are shown in the table below. C) Survival curves of am19MTmZ (M19Z) and am19MTmZpIL36G (M19Z_36G). Median survival numbers are shown in the table below.

Figure 7 depicts survival curves of C57BL/6 mice. Mice were inoculated with 1 million mouse CD19+ EL4 tumor cells (EL4-CD19) via tail vein injection. On the following day, the mice received (without any pre-conditioning chemotherapy): no CAR T cells (untreated), 2,500,000 m19m28mz (syngeneic anti-mouse CD19 CD28-based second generation CAR T cells), 2,500,000 m19mz-IL36Y (syngeneic anti-mouse CD19 first-generation IL36-gamma secreting CAR T cells) or m19m28mz-IL36Y (syngeneic anti-mouse CD19 CD28-based second generation IL36-gamma secreting CAR T cells).

Figure 8 depicts pro-inflammatory cytokine secretion in tumor-bearing mice. C57BL/6 mice were inoculated with 1 million mouse CD19+ EL4 tumor cells (EL4-CD19) via tail vein injection. On the following day, the mice received (without getting any pre-conditioning chemotherapy): no CAR T cells (untreated), 2,500,000 m19m28mz (syngeneic anti-mouse CD19 CD28-based second generation CAR T cells), 2,500,000 m19mz-IL36Y (syngeneic anti-mouse CD19 first-generation IL36-gamma secreting CAR T cells) or m19m28mz-IL36Y (syngeneic anti-mouse CD19 CD28-based second generation IL36-gamma secreting CAR T cells). Serum was collected and analyzed for cytokine levels using a Luminex bead-based multiplex assay.

Figure 9 depicts B cell aplasia in tumor-bearing mice. C57BL/6 mice were inoculated with 1 million mouse CD19+ EL4 tumor cells (EL4-CD19) via tail vein injection. On the following day, the mice received (without getting any pre-conditioning chemotherapy): no CAR T cells (untreated), 2,500,000 m19m28mz (syngeneic anti-mouse CD19 CD28-based second generation CAR T cells), 2,500,000 m19mz-IL36Y (syngeneic anti-mouse CD19 first-generation IL36-gamma secreting CAR T cells) or m19m28mz-IL36Y (syngeneic anti-mouse CD19 CD28-based second generation IL36-gamma secreting CAR T cells). Post RBC lysis, the percentage of peripheral B-cells (CD19+ cells as a percentage of CD45+ cells) was determined utilizing flow cytometry.

DETAILED DESCRIPTION OF THE INVENTION

The presently disclosed subject matter provides cells, including genetically modified immunoresponsive cells (e.g., T cells, NK cells, or CTL cells) comprising a combination of an antigen-recognizing receptor (e.g., TCR or CAR) and a secretable IL-36 polypeptide (e.g., an exogenous IL-36 polypeptide, or a nucleic acid encoding an IL-36 polypeptide). The presently disclosed subject matter also provides methods of using such cells for inducing and/or enhancing an immune response to a target antigen, and/or treating and/or preventing neoplasia or other diseases/disorders where an increase in an antigen-specific immune response is desired. The presently disclosed subject matter is based, at least in part, on the discovery that a secretable IL-36 polypeptide enhances the anti-tumor effect of an immunoresponsive cell comprising an antigen-recognizing receptor (e.g., a CAR-expressing T cell or a TCR-expressing T cell). It was observed that the co-expression of an IL-36 polypeptide and an antigen-recognizing receptor (e.g., a CAR, such as 19z CAR) on T cells led to increased cytokine secretion.

Malignant cells have developed a series of mechanisms to protect themselves from immune recognition and elimination. The presently disclosed subject matter provides immunogenicity within the tumor microenvironment for tumor eradication, and represents a significant advance over conventional adoptive T cell therapy. The presently disclosed subject matter provides an option of foregoing some or all ancillary treatments such as prior conditioning of the host with total body irradiation, high-dose chemotherapy, and/or post-infusion cytokine support.

1. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art. The following references provide one of skill with a general definition of many of the terms used in the presently

disclosed subject matter: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

As used herein, the term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" can mean within 3 or more than 3 standard deviations, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, e.g., up to 10%, up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, e.g., within 5-fold or within 2-fold, of a value.

By "activates an immunoresponsive cell" is meant induction of signal transduction or changes in protein expression in the cell resulting in initiation of an immune response. For example, when CD3 Chains cluster in response to ligand binding and immunoreceptor tyrosine-based inhibition motifs (ITAMs) a signal transduction cascade is produced. In certain embodiments, when an endogenous TCR or an exogenous CAR binds to an antigen, a formation of an immunological synapse occurs that includes clustering of many molecules near the bound receptor (e.g. CD4 or CD8, CD3γ/δ/ε/ζ, etc.). This clustering of membrane bound signaling molecules allows for ITAM motifs contained within the CD3 chains to become phosphorylated. This phosphorylation in turn initiates a T cell activation pathway ultimately activating transcription factors, such as NF-κB and AP-1. These transcription factors induce global gene expression of the T cell to increase IL-2 production for proliferation and expression of master regulator T cell proteins in order to initiate a T cell mediated immune response.

By "stimulates an immunoresponsive cell" is meant a signal that results in a robust and sustained immune response. In various embodiments, this occurs after immune cell (e.g., T-cell) activation or concomitantly mediated through receptors including, but not limited to, CD28, CD137 (4-lBB), OX40, CD40 and ICOS. Receiving multiple stimulatory signals can be important to mount a robust and long-term T cell mediated immune response. T cells can quickly become inhibited and unresponsive to

antigen. While the effects of these co-stimulatory signals may vary, they generally result in increased gene expression in order to generate long lived, proliferative, and anti-apoptotic T cells that robustly respond to antigen for complete and sustained eradication.

The term "antigen-recognizing receptor" as used herein refers to a receptor that is capable of activating an immune or immunoresponsive cell (e.g., a T-cell) in response to its binding to an antigen. Non-limiting examples of antigen-recognizing receptors include native or endogenous T cell receptors ("TCRs"), and chimeric antigen receptors ("CARs").

As used herein, the term "antibody" means not only intact antibody molecules, but also fragments of antibody molecules that retain immunogen-binding ability. Such fragments are also well known in the art and are regularly employed both *in vitro* and *in vivo*. Accordingly, as used herein, the term "antibody" means not only intact immunoglobulin molecules but also the well-known active fragments $F(ab')_2$, and Fab. $F(ab')_2$, and Fab fragments that lack the Fe fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl et al., *J. Nucl. Med.* 24:316-325 (1983). As used herein, antibodies include whole native antibodies, bispecific antibodies; chimeric antibodies; Fab, Fab', single chain V region fragments (scFv), fusion polypeptides, and unconventional antibodies. In certain embodiments, an antibody is a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant (C_H) region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant C_L region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further sub-divided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1 q) of the classical complement system.

As used herein, "CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. *See, e.g.*, Kabat et al., Sequences of Proteins of Immunological Interest, 4th U. S. Department of Health and Human Services, National Institutes of Health (1987). Generally, antibodies comprise three heavy chain and three light chain CDRs or CDR regions in the variable region. CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. In certain embodiments, the CDRs regions are delineated using the Kabat system (Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242).

As used herein, the term "single-chain variable fragment" or "scFv" is a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of an immunoglobulin covalently linked to form a V_H::V_L heterodimer. The V_H and V_L are either joined directly or joined by a peptide-encoding linker (e.g., 10, 15, 20, 25 amino acids), which connects the N-terminus of the V_H with the C-terminus of the V_L, or the C-terminus of the V_H with the N-terminus of the V_L. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility. Despite removal of the constant regions and the introduction of a linker, scFv proteins retain the specificity of the original immunoglobulin. Single chain Fv polypeptide antibodies can be expressed from a nucleic acid including V_H - and V_L -encoding sequences as described by Huston, et al. (Proc. Nat. Acad. Sci. USA, 85:5879-5883, 1988). *See, also*, U.S. Patent Nos. 5,091,513, 5,132,405 and 4,956,778; and U.S. Patent Publication Nos. 20050196754 and 20050196754. Antagonistic scFvs having inhibitory activity have been described (see, e.g., Zhao et al., Hyrbidoma (Larchmt) 2008 27(6):455-51; Peter et al., J Cachexia Sarcopenia Muscle 2012 August 12; Shieh et al., J Imunol2009 183(4):2277-85; Giomarelli et al., Thromb Haemost 2007 97(6):955-63; Fife eta., J Clin Invst 2006 116(8):2252-61; Brocks et al., Immunotechnology 1997 3(3):173-84; Moosmayer et al., Ther Immunol 1995 2(10:31-40). Agonistic scFvs having stimulatory activity have been described (see, e.g., Peter et al., J Bioi Chern 2003 25278(38):36740-7; Xie et al., Nat Biotech 1997 15(8):768-71; Ledbetter et al., Crit Rev Immunol1997 17(5-6):427-55; Ho et al., BioChim Biophys Acta 2003 1638(3):257-66).

As used herein, the term "affinity" is meant a measure of binding strength. Affinity can depend on the closeness of stereochemical fit between antibody combining sites and antigen determinants, on the size of the area of contact between them, and/or on

the distribution of charged and hydrophobic groups. As used herein, the term "affinity" also includes "avidity", which refers to the strength of the antigen-antibody bond after formation of reversible complexes. Methods for calculating the affinity of an antibody for an antigen are known in the art, including, but not limited to, various antigen-binding experiments, e.g., functional assays (e.g., flow cytometry assay).

The term "chimeric antigen receptor" or "CAR" as used herein refers to a molecule comprising an extracellular antigen-binding domain that is fused to an intracellular signaling domain that is capable of activating or stimulating an immunoresponsive cell, and a transmembrane domain. In certain embodiments, the extracellular antigen-binding domain of a CAR comprises a scFv. The scFv can be derived from fusing the variable heavy and light regions of an antibody. Alternatively or additionally, the scFv may be derived from Fab's (instead of from an antibody, e.g., obtained from Fab libraries). In certain embodiments, the scFv is fused to the transmembrane domain and then to the intracellular signaling domain. In certain embodiments, the CAR is selected to have high binding affinity or avidity for the antigen.

As used herein, the term "nucleic acid molecules" include any nucleic acid molecule that encodes a polypeptide of interest (e.g., an IL-36 polypeptide) or a fragment thereof. Such nucleic acid molecules need not be 100% homologous or identical with an endogenous nucleic acid sequence, but may exhibit substantial identity. Polynucleotides having "substantial identity" or "substantial homology" to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. By "hybridize" is meant a pair to form a double-stranded molecule between complementary polynucleotide sequences (e.g., a gene described herein), or portions thereof, under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) Methods Enzymol. 152:399; Kimmel, A. R. (1987) Methods Enzymol. 152:507).

For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, e.g., less than about 500 mM NaCl and 50 mM trisodium citrate, or less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, e.g., at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30° C, at least about 37° C, or at least about 42° C. Varying additional parameters, such as hybridization time, the

concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In certain embodiments, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In certain embodiments, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 µg/ml denatured salmon sperm DNA (ssDNA). In certain embodiments, hybridization will occur at 42° C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps can be less than about 30 mM NaCl and 3 mM trisodium citrate, e.g., less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25° C, of at least about 42° C, or of at least about 68° C. In certain embodiments, wash steps will occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In certain embodiments, wash steps will occur at 42° C. in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In certain embodiments, wash steps will occur at 68° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art. Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (Science 196:180, 1977); Grunstein and Rogness (Proc. Natl. Acad. Sci., USA 72:3961, 1975); Ausubel et al. (Current Protocols in Molecular Biology, Wiley Interscience, New York, 2001); Berger and Kimmel (Guide to Molecular Cloning Techniques, 1987, Academic Press, New York); and Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.

By "substantially identical" or "substantially homologous" is meant a polypeptide or nucleic acid molecule exhibiting at least about 50% homologous or identical to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). In certain embodiments, such a sequence is at least about

60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or at least about 100% homologous or identical to the sequence of the amino acid or nucleic acid used for comparison.

Sequence identity can be measured by using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e-3 and e-100 indicating a closely related sequence.

By "analog" is meant a structurally related polypeptide or nucleic acid molecule having the function of a reference polypeptide or nucleic acid molecule.

The term "ligand" as used herein refers to a molecule that binds to a receptor. In certain embodiments, the ligand binds to a receptor on another cell, allowing for cell-to-cell recognition and/or interaction.

The term "constitutive expression" or "constitutively expressed" as used herein refers to expression or expressed under all physiological conditions.

By "disease" is meant any condition, disease or disorder that damages or interferes with the normal function of a cell, tissue, or organ, e.g., neoplasia, and pathogen infection of cell.

By "effective amount" is meant an amount sufficient to have a therapeutic effect. In certain embodiments, an "effective amount" is an amount sufficient to arrest, ameliorate, or inhibit the continued proliferation, growth, or metastasis (e.g., invasion, or migration) of a neoplasm.

By "enforcing tolerance" is meant preventing the activity of self-reactive cells or immunoresponsive cells that target transplanted organs or tissues.

By "endogenous" is meant a nucleic acid molecule or polypeptide that is normally expressed in a cell or tissue.

By "exogenous" is meant a nucleic acid molecule or polypeptide that is not endogenously present in a cell. The term "exogenous" would therefore encompass any recombinant nucleic acid molecule or polypeptide expressed in a cell, such as foreign, heterologous, and over-expressed nucleic acid molecules and polypeptides. By "exogenous" nucleic acid is meant a nucleic acid not present in a native wild-type cell; for example, an exogenous nucleic acid may vary from an endogenous counterpart by sequence, by position/location, or both. For clarity, an exogenous nucleic acid may have the same or different sequence relative to its native endogenous counterpart; it may be introduced by genetic engineering into the cell itself or a progenitor thereof, and may optionally be linked to alternative control sequences, such as a non-native promoter or secretory sequence.

By a "heterologous nucleic acid molecule or polypeptide" is meant a nucleic acid molecule (e.g., a cDNA, DNA or RNA molecule) or polypeptide that is not normally present in a cell or sample obtained from a cell. This nucleic acid may be from another organism, or it may be, for example, an mRNA molecule that is not normally expressed in a cell or sample.

By "immunoresponsive cell" is meant a cell that functions in an immune response or a progenitor, or progeny thereof.

By "modulate" is meant positively or negatively alter. Exemplary modulations include a about 1%, about 2%, about 5%, about 10%, about 25%, about 50%, about 75%, or about 100% change.

By "increase" is meant to alter positively by at least about 5%. An alteration may be by about 5%, about 10%, about 25%, about 30%, about 50%, about 75%, about 100% or more.

By "reduce" is meant to alter negatively by at least about 5%. An alteration may be by about 5%, about 10%, about 25%, about 30%, about 50%, about 75%, or even by about 100%.

By "isolated cell" is meant a cell that is separated from the molecular and/or cellular components that naturally accompany the cell.

The terms "isolated," "purified," or "biologically pure" refer to material that is free to varying degrees from components which normally accompany it as found in its native state. "Isolate" denotes a degree of separation from original source or surroundings. "Purify" denotes a degree of separation that is higher than isolation. A "purified" or "biologically pure" protein is sufficiently free of other materials such that

any impurities do not materially affect the biological properties of the protein or cause other adverse consequences. That is, a nucleic acid or peptide is purified if it is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Purity and homogeneity are typically determined using analytical chemistry techniques, for example, polyacrylamide gel electrophoresis or high performance liquid chromatography. The term "purified" can denote that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. For a protein that can be subjected to modifications, for example, phosphorylation or glycosylation, different modifications may give rise to different isolated proteins, which can be separately purified.

The term "antigen-binding domain" as used herein refers to a domain capable of specifically binding a particular antigenic determinant or set of antigenic determinants present on a cell.

"Linker", as used herein, shall mean a functional group (e.g., chemical or polypeptide) that covalently attaches two or more polypeptides or nucleic acids so that they are connected to one another. As used herein, a "peptide linker" refers to one or more amino acids used to couple two proteins together (e.g., to couple V_H and V_L domains). In certain embodiments, the linker comprises a sequence set forth in GGGGSGGGGSGGGGS [SEQ ID NO: 23].

By "neoplasm" is meant a disease characterized by the pathological proliferation of a cell or tissue and its subsequent migration to or invasion of other tissues or organs. Neoplasia growth is typically uncontrolled and progressive, and occurs under conditions that would not elicit, or would cause cessation of, multiplication of normal cells. Neoplasia can affect a variety of cell types, tissues, or organs, including but not limited to an organ selected from the group consisting of bladder, bone, brain, breast, cartilage, glia, esophagus, fallopian tube, gallbladder, heart, intestines, kidney, liver, lung, lymph node, nervous tissue, ovaries, pancreas, prostate, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, uterus, and vagina, or a tissue or cell type thereof. Neoplasia include cancers, such as sarcomas, carcinomas, or plasmacytomas (malignant tumor of the plasma cells).

By "receptor" is meant a polypeptide, or portion thereof, present on a cell membrane that selectively binds one or more ligand.

By "recognize" is meant selectively binds to a target. A T cell that recognizes a tumor can expresses a receptor (e.g., a TCR or CAR) that binds to a tumor antigen.

By "reference" or "control" is meant a standard of comparison. For example, the level of scFv-antigen binding by a cell expressing a CAR and an scFv may be compared to the level of scFv-antigen binding in a corresponding cell expressing CAR alone.

By "secreted" is meant a polypeptide that is released from a cell via the secretory pathway through the endoplasmic reticulum, Golgi apparatus, and as a vesicle that transiently fuses at the cell plasma membrane, releasing the proteins outside of the cell.

By "signal sequence" or "leader sequence" is meant a peptide sequence (e.g., 5, 10, 15, 20, 25 or 30 amino acids) present at the N-terminus of newly synthesized proteins that directs their entry to the secretory pathway. Exemplary leader sequences include, but is not limited to, the IL-2 signal sequence: MYRMQLLSCIALSLALVTNS [SEQ ID NO: 8] (human), MYSMQLASCVTLTLVLLVNS [SEQ ID NO: 24] (mouse); the kappa leader sequence: METPAQLLFLLLLWLPDTTG [SEQ ID NO: 25] (human), METDTLLLWVLLLWVPGSTG [SEQ ID NO: 26] (mouse); the CD8 leader sequence: MALPVTALLLPLALLLHAARP [SEQ ID NO: 27] (human); the truncated human CD8 signal peptide: MALPVTALLLPLALLLHA [SEQ ID NO: 80] (human); the albumin signal sequence: MKWVTFISLLFSSAYS [SEQ ID NO: 28] (human); and the prolactin signal sequence: MDSKGSSQKGSRLLLLLVVSNLLLCQGVVS [SEQ ID NO: 29] (human). By "soluble" is meant a polypeptide that is freely diffusible in an aqueous environment (e.g., not membrane bound).

By "specifically binds" is meant a polypeptide or fragment thereof that recognizes and binds to a biological molecule of interest (e.g., a polypeptide), but which does not substantially recognize and bind other molecules in a sample, for example, a biological sample, which naturally includes a presently disclosed polypeptide.

The term "tumor antigen" as used herein refers to an antigen (e.g., a polypeptide) that is uniquely or differentially expressed on a tumor cell compared to a normal or non-IS neoplastic cell. In certain embodiments, a tumor antigen includes any polypeptide expressed by a tumor that is capable of activating or inducing an immune response via an antigen recognizing receptor (e.g., CD19, MUC-16) or capable of suppressing an immune response via receptor-ligand binding (e.g., CD47, PD-Ll/L2, B7.1/2).

The terms "comprises", "comprising", and are intended to have the broad meaning ascribed to them in U.S. Patent Law and can mean "includes", "including" and the like.

As used herein, "treatment" refers to clinical intervention in an attempt to alter the disease course of the individual or cell being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Therapeutic effects of treatment include, without limitation, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastases, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. By preventing progression of a disease or disorder, a treatment can prevent deterioration due to a disorder in an affected or diagnosed subject or a subject suspected of having the disorder, but also a treatment may prevent the onset of the disorder or a symptom of the disorder in a subject at risk for the disorder or suspected of having the disorder.

An "individual" or "subject" herein is a vertebrate, such as a human or non-human animal, for example, a mammal. Mammals include, but are not limited to, humans, primates, farm animals, sport animals, rodents and pets. Non-limiting examples of non-human animal subjects include rodents such as mice, rats, hamsters, and guinea pigs; rabbits; dogs; cats; sheep; pigs; goats; cattle; horses; and non-human primates such as apes and monkeys. The term "immunocompromised" as used herein refers to a subject who has an immunodeficiency. The subject is very vulnerable to opportunistic infections, infections caused by organisms that usually do not cause disease in a person with a healthy immune system, but can affect people with a poorly functioning or suppressed immune system.

Other aspects of the presently disclosed subject matter are described in the following disclosure and are within the ambit of the presently disclosed subject matter.

2. Antigen-Recognizing Receptors

The present disclosure provides antigen-recognizing receptors that bind to an antigen of interest. In certain embodiments, the antigen-recognizing receptor is a chimeric antigen receptor (CAR). In certain embodiments, the antigen-recognizing receptor is a T-cell receptor (TCR). The antigen-recognizing receptor can bind to a tumor antigen or a pathogen antigen.

2.1. Antigens

In certain embodiments, the antigen-recognizing receptor binds to a tumor antigen. Any tumor antigen (antigenic peptide) can be used in the tumor-related embodiments described herein. Sources of antigen include, but are not limited to, cancer proteins. The antigen can be expressed as a peptide or as an intact protein or portion

thereof. The intact protein or a portion thereof can be native or mutagenized. Non-limiting examples of tumor antigens include carbonic anhydrase IX (CAlX), carcinoembryonic antigen (CEA), CD8, CD7, CD10, CD19, CD20, CD22, CD30, CD33, CLL1, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD133, CD138, CD123, CD44V6, an antigen of a cytomegalovirus (CMV) infected cell (e.g., a cell surface antigen), epithelial glycoprotein-2 (EGP-2), epithelial glycoprotein-40 (EGP-40), epithelial cell adhesion molecule (EpCAM), receptor tyrosine-protein kinases erb-B2,3,4 (erb-B2,3,4), folate-binding protein (FBP), fetal acetylcholine receptor (AChR), folate receptor-α, Ganglioside G2 (GD2), Ganglioside G3 (GD3), human Epidermal Growth Factor Receptor 2 (HER-2), human telomerase reverse transcriptase (hTERT), Interleukin-13 receptor subunit alpha-2 (IL-13Rα2), κ-light chain, kinase insert domain receptor (KDR), Lewis Y (LeY), L1 cell adhesion molecule (L1CAM), melanoma antigen family A, 1 (MAGE-A1), Mucin 16 (MUC16), Mucin 1 (MUC1), Mesothelin (MSLN), ERBB2, MAGEA3, p53, MART1,GP100, Proteinase3 (PR1), Tyrosinase, Survivin, hTERT, EphA2, NKG2D ligands, cancer-testis antigen NY-ES0-1, oncofetal antigen (h5T4), prostate stem cell antigen (PSCA), prostate-specific membrane antigen (PSMA), ROR1, tumor-associated glycoprotein 72 (TAG-72), vascular endothelial growth factor R2 (VEGF-R2), and Wilms tumor protein (WT-1), BCMA, NKCS1, EGF1R, EGFR-VIII, CD99, CD70, ADGRE2, CCR1, LILRB2, PRAME and ERBB.

In certain embodiments, the antigen-recognizing receptor binds to CD19. In certain embodiments, the antigen-recognizing receptor binds to a murine CD19 polypeptide. In certain embodiments, the murine CD19 polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 61.

RPQKSLLVEVEEGGNVVLPCLPDSSPVSSEKLAWYRGNQSTPFLELSPGSPGLGLHVGS
LGILLVIVNVSDHMGGFYLCQKRPPFKDIWQPAWTVNVEDSGEMFRWNASDVRDLDCDL
RNRSSGSHRSTSGSQLYVWAKDHPKVWGTKPVCAPRGSSLNQSLINQDLTVAPGSTLWL
SCGVPPVPVAKGSISWTHVHPRRPNVSLLSLSLGGEHPVREMWVWGSLLLLPQATALDE
GTYYCLRGNLTIERHVKVIARSAVWLWLLRTGG [SEQ ID NO:61]

In certain embodiments, the antigen-recognizing receptor binds to a human CD19 polypeptide. In certain embodiments, the human CD19 polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 62.

PEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRESPLKPFLKLSLGLPGLGIHMRPL
AIWLFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSGELFRWNVSDLGGLGCGLK
NRSSEGPSSPSGKLMSPKLYVWAKDRPEIWEGEPPCLPPRDSLNQSLSQDLTMAPGSTL

WLSCGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPARDMWVMETGLLLPRATAQD AGKYYCHRGNLTMSFHLEITARPVLWHWLLRTGGWK [SEQ ID NO:62]

In certain embodiments, the antigen-recognizing receptor binds to the extracellular domain of a human or murine CD19 protein.

In certain embodiments, the antigen-recognizing receptor binds to a pathogen antigen, e.g., for use in treating and/or preventing a pathogen infection or other infectious disease, for example, in an immunocompromised subject. Non-limiting examples of pathogen includes a virus, bacteria, fungi, parasite and protozoa capable of causing disease.

Non-limiting examples of viruses include, *Retroviridae* (e.g. human immunodeficiency viruses, such as HIV-1 (also referred to as HDTV-III, LAVE or HTLV-III/LAV, or HIV-III; and other isolates, such as HIV-LP; *Picornaviridae* (e.g. polio viruses, hepatitis A virus; enteroviruses, human Coxsackie viruses, rhinoviruses, echoviruses); *Calciviridae* (e.g. strains that cause gastroenteritis); *Togaviridae* (e.g. equine encephalitis viruses, rubella viruses); *Flaviridae* (e.g. dengue viruses, encephalitis viruses, yellow fever viruses); *Coronoviridae* (e.g. coronaviruses); Rhabdoviridae (e.g. vesicular stomatitis viruses, rabies viruses); *Filoviridae* (e.g. ebola viruses); *Paramyxoviridae* (e.g. parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus); *Orthomyxoviridae* (e.g. influenza viruses); *Bungaviridae* (e.g. Hantaan viruses, bunga viruses, phleboviruses and Naira viruses); Arena viridae (hemorrhagic fever viruses); *Reoviridae* (e.g. reoviruses, orbiviurses and rotaviruses); *Birnaviridae*; *Hepadnaviridae* (Hepatitis B virus); *Parvovirida* (parvoviruses); *Papovaviridae* (papilloma viruses, polyoma viruses); *Adenoviridae* (most adenoviruses); *Herpesviridae* (herpes simplex virus (HSV) 1 and 2, varicella zoster virus, cytomegalovirus (CMV), herpes virus; *Poxviridae* (variola viruses, vaccinia viruses, pox viruses); and *Iridoviridae* (e.g. African swine fever virus); and unclassified viruses (e.g. the agent of delta hepatitis (thought to be a defective satellite of hepatitis B virus), the agents of non-A, non-B hepatitis (class 1 =internally transmitted; class 2 =parenterally transmitted (i.e. Hepatitis C); Norwalk and related viruses, and astroviruses).

Non-limiting examples of bacteria include *Pasteurella*, *Staphylococci*, *Streptococcus*, *Escherichia coli*, *Pseudomonas* species, and *Salmonella* species. Specific examples of infectious bacteria include but are not limited to, *Helicobacter pyloris*, *Borelia burgdorferi*, *Legionella pneumophilia*, *Mycobacteria sps* (e.g. *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. kansaii*, *M. gordonae*), *Staphylococcus aureus*, *Neisseria*

gonorrhoeae, *Neisseria meningitidis*, *Listeria monocytogenes*, *Streptococcus pyogenes* (Group A Streptococcus), *Streptococcus agalactiae* (Group B Streptococcus), *Streptococcus* (viridans group), *Streptococcus faecalis*, *Streptococcus bovis*, *Streptococcus* (anaerobic sps.), *Streptococcus pneumoniae*, pathogenic *Campylobacter sp.*, *Enterococcus sp.*, *Haemophilus influenzae*, *Bacillus antracis*, *corynebacterium diphtheriae*, *corynebacterium sp.*, *Erysipelothrix rhusiopathiae*, *Clostridium perfringers*, *Clostridium tetani*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pasturella multocida*, *Bacteroides sp.*, *Fusobacterium nucleatum*, *Streptobacillus moniliformis*, *Treponema pallidium*, *Treponema pertenue*, *Leptospira*, *Rickettsia*, and *Actinomyces israelli*.

In certain embodiments, the pathogen antigen is a viral antigen present in Cytomegalovirus (CMV), a viral antigen present in Epstein Barr Virus (EBV), a viral antigen present in Human Immunodeficiency Virus (HIV), or a viral antigen present in influenza virus.

2.2. T-cell receptor (TCR)

In certain embodiments, the antigen-recognizing receptor is a TCR. A TCR is a disulfide-linked heterodimeric protein consisting of two variable chains expressed as part of a complex with the invariant CD3 chain molecules. A TCR is found on the surface of T cells, and is responsible for recognizing antigens as peptides bound to major histocompatibility complex (MHC) molecules. In certain embodiments, a TCR comprises an alpha chain and a beta chain (encoded by TRA and TRB, respectively). In certain embodiments, a TCR comprises a gamma chain and a delta chain (encoded by TRG and TRD, respectively).

Each chain of a TCR is composed of two extracellular domains: Variable (V) region and a Constant (C) region. The Constant region is proximal to the cell membrane, followed by a transmembrane region and a short cytoplasmic tail. The Variable region binds to the peptide/MHC complex. The variable domain of both chains each has three complementarity determining regions (CDRs).

In certain embodiments, a TCR can form a receptor complex with three dimeric signaling modules CD3δ/ε, CD3γ/ε and CD247 ζ/ζ or ζ/η. When a TCR complex engages with its antigen and MHC (peptide/MHC), the T cell expressing the TCR complex is activated.

In certain embodiments, the antigen-recognizing receptor is a recombinant TCR. In certain embodiments, the antigen-recognizing receptor is a non-naturally occurring

TCR. In certain embodiments, the non-naturally occurring TCR differs from any naturally occurring TCR by at least one amino acid residue. In certain embodiments, the non-naturally occurring TCR differs from any naturally occurring TCR by at least about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 20, about 25, about 30, about 40, about 50, about 60, about 70, about 80, about 90, about 100 or more amino acid residues. In certain embodiments, the non-naturally occurring TCR is modified from a naturally occurring TCR by at least one amino acid residue. In certain embodiments, the non-naturally occurring TCR is modified from a naturally occurring TCR by at least about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 20, about 25, about 30, about 40, about 50, about 60, about 70, about 80, about 90, about 100 or more amino acid residues.

2.3. Chimeric Antigen Receptor (CAR)

In certain embodiments, the antigen-recognizing receptor is a CAR. CARs are engineered receptors, which graft or confer a specificity of interest onto an immune effector cell. CARs can be used to graft the specificity of a monoclonal antibody onto a T cell; with transfer of their coding sequence facilitated by retroviral vectors.

There are three generations of CARs. "First generation" CARs are typically composed of an extracellular antigen-binding domain (e.g., a scFv), which is fused to a transmembrane domain, which is fused to cytoplasmic/intracellular signaling domain. "First generation" CARs can provide *de novo* antigen recognition and cause activation of both $CD4^+$ and $CD8^+$ T cells through their CD3ζ chain signaling domain in a single fusion molecule, independent of HLA-mediated antigen presentation. "Second generation" CARs add intracellular signaling domains from various co-stimulatory molecules (e.g., CD28, 4-1BB, ICOS, OX40) to the cytoplasmic tail of the CAR to provide additional signals to the T cell. "Second generation" CARs comprise those that provide both co-stimulation (e.g., CD28 or 4-1BB) and activation (CD3ζ). "Third generation" CARs comprise those that provide multiple co-stimulation (e.g., CD28 and 4-1BB) and activation (CD3ζ). In certain embodiments, the antigen-recognizing receptor is a first generation CAR. In certain embodiments, the antigen-recognizing receptor is a second generation CAR.

In certain non-limiting embodiments, the extracellular antigen-binding domain of the CAR (embodied, for example, an scFv or an analog thereof) binds to an antigen with a dissociation constant (K_d) of about 2×10^{-7} M or less. In certain embodiments, the K_d

is about 2 x 10^{-7} M or less, about 1 x 10^{-7} M or less, about 9 x 10^{-8} M or less, about 1 x 10^{-8} M or less, about 9 x 10^{-9} M or less, about 5 x 10^{-9} M or less, about 4 x 10^{-9} M or less, about 3 x 10^{-9} or less, about 2 x 10^{-9} M or less, or about 1 x 10^{-9} M or less. In certain non-limiting embodiments, the K_d is about 3 x 10^{-9} M or less. In certain non-limiting embodiments, the K_d is from about 1 x 10^{-9} M to about 3 x 10^{-7} M. In certain non-limiting embodiments, the K_d is from about 1.5 x 10^{-9} M to about 3 x 10^{-7} M. In certain non-limiting embodiments, the K_d is from about 1.5 x 10^{-9} M to about 2.7 x 10^{-7} M.

Binding of the extracellular antigen-binding domain (for example, in an scFv or an analog thereof) can be confirmed by, for example, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), FACS analysis, bioassay (*e.g.*, growth inhibition), or Western Blot assay. Each of these assays generally detect the presence of protein-antibody complexes of particular interest by employing a labeled reagent (*e.g.*, an antibody, or an scFv) specific for the complex of interest. For example, the scFv can be radioactively labeled and used in a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by such means as the use of a γ counter or a scintillation counter or by autoradiography. In certain embodiments, the extracellular antigen-binding domain of the CAR is labeled with a fluorescent marker. Non-limiting examples of fluorescent markers include green fluorescent protein (GFP), blue fluorescent protein (*e.g.*, EBFP, EBFP2, Azurite, and mKalama1), cyan fluorescent protein (*e.g.*, ECFP, Cerulean, and CyPet), and yellow fluorescent protein (*e.g.*, YFP, Citrine, Venus, and YPet).

In accordance with the presently disclosed subject matter, a CARs can comprise an extracellular antigen-binding domain, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen-binding domain specifically binds to an antigen, e.g., a tumor antigen or a pathogen antigen.

In certain embodiments, the extracellular antigen-binding domain of a presently disclosed CAR is a murine scFv. In certain embodiments, the extracellular antigen-binding domain of a presently disclosed CAR is a murine scFv that binds to a murine CD19 polypeptide. In certain embodiments, the extracellular antigen-binding domain is a murine scFv, which comprises the amino acid sequence of SEQ ID NO: 59 and specifically binds to a murine CD19 polypeptide (e.g., a murine CD19 polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 61). In certain

embodiments, the nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 59 is set forth in SEQ ID NO: 60. In certain embodiments, the murine scFv comprises a heavy chain variable region (V_H) comprising the amino acid sequence set forth in SEQ ID NO: 49. In certain embodiments, the murine scFv comprises a light chain variable region (V_L) comprising the amino acid sequence set forth in SEQ ID NO: 50. In certain embodiments, the murine scFv comprises a V_H comprising the amino acid sequence set forth in SEQ ID NO: 49 and a V_L comprising the amino acid sequence set forth in SEQ ID NO: 50 , optionally with (iii) a linker sequence, for example a linker peptide, between the V_H and the V_L. In certain embodiments, the linker comprises amino acids having the sequence set forth in SEQ ID NO: 23. In certain embodiments, the extracellular antigen-binding domain comprises a V_H comprising an amino acid sequence that is at least about 80% (*e.g.*, at least about 85%, at least about 90%, or at least about 95%) homologous to SEQ ID NO: 49. For example, the extracellular antigen-binding domain comprises a V_H comprising an amino acid sequence that is about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% homologous to SEQ ID NO: 49. In certain embodiments, the extracellular antigen-binding domain comprises a V_H comprising the amino acid sequence set forth in SEQ ID NO: 49. In certain embodiments, the extracellular antigen-binding domain comprises a V_L comprising an amino acid sequence that is at least about 80% (*e.g.*, at least about 85%, at least about 90%, or at least about 95%) homologous to SEQ ID NO: 50. For example, the extracellular antigen-binding domain comprises a V_L comprising an amino acid sequence that is about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% homologous to SEQ ID NO: 50. In certain embodiments, the extracellular antigen-binding domain comprises a V_L comprising the amino acid sequence set forth in SEQ ID NO: 50. In certain embodiments, the extracellular antigen-binding domain comprises a V_H comprising an amino acid sequence that is at least about 80% (*e.g.*, at least about 85%, at least about 90%, or at least about 95%) homologous to SEQ ID NO: 49, and a V_L comprising an amino acid sequence that is at least about 80% (*e.g.*, at least about 85%, at least about 90%, or at least about 95%) homologous to SEQ ID NO: 50. In certain embodiments, the extracellular antigen-binding domain comprises a V_H comprising the

amino acid sequence set forth in SEQ ID NO: 49 and a V_L comprising the amino acid sequence set forth in SEQ ID NO: 50. In certain embodiments, the extracellular antigen-binding domain comprises a V_H CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 43, or a conservative modification thereof, a V_H CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 44 or a conservative modification thereof, and a V_H CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 45, a conservative modification thereof. In certain embodiments, the extracellular antigen-binding domain comprises a V_H CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 43, a V_H CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 44, and a V_H CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 45. In certain embodiments, the extracellular antigen-binding domain comprises a V_L CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 46 or a conservative modification thereof, a V_L CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 47 or a conservative modification thereof, and a V_L CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 48 or a conservative modification thereof. In certain embodiments, the extracellular antigen-binding domain comprises a V_L CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 46, a V_L CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 47, and a V_L CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 48. In certain embodiments, the extracellular antigen-binding domain comprises a V_H CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 43 or a conservative modification thereof, a V_H CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 44 or a conservative modification thereof, a V_H CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 45, a conservative modification thereof, a V_L CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 46 or a conservative modification thereof, a V_L CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 47 or a conservative modification thereof, and a V_L CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 48 or a conservative modification thereof. In certain embodiments, the extracellular antigen-binding domain comprises a V_H CDR1 comprising amino acids having the sequence set forth in SEQ ID NO: 43, a V_H CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 44, a V_H CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 45, a V_L CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 46, a V_L CDR2 comprising the amino acid sequence set forth in SEQ ID

NO: 47, and a V_L CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 48.

In certain embodiments, the extracellular antigen-binding domain of a presently disclosed CAR is a murine scFv that binds to a human CD19 polypeptide. In certain embodiments, the extracellular antigen-binding domain is a murine scFv, which comprises the amino acid sequence of SEQ ID NO: 63 and specifically binds to a human CD19 polypeptide (e.g., a human CD19 polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 62). In certain embodiments, the nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 63 is set forth in SEQ ID NO: 64. In certain embodiments, the murine scFv comprises a heavy chain variable region (V_H) comprising the amino acid sequence set forth in SEQ ID NO: 57. In certain embodiments, the murine scFV comprises a light chain variable region (V_L) comprising the amino acid sequence set forth in SEQ ID NO: 58. In certain embodiments, the murine scFv comprises V_H comprising the amino acid sequence set forth in SEQ ID NO: 57 and a V_L comprising the amino acid sequence set forth in SEQ ID NO: 58 , optionally with (iii) a linker sequence, for example a linker peptide, between the V_H and the V_L. In certain embodiments, the linker comprises amino acids having the sequence set forth in SEQ ID NO: 23. In certain embodiments, the extracellular antigen-binding domain comprises a V_H comprising an amino acid sequence that is at least about 80% (*e.g.*, at least about 85%, at least about 90%, or at least about 95%) homologous to SEQ ID NO: 57. For example, the extracellular antigen-binding domain comprises a V_H comprising an amino acid sequence that is about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% homologous to SEQ ID NO: 57. In certain embodiments, the extracellular antigen-binding domain comprises a V_H comprising the amino sequence set forth in SEQ ID NO: 57. In certain embodiments, the extracellular antigen-binding domain comprises a V_L comprising an amino acid sequence that is at least about 80% (*e.g.*, at least about 85%, at least about 90%, or at least about 95%) homologous to SEQ ID NO: 58. For example, the extracellular antigen-binding domain comprises a V_L comprising an amino acid sequence that is about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% homologous to SEQ ID NO: 58. In certain embodiments, the extracellular antigen-

binding domain comprises a V_L comprising the amino acid sequence set forth in SEQ ID NO: 58. certain embodiments, the extracellular antigen-binding domain comprises a V_H comprising an amino acid sequence that is at least about 80% (*e.g.*, at least about 85%, at least about 90%, or at least about 95%) homologous to SEQ ID NO: 57, and a V_L comprising an amino acid sequence that is at least about 80% (*e.g.*, at least about 85%, at least about 90%, or at least about 95%) homologous to SEQ ID NO: 58. In certain embodiments, the extracellular antigen-binding domain comprises a V_H comprising the amino acid sequence set forth in SEQ ID NO: 57 and a V_L comprising the amino acid sequence set forth in SEQ ID NO: 58. In certain embodiments, the extracellular antigen-binding domain comprises a V_H CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 51, or a conservative modification thereof, a V_H CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 52 or a conservative modification thereof, and a V_H CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 53, a conservative modification thereof. In certain embodiments, the extracellular antigen-binding domain comprises a V_H CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 51, a V_H CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 52, and a V_H CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 53. In certain embodiments, the extracellular antigen-binding domain comprises a V_L CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 54 or a conservative modification thereof, a V_L CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 55 or a conservative modification thereof, and a V_L CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 56 or a conservative modification thereof. In certain embodiments, the extracellular antigen-binding domain comprises a V_L CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 54, a V_L CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 55, and a V_L CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 56. In certain embodiments, the extracellular antigen-binding domain comprises a V_H CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 51 or a conservative modification thereof, a V_H CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 52 or a conservative modification thereof, a V_H CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 53, a conservative modification thereof, a V_L CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 54 or a conservative modification thereof, a V_L CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 55 or a conservative modification thereof, and a V_L CDR3 comprising the amino acid sequence set forth in

SEQ ID NO: 56 or a conservative modification thereof. In certain embodiments, the extracellular antigen-binding domain comprises a V_H CDR1 comprising amino acids having the sequence set forth in SEQ ID NO: 51, a V_H CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 52, a V_H CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 53, a V_L CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 54, a V_L CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 55 and a V_L CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 56.

Table 1

		anti-mouse CD19 scFv (1D3)		
CDRs		1	2	3
V_H	a.a.	FYYMH [SEQ ID NO: 43]	RIDPEDESTK YSEKFKN [SEQ ID NO: 44]	GGYYFDY [SEQ ID NO: 45]
V_L	a.a.	QASEDIYSGL A [SEQ ID NO: 46]	GASDLQD [SEQ ID NO: 47]	QQGLTYPRT [SEQ ID NO: 48]
Full V_H		EVQLQQSGAE LVRPGTSVKL SCKVSGDTIT FYYMHFVKQR PGQGLEWIGR IDPEDESTKY SEKFKNKATL TADTSSNTAY LKLSSLTSED TATYFCIYGG YYFDYWGQGV MVTVSS [SEQ ID NO: 49]		
Full V_L		DIQMTQSPAS LSTSLGETVT IQCQASEDIY SGLAWYQQKP GKSPQLLIYG ASDLQDGVPS RFSGSGSGTQ YSLKITSMQT EDEGVYFCQQ GLTYPRTFGG GTKLELKR [SEQ ID NO: 50]		
scFv		MASPLTRFLS LNLLLLGESI ILGSGEAEVQ LQQSGAELVR PGTSVKLSCK VSGDTITFYY MHFVKQRPGQ GLEWIGRIDP EDESTKYSEK FKNKATLTAD TSSNTAYLKL SSLTSEDTAT YFCIYGGYYF DYWGQGVMVT VSSGGGGSGG GGSGGGGSDI QMTQSPASLS TSLGETVTIQ CQASEDIYSG LAWYQQKPGK SPQLLIYGAS DLQDGVPSRF SGSGSGTQYS LKITSMQTED EGVYFCQQGL TYPRTFGGGT KLELKR [SEQ ID NO:59]		
DNA		ATGGCCTCACCGTTGACCCGCTTTCTGTCGCTGAACCTGCTGCTGCTGGGTGAGTCG ATTATCCTGGGGAGTGGAGAAGCTGAAGTCCAGCTGCAGCAGTCTGGGGCTGAGCTT GTGAGACCTGGGACCTCTGTGAAGTTATCTTGCAAAGTTTCTGGCGATACCATTACA TTTTACTACATGCACTTTGTGAAGCAAAGGCCTGGACAGGGTCTGGAATGGATAGGA AGGATTGATCCTGAGGATGAAAGTACTAAATATTCTGAGAAGTTCAAAAACAAGGCG ACACTCACTGCAGATACATCTTCCAACACAGCCTACCTGAAGCTCAGCAGCCTGACC TCTGAGGACACTGCAACCTATTTTTGTATCTACGGAGGATACTACTTTGATTACTGG GGCCAAGGGGTCATGGTCACAGTCTCCTCAGGTGGAGGTGGATCAGGTGGAGGTGGA TCTGGTGGAGGTGGATCTGACATCCAGATGACACAGTCTCCAGCTTCCCTGTCTACA TCTCTGGGAGAAACTGTCACCATCCAATGTCAAGCAAGTGAGGACATTTACAGTGGT TTAGCGTGGTATCAGCAGAAGCCAGGGAAATCTCCTCAGCTCCTGATCTATGGTGCA AGTGACTTACAAGACGGCGTCCCATCACGATTCAGTGGCAGTGGATCTGGCACACAG TATTCTCTCAAGATCACCAGCATGCAAACTGAAGATGAAGGGGTTTATTTCTGTCAA CAGGGTTTAACGTATCCTCGGACGTTCGGTGGCGGCACCAAGCTGGAATTGAAACGG [SEQ ID NO: 60]		

Table 2

		anti-human CD19 scFv (SJ25C1)		
CDRs		1	2	3
V_H	a.a.	GYAFSS [SEQ ID NO: 51]	YPGDGD [SEQ ID NO: 52]	KTISSVVDF [SEQ ID NO: 53]
V_L	a.a.	NVGTNVA [SEQ ID	SATYRN [SEQ ID NO:	FCQQYNRY [SEQ ID

	NO: 54]	55]	NO: 56]
Full V_H	EVKLQQSGAE LVRPGSSVKI SCKASGYAFS SYWMNWVKQR PGQGLEWIGQ IYPGDGDTNY NGKFKGQATL TADKSSSTAY MQLSGLTSED SAVYFCARKT ISSVVDFYFD YWGQGTTVTV SS [SEQ ID NO: 57]		
Full V_L	DIELTQSPKF MSTSVGDRVS VTCKASQNVG TNVAWYQQKP GQSPKPLIYS ATYRNSGVPD RFTGSGSGTD FTLTITNVQS KDLADYFCQQ YNRYPYTSGG GTKLEIKR [SEQ ID NO: 58]		
scFv	MALPVTALLL PLALLLHAEV KLQQSGAELV RPGSSVKISC KASGYAFSSY WMNWVKQRPG QGLEWIGQIY PGDGDTNYNG KFKGQATLTA DKSSSTAYMQ LSGLTSEDSA VYFCARKTIS SVVDFYFDYW GQGTTVTVSS GGGGSGGGGS GGGGSDIELT QSPKFMSTSV GDRVSVTCKA SQNVGTNVAW YQQKPGQSPK PLIYSATYRN SGVPDRFTGS GSGTDFTLTI TNVQSKDLAD YFCQQYNRYP YTSGGGTKLE IKR [SEQ ID NO: 63]		
DNA	ATGGCTCTCCCAGTGACTGCCCTACTGCTTCCCCTAGCGCTTCTCCTGCATGCAGAG GTGAAGCTGCAGCAGTCTGGGGCTGAGCTGGTGAGGCCTGGGTCCTCAGTGAAGATT TCCTGCAAGGCTTCTGGCTATGCATTCAGTAGCTACTGGATGAACTGGGTGAAGCAG AGGCCTGGACAGGGTCTTGAGTGGATTGGACAGATTTATCCTGGAGATGGTGATACT AACTACAATGGAAAGTTCAAGGGTCAAGCCACACTGACTGCAGACAAATCCTCCAGC ACAGCCTACATGCAGCTCAGCGGCCTAACATCTGAGGACTCTGCGGTCTATTTCTGT GCAAGAAAGACCATTAGTTCGGTAGTAGATTTCTACTTTGACTACTGGGGCCAAGGG ACCACGGTCACCGTCTCCTCAGGTGGAGGTGGATCAGGTGGAGGTGGATCTGGTGGA GGTGGATCTGACATTGAGCTCACCCAGTCTCCAAAATTCATGTCCACATCAGTAGGA GACAGGGTCAGCGTCACCTGCAAGGCCAGTCAGAATGTGGGTACTAATGTAGCCTGG TATCAACAGAAACCAGGACAATCTCCTAAACCACTGATTTACTCGGCAACCTACCGG AACAGTGGAGTCCCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTTCACTCTC ACCATCACTAACGTGCAGTCTAAAGACTTGGCAGACTATTTCTGTCAACAATATAAC AGGTATCCGTACACGTCCGGAGGGGGGACCAAGCTGGAGATCAAACGG [SEQ ID NO: 64]		

As used herein, the term "a conservative sequence modification" refers to an amino acid modification that does not significantly affect or alter the binding characteristics of the presently disclosed CAR (*e.g.*, the extracellular antigen-binding domain of the CAR) comprising the amino acid sequence. Conservative modifications can include amino acid substitutions, additions and deletions. Modifications can be introduced into the human scFv of the presently disclosed CAR by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Amino acids can be classified into groups according to their physicochemical properties such as charge and polarity. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid within the same group. For example, amino acids can be classified by charge: positively-charged amino acids include lysine, arginine, histidine, negatively-charged amino acids include aspartic acid, glutamic acid, neutral charge amino acids include alanine, asparagine, cysteine, glutamine, glycine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. In addition, amino acids can be classified by polarity: polar amino acids include arginine (basic polar), asparagine, aspartic acid (acidic polar), glutamic acid (acidic polar), glutamine, histidine (basic polar), lysine (basic polar), serine,

threonine, and tyrosine; non-polar amino acids include alanine, cysteine, glycine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, and valine. Thus, one or more amino acid residues within a CDR region can be replaced with other amino acid residues from the same group and the altered antibody can be tested for retained function (*i.e.*, the functions set forth in (c) through (l) above) using the functional assays described herein. In certain embodiments, no more than one, no more than two, no more than three, no more than four, no more than five residues within a specified sequence or a CDR region are altered.

The V_H and/or V_L amino acid sequences having at least about 80%, at least about 85%, at least about 90%, or at least about 95% (*e.g.*, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99%) homology to a specific sequence (*e.g.*, SEQ ID NOs: 49, 50, 57, and 58) may contain substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the specified sequence(s), but retain the ability to bind to a target antigen (e.g., CD19). In certain embodiments, a total of 1 to 10 amino acids are substituted, inserted and/or deleted in a specific sequence (*e.g.*, SEQ ID NOs: 49, 50, 57, and 58). In certain embodiments, substitutions, insertions, or deletions occur in regions outside the CDRs (*e.g.*, in the FRs) of the extracellular antigen-binding domain. In certain embodiments, the extracellular antigen-binding domain comprises V_H and/or V_L sequence selected from the group consisting of SEQ ID NOs: 49, 50, 57, and 58, including post-translational modifications of that sequence (SEQ ID NO: 49, 50, 57, and 58).

As used herein, the percent homology between two amino acid sequences is equivalent to the percent identity between the two sequences. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm.

The percent homology between two amino acid sequences can be determined using the algorithm of E. Meyers and W. Miller (Comput. Appl. Biosci., 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120

weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent homology between two amino acid sequences can be determined using the Needleman and Wunsch (J. Mol. Biol. 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

Additionally or alternatively, the amino acids sequences of the presently disclosed subject matter can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the XBLAST program (version 2.0) of Altschul, et al. (1990) J. Mol. Biol. 215:403-10. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to the specified sequences (*e.g.*, heavy and light chain variable region sequences of scFv m903, m904, m905, m906, and m900) disclosed herein. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) Nucleic Acids Res. 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used.

2.3.1. Extracellular Antigen-Binding Domain of A CAR

In certain embodiments, the extracellular antigen-binding domain specifically binds to an antigen. In certain embodiments, the extracellular antigen-binding domain is an scFv. In certain embodiments, the scFv is a human scFv. In certain embodiments, the scFv is a humanized scFv. In certain embodiments, the scFv is a murine scFv. In certain embodiments, the extracellular antigen-binding domain is a Fab, which is optionally crosslinked. In certain embodiments, the extracellular antigen-binding domain is a $F(ab)_2$. In certain embodiments, any of the foregoing molecules may be comprised in a fusion protein with a heterologous sequence to form the extracellular antigen-binding domain. In certain embodiments, the scFv is identified by screening scFv phage library with an antigen-Fc fusion protein. In certain embodiments, the antigen is a tumor antigen. In certain embodiments, the antigen is a pathogen antigen.

2.3.2. Transmembrane Domain of a CAR

In certain non-limiting embodiments, the transmembrane domain of the CAR comprises a hydrophobic alpha helix that spans at least a portion of the membrane. Different transmembrane domains result in different receptor stability. After antigen

recognition, receptors cluster and a signal is transmitted to the cell. In accordance with the presently disclosed subject matter, the transmembrane domain of the CAR can comprise a CD8 polypeptide, a CD28 polypeptide, a CD3ζ polypeptide, a CD4 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an ICOS polypeptide, a synthetic peptide (not based on a protein associated with the immune response), or a combination thereof.

In certain embodiments, the transmembrane domain comprises a CD8 polypeptide. In certain embodiments, the CD8 polypeptide comprises or has an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to the sequence having a NCBI Reference No: NP_001139345.1 (SEQ ID NO: 9) (homology herein may be determined using standard software such as BLAST or FASTA) as provided below, or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. In certain embodiments, the CD8 polypeptide comprises or has an amino acid sequence that is a consecutive portion of SEQ ID NO: 9 which is at least 20, or at least 30, or at least 40, or at least 50, and up to 235 amino acids in length. Alternatively or additionally, in non-limiting various embodiments, the CD8 polypeptide comprises or has an amino acid sequence of amino acids 1 to 235, 1 to 50, 50 to 100, 100 to 150, 150 to 200, or 200 to 235 of SEQ ID NO: 9. In certain embodiments, the CAR of the presently disclosed comprises a transmembrane domain comprising a CD8 polypeptide that comprises or has an amino acid sequence of amino acids 137 to 209 of SEQ ID NO: 9.

MALPVTALLLPLALLLHAARPSQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLFQPRGAAASPTFLL
YLSQNKPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRENEGYYFCSALSNSIMYFSHFVPVFLPAKPTTTPA
PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRRR
VCKCPRPVVKSGDKPSLSARYV [SEQ ID NO: 9]

In certain embodiments, the CD8 polypeptide comprises or has an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to the sequence having a NCBI Reference No: AAA92533.1 (SEQ ID NO: 10) (homology herein may be determined using standard software such as BLAST or FASTA) as provided below, or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. In certain embodiments, the CD8 polypeptide comprises or has an amino acid sequence that is a consecutive portion of SEQ ID NO: 10

which is at least about 20, or at least about 30, or at least about 40, or at least about 50, or at least about 60, or at least about 70, or at least about 100, or at least about 200, and up to 247 amino acids in length. Alternatively or additionally, in non-limiting various embodiments, the CD8 polypeptide comprises or has an amino acid sequence of amino acids 1 to 247, 1 to 50, 50 to 100, 100 to 150, 150 to 200, 151 to 219, or 200 to 247 of SEQ ID NO: 10. In certain embodiments, the CAR of the presently disclosed comprises a transmembrane domain comprising a CD8 polypeptide that comprises or has an amino acid sequence of amino acids 151 to 219 of SEQ ID NO: 10.

1 MASPLTRFLS LNLLLMGESI ILGSGEAKPQ APELRIFPKK MDAELGQKVD LVCEVLGSVS
61 QGCSWLFQNS SSKLPQPTFV VYMASSHNKI TWDEKLNSSK LFSAVRDTNN KYVLTLNKFS
121 KENEGYYFCS VISNSVMYFS SVVPVLQKVN STTTKPVLRT PSPVHPTGTS QPQRPEDCRP
181 RGSVKGTGLD FACDIYIWAP LAGICVAPLL SLIITLICYH RSRKRVCKCP RPLVRQEGKP
241 RPSEKIV [SEQ ID NO: 10]

In certain embodiments, the CD8 polypeptide comprises or has the amino acid sequence set forth in SEQ ID NO: 11, which is provided below:

STTTKPVLRTPSPVHPTGTSQPQRPEDCRPRGSVKGTGLDFACDIYIWAPLAGICVALLLSLIITLICY [SEQ ID NO: 11]

In accordance with the presently disclosed subject matter, a "CD8 nucleic acid molecule" refers to a polynucleotide encoding a CD8 polypeptide.

In certain embodiments, the CD8 nucleic acid molecule encoding the CD8 polypeptide having the amino acid sequence set forth in SEQ ID NO: 11 comprises or has nucleic acids having the sequence set forth in SEQ ID NO: 12 as provided below.

TCTACTACTACCAAGCCAGTGCTGCGAACTCCCTCACCTGTGCACCCTACCGGGACATCTCAGCCCCAGAG ACCAGAAGATTGTCGGCCCCGTGGCTCAGTGAAGGGGACCGGATTGGACTTCGCCTGTGATATTTACATCT GGGCACCCTTGGCCGGAATCTGCGTGGCCCTTCTGCTGTCCTTGATCATCACTCTCATCTGCTAC [SEQ ID NO: 12]

In certain embodiments, the transmembrane domain of a presently disclosed CAR comprises a CD28 polypeptide. The CD28 polypeptide can have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or 100% homologous to the sequence having a NCBI Reference No: P10747 or NP_006130 (SEQ ID No: 2), or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. In non-limiting certain embodiments, the CD28 polypeptide comprises or has an amino acid sequence that is a consecutive portion of SEQ ID NO: 2 which is at least 20, or at least 30, or at least 40, or at least 50, and up to 220 amino acids in length. Alternatively or

additionally, in non-limiting various embodiments, the CD28 polypeptide comprises or has an amino acid sequence of amino acids 1 to 220, 1 to 50, 50 to 100, 100 to 150, 114 to 220, 150 to 200, or 200 to 220 of SEQ ID NO: 2. In certain embodiments, the CD28 polypeptide comprised in the transmembrane domain of a presently disclosed CAR comprises or has an amino acid sequence of amino acids 153 to 179 of SEQ ID NO: 2.

SEQ ID NO: 2 is provided below:

1 MLRLLLALNL FPSIQVTGNK ILVKQSPMLV AYDNAVNLSC KYSYNLFSRE FRASLHKGLD
61 SAVEVCVVYG NYSQQLQVYS KTGFNCDGKL GNESVTFYLQ NLYVNQTDIY FCKIEVMYPP
121 PYLDNEKSNG TIIHVKGKHL CPSPLFPGPS KPFWVLVVVG GVLACYSLLV TVAFIIFWVR
181 SKRSRLLHSD YMNMTPRRPG PTRKHYQPYA PPRDFAAYRS [SEQ ID NO: 2]

In accordance with the presently disclosed subject matter, a "CD28 nucleic acid molecule" refers to a polynucleotide encoding a CD28 polypeptide. In certain embodiments, the CD28 nucleic acid molecule encoding the CD28 polypeptide having amino acids 153 to 179 of SEQ ID NO: 2 comprises or has nucleic acids having the sequence set forth in SEQ ID NO: 22 as provided below.

ttttgggtgctggtggtggttggtggagtcctggcttgctatagcttgctagtaacagtggcctttattat tttctgggt**g** [SEQ ID NO: 22]

In certain embodiments, the intracellular signaling domain of the CAR comprises a murine CD28 transmembrane domain. The murine CD28 transmembrane domain can comprise or have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to SEQ ID NO: 76 or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. SEQ ID NO: 76 is provided below:

FWALVVVAGV LFCYGLLVTV ALCVIWT [SEQ ID NO: 76].

An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 76 is set forth in SEQ ID NO: 77, which is provided below.

TTTTGGGCACTGGTCGTGGTTGCTGGAGTCCTGTTTTGTTATGGCTTGCTAGTGACAGTGGCTCTTTGTGT TATCTGGACA [SEQ ID NO: 77]

In certain embodiments, the intracellular signaling domain of the CAR comprises a human CD28 transmembrane domain. The human CD28 transmembrane domain can comprise or have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to SEQ ID NO: 78 or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. SEQ ID NO: 78 is provided below:

FWVLVVVGGV LACYSLLVTV AFIIFWV [SEQ ID NO: 78]

An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 78 is set forth in SEQ ID NO: 79, which is provided below.

TTTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTAT TTTCTGGGTG [SEQ ID NO: 79]

In certain non-limiting embodiments, a CAR can also comprise a spacer region that links the extracellular antigen-binding domain to the transmembrane domain. The spacer region can be flexible enough to allow the antigen binding domain to orient in different directions to facilitate antigen recognition. The spacer region can be the hinge region from IgG1, or the CH_2CH_3 region of immunoglobulin and portions of CD3, a portion of a CD28 polypeptide (e.g., a portion of SEQ ID NO: 2), a portion of a CD8 polypeptide (e.g., a portion of SEQ ID NO: 9, or a portion of SEQ ID NO: 10), a variation of any of the foregoing which is at least about 80%, at least about 85%, at least about 90%, or at least about 95% homologous thereto, or a synthetic spacer sequence.

2.3.3. Intracellular Signaling Domain of a CAR

In certain non-limiting embodiments, an intracellular signaling domain of the CAR comprises a CD3ζ polypeptide, which can activate or stimulate a cell (*e.g.*, a cell of the lymphoid lineage, *e.g.*, a T cell). CD3ζ comprises 3 ITAMs, and transmits an activation signal to the cell (*e.g.*, a cell of the lymphoid lineage, *e.g.*, a T cell) after antigen is bound. The intracellular signaling domain of the CD3ζ-chain is the primary transmitter of signals from endogenous TCRs. In certain embodiments, the CD3ζ polypeptide comprises or has an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to the sequence having a NCBI Reference No: NP_932170 (SEQ ID No: 1), or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. In certain non-limiting embodiments, the CD3ζ polypeptide comprises or has an amino acid sequence that is a consecutive portion of SEQ ID NO: 1, which is at least 20, or at least 30, or at least 40, or at least 50, and up to 164 amino acids in length. Alternatively or additionally, in non-limiting various embodiments, the CD3ζ polypeptide comprises or has an amino acid sequence of amino acids 1 to 164, 1 to 50, 50 to 100, 100 to 150, or 150 to 164 of SEQ ID NO: 1. In certain embodiments, the CD3ζ polypeptide comprises or has an amino acid sequence of amino acids 52 to 164 of SEQ ID NO: 1.

SEQ ID NO: 1 is provided below:

1 MKWKALFTAA ILQAQLPITE AQSFGLLDPK LCYLLDGILF IYGVILTALF LRVKFSRSAD

61 APAYQQGQNQ LYNELNLGRR EEYDVLDKRR GRDPEMGGKP QRRKNPQEGL YNELQKDKMA
121 EAYSEIGMKG ERRRGKGHDG LYQGLSTATK DTYDALHMQA LPPR [SEQ ID NO: 1]

In certain embodiments, the CD3ζ polypeptide comprises or has an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to the sequence having a NCBI Reference No: NP_001106864.2 (SEQ ID No: 13), or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. In certain non-limiting embodiments, the CD3ζ polypeptide comprises or has an amino acid sequence that is a consecutive portion of SEQ ID NO: 13, which is at least about 20, or at least about 30, or at least about 40, or at least about 50, or at least about 90, or at least about 100, and up to 188 amino acids in length. Alternatively or additionally, in non-limiting various embodiments, the CD3ζ polypeptide comprises or has an amino acid sequence of amino acids 1 to 164, 1 to 50, 50 to 100, 52 to 142, 100 to 150, or 150 to 188 of SEQ ID NO: 13. In certain embodiments, the CD3ζ polypeptide comprises or has an amino acid sequence of amino acids 52 to 142 of SEQ ID NO: 13.

SEQ ID NO: 13 is provided below:

1 MKWKVSVLAC ILHVRFPGAE AQSFGLLDPK LCYLLDGILF IYGVIITALY LRAKFSRSAE
61 TAANLQDPNQ LYNELNLGRR EEYDVLEKKR ARDPEMGGKQ RRRNPQEGVY NALQKDKMAE
121 AYSEIGTKGE RRRGKGHDGL YQDSHFQAVQ FGNRREREGS ELTRTLGLRA RPKACRHKKP
181 LSLPAAVS [SEQ ID NO: 13]

In certain embodiments, the CD3ζ polypeptide comprises or has the amino acid sequence set forth in SEQ ID NO: 14, which is provided below:

RAKFSRSAETAANLQDPNQLYNELNLGRREEYDVLEKKRARDPEMGGKQQRRRNPQEGVYNALQKDKMAEA
YSEIGTKGERRRGKGHDGLYQGLSTATKDTYDALHMQTLAPR [SEQ ID NO: 14]

In accordance with the presently disclosed subject matter, a "CD3ζ nucleic acid molecule" refers to a polynucleotide encoding a CD3ζ polypeptide. In certain embodiments, the CD3ζ nucleic acid molecule encoding the CD3ζ polypeptide having the amino acid sequence set forth in SEQ ID NO: 14 comprises or has the nucleotide sequence set forth in SEQ ID NO: 15 as provided below.

AGAGCAAAATTCAGCAGGAGTGCAGAGACTGCTGCCAACCTGCAGGACCCCAACCAGCTCTACAATGAGCT
CAATCTAGGGCGAAGAGAGGAATATGACGTCTTGGAGAAGAAGCGGGCTCGGGATCCAGAGATGGGAGGCA
AACAGCAGAGGAGGAGGAACCCCCAGGAAGGCGTATACAATGCACTGCAGAAAGACAAGATGGCAGAAGCC
TACAGTGAGATCGGCACAAAAGGCGAGAGGCGGAGAGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAG
CACTGCCACCAAGGACACCTATGATGCCCTGCATATGCAGACCCTGGCCCCTCGCTAA [SEQ ID NO:
15]

In certain embodiments, the intracellular signaling domain of the CAR comprises a murine CD3ζ polypeptide. The murine CD3ζ polypeptide can comprise or have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to SEQ ID NO: 72 or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. SEQ ID NO: 72 is provided below:

RAKFSRSAET AANLQDPNQL YNELNLGRRE EYDVLEKKRA RDPEMGGKQQ RRRNPQEGVY NALQKDKMAE AYSEIGTKGE RRRGKGHDGL YQGLSTATKD TYDALHMQTL APR [SEQ ID NO: 72].

An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 72 is set forth in SEQ ID NO: 73, which is provided below.

AGAGCAAAATTCAGCAGGAGTGCAGAGACTGCTGCCAACCTGCAGGACCCCAACCAGCTCTACAATGAGCT CAATCTAGGGCGAAGAGAGGAATATGACGTCTTGGAGAAGAAGCGGGCTCGGGATCCAGAGATGGGAGGCA AACAGCAGAGGAGGAGGAACCCCCAGGAAGGCGTATACAATGCACTGCAGAAAGACAAGATGGCAGAAGCC TACAGTGAGATCGGCACAAAAGGCGAGAGGCGGAGAGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAG CACTGCCACCAAGGACACCTATGATGCCCTGCATATGCAGACCCTGGCCCCTCGC [SEQ ID NO: 73]

In certain embodiments, the intracellular signaling domain of the CAR comprises a human CD3ζ polypeptide. The human CD3ζ polypeptide can comprise or have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to SEQ ID NO: 74 or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. SEQ ID NO: 74 is provided below:

RVKFSRSADA PAYQQGQNQL YNELNLGRRE EYDVLDKRRG RDPEMGGKPR RKNPQEGLYN ELQKDKMAEA YSEIGMKGER RRGKGHDGLY QGLSTATKDT YDALHMQALP PR [SEQ ID NO: 74].

An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 74 is set forth in SEQ ID NO: 75, which is provided below.

AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCT CAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAA AGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTAC AGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTAC AGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGC [SEQ ID NO: 75]

In certain non-limiting embodiments, an intracellular signaling domain of the CAR does not comprise a co-stimulatory signaling region, i.e., the CAR is a first generation CAR.

In certain non-limiting embodiments, an intracellular signaling domain of the CAR further comprises at least a co-stimulatory signaling region. In certain embodiments, the co-stimulatory region comprises at least one co-stimulatory molecule, which can provide optimal lymphocyte activation. As used herein, "co-stimulatory molecules" refer to cell surface molecules other than antigen receptors or their ligands that are required for an efficient response of lymphocytes to antigen. The at least one co-stimulatory signaling region can include a CD28 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an ICOS polypeptide, a DAP-10 polypeptide, or a combination thereof. The co-stimulatory molecule can bind to a co-stimulatory ligand, which is a protein expressed on cell surface that upon binding to its receptor produces a co-stimulatory response, *i.e.*, an intracellular response that effects the stimulation provided when an antigen binds to its CAR molecule. Co-stimulatory ligands, include, but are not limited to CD80, CD86, CD70, OX40L, and 4-1BBL. As one example, a 4-1BB ligand (*i.e.*, 4-1BBL) may bind to 4-1BB (also known as "CD137") for providing an intracellular signal that in combination with a CAR signal induces an effector cell function of the CAR^+ T cell. CARs comprising an intracellular signaling domain that comprises a co-stimulatory signaling region comprising 4-1BB, ICOS or DAP-10 are disclosed in U.S. 7,446,190, which is herein incorporated by reference in its entirety.

In certain embodiments, the intracellular signaling domain of the CAR comprises a co-stimulatory signaling region that comprises a CD28 polypeptide. The CD28 polypeptide can comprise or have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or 100% homologous to the sequence having a NCBI Reference No: P10747 or NP_006130 (SEQ ID NO: 2), or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. In non-limiting certain embodiments, the CD28 polypeptide comprises or has an amino acid sequence that is a consecutive portion of SEQ ID NO: 2 which is at least 20, or at least 30, or at least 40, or at least 50, and up to 220 amino acids in length. Alternatively or additionally, in non-limiting various embodiments, the CD28 polypeptide comprises or has an amino acid sequence of amino acids 1 to 220, 1 to 50, 50 to 100, 100 to 150, 114 to 220, 150 to 200, or 200 to 220 of SEQ ID NO: 2. In certain embodiments, the intracellular signaling domain of the CAR comprises a co-stimulatory signaling region that comprises a CD28 polypeptide comprising or having an amino acid sequence of amino acids 180 to 220 of SEQ ID NO: 2.

In certain embodiments, the CD28 polypeptide comprises or has an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to the sequence having a NCBI Reference No: NP_031668.3 (SEQ ID NO: 16), or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. In non-limiting certain embodiments, the CD28 polypeptide comprises or has an amino acid sequence that is a consecutive portion of SEQ ID NO: 16 which is at least about 20, or at least about 30, or at least about 40, or at least about 50, and up to 218 amino acids in length. Alternatively or additionally, in non-limiting various embodiments, the CD28 polypeptide comprises or has an amino acid sequence of amino acids 1 to 218, 1 to 50, 50 to 100, 100 to 150, 114 to 220, 150 to 200, 178 to 218, or 200 to 220 of SEQ ID NO: 16. In certain embodiments, the co-stimulatory signaling region of a presently disclosed CAR comprises a CD28 polypeptide that comprises or has the amino acids 178 to 218 of SEQ ID NO: 16.

SEQ ID NO: 16 is provided below:

1 MTLRLLFLAL NFFSVQVTEN KILVKQSPLL VVDSNEVSLS CRYSYNLLAK EFRASLYKGV
61 NSDVEVCVGN GNFTYQPQFR SNAEFNCDGD FDNETVTFRL WNLHVNHTDI YFCKIEFMYP
121 PPYLDNERSN GTIIHIKEKH LCHTQSSPKL FWALVVVAGV LFCYGLLVTV ALCVIWTNSR
181 RNRLLQSDYM NMTPRRPGLT RKPYQPYAPA RDFAAYRP [SEQ ID NO: 16]

In accordance with the presently disclosed subject matter, a "CD28 nucleic acid molecule" refers to a polynucleotide encoding a CD28 polypeptide. In certain embodiments, a CD28 nucleic acid molecule that encodes a CD28 polypeptide comprised in the co-stimulatory signaling region of a presently disclosed CAR (e.g., amino acids 178 to 218 of SEQ ID NO: 16) comprises or has a nucleotide sequence set forth in SEQ ID NO: 17, which is provided below.

AATAGTAGAAGGAACAGACTCCTTCAAAGTGACTACATGAACATGACTCCCCGGAGGCCTGGGCTCACTCG
AAAGCCTTACCAGCCCTACGCCCCTGCCAGAGACTTTGCAGCGTACCGCCCC [SEQ ID NO: 17]

In certain embodiments, the intracellular signaling domain of the CAR comprises a murine intracellular signaling domain of CD28. The murine intracellular signaling domain of CD28 can comprise or have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to SEQ ID NO: 68 or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. SEQ ID NO: 68 is provided below:

NSRRNRLLQS DYMNMTPRRP GLTRKPYQPY APARDFAAYR P [SEQ ID NO: 68]

An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 68 is set forth in SEQ ID NO: 69, which is provided below.

AATAGTAGAAGGAACAGACTCCTTCAAAGTGACTACATGAACATGACTCCCCGGAGGCCTGGGCTCACTCG
AAAGCCTTACCAGCCCTACGCCCCTGCCAGAGACTTTGCAGCGTACCGCCCC [SEQ ID NO: 69]

In certain embodiments, the intracellular signaling domain of the CAR comprises a human intracellular signaling domain of CD28. The human intracellular signaling domain of CD28 can comprise or have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to SEQ ID NO: 70 or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. SEQ ID NO: 70 is provided below:

RSKRSRLLHS DYMNMTPRRP GPTRKHYQPY APPRDFAAYR S [SEQ ID NO: 70]

An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 70 is set forth in SEQ ID NO: 71, which is provided below.

AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCCGGGCCCACCCG
CAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCCTATCGCTCC [SEQ ID NO: 71]

In certain embodiments, the intracellular signaling domain of the CAR comprises a co-stimulatory signaling region that comprises two co-stimulatory molecules: CD28 and 4-1BB or CD28 and OX40.

4-1BB can act as a tumor necrosis factor (TNF) ligand and have stimulatory activity. The 4-1BB polypeptide can comprise or have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to the sequence having a NCBI Reference No: P41273 or NP_001552 (SEQ ID NO: 3) or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

SEQ ID NO: 3 is provided below:

1 MGNSCYNIVA TLLLVLNFER TRSLQDPCSN CPAGTFCDNN RNQICSPCPP NSFSSAGGQR
61 TCDICRQCKG VFRTRKECSS TSNAECDCTP GFHCLGAGCS MCEQDCKQGQ ELTKKGCKDC
121 CFGTFNDQKR GICRPWTNCS LDGKSVLVNG TKERDVVCGP SPADLSPGAS SVTPPAPARE
181 PGHSPQIISF FLALTSTALL FLLFFLTLRF SVVKRGRKKL LYIFKQPFMR PVQTTQEEDG
241 CSCRFPEEEE GGCEL [SEQ ID NO: 3]

In accordance with the presently disclosed subject matter, a "4-1BB nucleic acid molecule" refers to a polynucleotide encoding a 4-1BB polypeptide.

In certain embodiments, the intracellular signaling domain of the CAR comprises an intracellular signaling domain of 4-1BB. The intracellular signaling domain of 4-1BB

can comprise or have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to SEQ ID NO: 66 or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. SEQ ID NO: 66 is provided below:

KRGRKKLLYI FKQPFMRPVQ TTQEEDGCSC RFPEEEEGGC EL [SEQ ID NO: 66]

An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 66 is set forth in SEQ ID NO: 67, which is provided below.

AAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATTTATGAGACCAGTACAAACTACTCA AGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTG [SEQ ID NO: 67]

An OX40 polypeptide can comprise or have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to the sequence having a NCBI Reference No: P43489 or NP_003318 (SEQ ID NO: 18), or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

SEQ ID NO: 18 is provided below:

1 MCVGARRLGR GPCAALLLLG LGLSTVTGLH CVGDTYPSND RCCHECRPGN GMVSRCSRSQ
61 NTVCRPCGPG FYNDVVSSKP CKPCTWCNLR SGSERKQLCT ATQDTVCRCR AGTQPLDSYK
121 PGVDCAPCPP GHFSPGDNQA CKPWTNCTLA GKHTLQPASN SSDAICEDRD PPATQPQETQ
181 GPPARPITVQ PTEAWPRTSQ GPSTRPVEVP GGRAVAAILG LGLVLGLLGP LAILLALYLL
241 RRDQRLPPDA HKPPGGGSFR TPIQEEQADA HSTLAKI [SEQ ID NO: 18]

In accordance with the presently disclosed subject matter, an "OX40 nucleic acid molecule" refers to a polynucleotide encoding an OX40 polypeptide.

An ICOS polypeptide can comprise or have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to the sequence having a NCBI Reference No: NP_036224 (SEQ ID NO: 19) or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

SEQ ID NO: 19 is provided below:

1 MKSGLWYFFL FCLRIKVLTG EINGSANYEM FIFHNGGVQI LCKYPDIVQQ FKMQLLKGGQ
61 ILCDLTKTKG SGNTVSIKSL KFCHSQLSNN SVSFFLYNLD HSHANYYFCN LSIFDPPPFK
121 VTLTGGYLHI YESQLCCQLK FWLPIGCAAF VVVCILGCIL ICWLTKKKYS SSVHDPNGEY
181 MFMRAVNTAK KSRLTDVTL [SEQ ID NO: 19]

In accordance with the presently disclosed subject matter, an "ICOS nucleic acid molecule" refers to a polynucleotide encoding an ICOS polypeptide.

In certain embodiments, a presently disclosed CAR further comprises an inducible promoter, for expressing nucleic acid sequences in human cells. Promoters for use in expressing CAR genes can be a constitutive promoter, such as ubiquitin C (UbiC) promoter.

In certain embodiments, a presently disclosed CAR comprises an extracellular antigen-binding domain that binds to CD19 (e.g., murine CD19), a transmembrane domain comprising a CD28 polypeptide, and an intracellular signaling domain comprising a CD3ζ polypeptide (e.g., a murine CD3ζ polypeptide), wherein the intracellular signaling domain does not comprise a co-stimulatory signaling region, namely, the CAR is a first generation CAR. In certain embodiments, the CAR is designated as "m19mz" (or "am19mz"). In certain embodiments, the CAR (e.g., m19mz) comprises an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to the amino acid sequence set forth in SEQ ID NO: 5, which is provided below.

MASPLTRFLS LNLLLLGESI ILGSGEAEVQ LQQSGAELVR PGTSVKLSCK VSGDTITFYY
MHFVKQRPGQ GLEWIGRIDP EDESTKYSEK FKNKATLTAD TSSNTAYLKL SSLTSEDTAT
YFCIYGGYYF DYWGQGVMVT VSSGGGGSGG GGSGGGGSDI QMTQSPASLS TSLGETVTIQ
CQASEDIYSG LAWYQQKPGK SPQLLIYGAS DLQDGVPSRF SGSGSGTQYS LKITSMQTED
EGVYFCQQGL TYPRTFGGGT KLELKRAAAE QKLISEEDLI EFMYPPPYLD NERSNGTIIH
IKEKHLCHTQ SSPKLFWALV VVAGVLFCYG LLVTVALCVI WTRAKFSRSA ETAANLQDPN
QLYNELNLGR REEYDVLEKK RARDPEMGGK QQRRRNPQEG VYNALQKDKM AEAYSEIGTK
GERRRGKGHD GLYQGLSTAT KDTYDALHMQ TLAPR [SEQ ID NO:5]

SEQ ID NO: 5 includes a CD8 leader sequence at amino acids 1 to 27, and is able to bind to CD19 (e.g., murine CD19).

An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 5 is set forth in SEQ ID NO: 65, which is provided below.

ATGGCCTCACCGTTGACCCGCTTTCTGTCGCTGAACCTGCTGCTGCTGGGTGAGTCGATTATCCTGGG
GAGTGGAGAAGCTGAAGTCCAGCTGCAGCAGTCTGGGGCTGAGCTTGTGAGACCTGGGACCTCTGTGA
AGTTATCTTGCAAAGTTTCTGGCGATACCATTACATTTTACTACATGCACTTTGTGAAGCAAAGGCCT
GGACAGGGTCTGGAATGGATAGGAAGGATTGATCCTGAGGATGAAAGTACTAAATATTCTGAGAAGTT
CAAAAACAAGGCGACACTCACTGCAGATACATCTTCCAACACAGCCTACCTGAAGCTCAGCAGCCTGA
CCTCTGAGGACACTGCAACCTATTTTTGTATCTACGGAGGATACTACTTTGATTACTGGGGCCAAGGG
GTCATGGTCACAGTCTCCTCAGGTGGAGGTGGATCAGGTGGAGGTGGATCTGGTGGAGGTGGATCTGA
CATCCAGATGACACAGTCTCCAGCTTCCCTGTCTACATCTCTGGGAGAAACTGTCACCATCCAATGTC
AAGCAAGTGAGGACATTTACAGTGGTTTAGCGTGGTATCAGCAGAAGCCAGGGAAATCTCCTCAGCTC
CTGATCTATGGTGCAAGTGACTTACAAGACGGCGTCCCATCACGATTCAGTGGCAGTGGATCTGGCAC

ACAGTATTCTCTCAAGATCACCAGCATGCAAACTGAAGATGAAGGGGTTTATTTCTGTCAACAGGGTT
TAACGTATCCTCGGACGTTCGGTGGCGGCACCAAGCTGGAATTGAAACGGGCGGCCGCAGAACAGAAA
CTGATCTCTGAAGAAGACCTGATTGAGTTCATGTACCCTCCGCCTTACCTAGACAACGAGAGGAGCAA
TGGAACTATTATTCACATAAAAGAGAAACATCTTTGTCATACTCAGTCATCTCCTAAGCTGTTTTGGG
CACTGGTCGTGGTTGCTGGAGTCCTGTTTTGTTATGGCTTGCTAGTGACAGTGGCTCTTTGTGTTATC
TGGACAAGAGCAAAATTCAGCAGGAGTGCAGAGACTGCTGCCAACCTGCAGGACCCCAACCAGCTCTA
CAATGAGCTCAATCTAGGGCGAAGAGAGGAATATGACGTCTTGGAGAAGAAGCGGGCTCGGGATCCAG
AGATGGGAGGCAAACAGCAGAGGAGGAGGAACCCCCAGGAAGGCGTATACAATGCACTGCAGAAAGAC
AAGATGGCAGAAGCCTACAGTGAGATCGGCACAAAAGGCGAGAGGCGGAGAGGCAAGGGGCACGATGG
CCTTTACCAGGGTCTCAGCACTGCCACCAAGGACACCTATGATGCCCTGCATATGCAGACCCTGGCCC
CTCGCTAA [SEQ ID NO: 65]

In certain embodiments, a presently disclosed CAR comprises an extracellular antigen-binding domain that binds to CD19 (e.g., murine CD19), a transmembrane domain comprising a CD28 polypeptide, and an intracellular signaling domain comprising a CD3ζ polypeptide (e.g., a murine CD3ζ polypeptide) and a co-stimulatory signaling region comprising a CD28 polypeptide (e.g., a murine CD28 polypeptide). In certain embodiments, the CAR is designated as "m19m28z" (or "am19m28z"). In certain embodiments, the CAR (e.g., m19m28z) comprises an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to the amino acid sequence set forth in SEQ ID NO: 6, which is provided below.

MASPLTRFLS LNLLLLGESI ILGSGEAEVQ LQQSGAELVR PGTSVKLSCK VSGDTITFYY
MHFVKQRPGQ GLEWIGRIDP EDESTKYSEK FKNKATLTAD TSSNTAYLKL SSLTSEDTAT
YFCIYGGYYF DYWGQGVMVT VSSGGGGSGG GGSGGGGSDI QMTQSPASLS TSLGETVTIQ
CQASEDIYSG LAWYQQKPGK SPQLLIYGAS DLQDGVPSRF SGSGSGTQYS LKITSMQTED
EGVYFCQQGL TYPRTFGGGT KLELKRAAAE QKLISEEDLI EFMYPPPYLD NERSNGTIIH
IKEKHLCHTQ SSPKLFWALV VVAGVLFCYG LLVTVALCVI WTNSRRNRLL QSDYMNMTPR
RPGLTRKPYQ PYAPARDFAA YRPRAKFSRS AETAANLQDP NQLYNELNLG RREEYDVLEK
KRARDPEMGG KQQRRRNPQE GVYNALQKDK MAEAYSEIGT KGERRRGKGH DGLYQGLSTA
TKDTYDALHM QTLAPR [SEQ ID NO: 6]

SEQ ID NO: 6 includes a CD8 leader sequence at amino acids 1 to 27, and is able to bind to CD19 (e.g., murine CD19). An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 6 is set forth in SEQ ID NO:7, which is provided below.

ATGGCCTCACCGTTGACCCGCTTTCTGTCGCTGAACCTGCTGCTGCTGGGTGAGTCGATTATCCTGGG
GAGTGGAGAAGCTGAAGTCCAGCTGCAGCAGTCTGGGGCTGAGCTTGTGAGACCTGGGACCTCTGTGA
AGTTATCTTGCAAAGTTTCTGGCGATACCATTACATTTTACTACATGCACTTTGTGAAGCAAAGGCCT

GGACAGGGTCTGGAATGGATAGGAAGGATTGATCCTGAGGATGAAAGTACTAAATATTCTGAGAAGTT
CAAAAACAAGGCGACACTCACTGCAGATACATCTTCCAACACAGCCTACCTGAAGCTCAGCAGCCTGA
CCTCTGAGGACACTGCAACCTATTTTTGTATCTACGGAGGATACTACTTTGATTACTGGGGCCAAGGG
GTCATGGTCACAGTCTCCTCAGGTGGAGGTGGATCAGGTGGAGGTGGATCTGGTGGAGGTGGATCTGA
CATCCAGATGACACAGTCTCCAGCTTCCCTGTCTACATCTCTGGGAGAAACTGTCACCATCCAATGTC
AAGCAAGTGAGGACATTTACAGTGGTTTAGCGTGGTATCAGCAGAAGCCAGGGAAATCTCCTCAGCTC
CTGATCTATGGTGCAAGTGACTTACAAGACGGCGTCCCATCACGATTCAGTGGCAGTGGATCTGGCAC
ACAGTATTCTCTCAAGATCACCAGCATGCAAACTGAAGATGAAGGGGTTTATTTCTGTCAACAGGGTT
TAACGTATCCTCGGACGTTCGGTGGCGGCACCAAGCTGGAATTGAAACGGGCGGCCGCAGAACAGAAA
CTGATCTCTGAAGAAGACCTGATTGAGTTCATGTACCCTCCGCCTTACCTAGACAACGAGAGGAGCAA
TGGAACTATTATTCACATAAAAGAGAAACATCTTTGTCATACTCAGTCATCTCCTAAGCTGTTTTGGG
CACTGGTCGTGGTTGCTGGAGTCCTGTTTTGTTATGGCTTGCTAGTGACAGTGGCTCTTTGTGTTATC
TGGACAAATAGTAGAAGGAACAGACTCCTTCAAAGTGACTACATGAACATGACTCCCCGGAGGCCTGG
GCTCACTCGAAAGCCTTACCAGCCCTACGCCCCTGCCAGAGACTTTGCAGCGTACCGCCCCAGAGCAA
AATTCAGCAGGAGTGCAGAGACTGCTGCCAACCTGCAGGACCCCAACCAGCTCTACAATGAGCTCAAT
CTAGGGCGAAGAGAGGAATATGACGTCTTGGAGAAGAAGCGGGCTCGGGATCCAGAGATGGGAGGCAA
ACAGCAGAGGAGGAGGAACCCCCAGGAAGGCGTATACAATGCACTGCAGAAAGACAAGATGGCAGAAG
CCTACAGTGAGATCGGCACAAAAGGCGAGAGGCGGAGAGGCAAGGGGCACGATGGCCTTTACCAGGGT
CTCAGCACTGCCACCAAGGACACCTATGATGCCCTGCATATGCAGACCCTGGCCCCTCGCTAA [SEQ
ID NO: 7]

In certain embodiments, a presently disclosed CAR comprises an extracellular antigen-binding domain that binds to CD19 (e.g., human CD19), a transmembrane domain comprising a CD28 polypeptide, and an intracellular signaling domain comprising a CD3ζ polypeptide (e.g., a murine CD3ζ polypeptide), wherein the intracellular signaling domain does not comprise a co-stimulatory signaling region, namely, the CAR is a first generation CAR. In certain embodiments, the CAR is designated as "ah19mz". In certain embodiments, the CAR (e.g., ah19mz) comprises an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to the amino acid sequence set forth in SEQ ID NO: 33, which is provided below. SEQ ID NO: 33 includes a CD8 leader sequence at amino acids 1 to 18, and is able to bind to CD19 (e.g., human CD19)

MALPVTALLL PLALLLHAEV KLQQSGAELV RPGSSVKISC KASGYAFSSY WMNWVKQRPG
QGLEWIGQIY PGDGDTNYNG KFKGQATLTA DKSSSTAYMQ LSGLTSEDSA VYFCARKTIS
SVVDFYFDYW GQGTTVTVSS GGGGSGGGGS GGGGSDIELT QSPKFMSTSV GDRVSVTCKA
SQNVGTNVAW YQQKPGQSPK PLIYSATYRN SGVPDRFTGS GSGTDFTLTI TNVQSKDLAD
YFCQQYNRYP YTSGGGTKLE IKRAAAIEFM YPPPYLDNER SNGTIIHIKE KHLCHTQSSP
KLFWALVVVA GVLFCYGLLV TVALCVIWTR AKFSRSAETA ANLQDPNQLY NELNLGRREE
YDVLEKKRAR DPEMGGKQQR RRNPQEGVYN ALQKDKMAEA YSEIGTKGER RRGKGHDGLY
QGLSTATKDT YDALHMQTLA PR [SEQ ID NO: 33]

An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 33 is set forth in SEQ ID NO: 34, which is provided below.

ATGGCTCTCCCAGTGACTGCCCTACTGCTTCCCCTAGCGCTTCTCCTGCATGCAGAGGTGAAGCTGCA
GCAGTCTGGGGCTGAGCTGGTGAGGCCTGGGTCCTCAGTGAAGATTTCCTGCAAGGCTTCTGGCTATG
CATTCAGTAGCTACTGGATGAACTGGGTGAAGCAGAGGCCTGGACAGGGTCTTGAGTGGATTGGACAG
ATTTATCCTGGAGATGGTGATACTAACTACAATGGAAAGTTCAAGGGTCAAGCCACACTGACTGCAGA
CAAATCCTCCAGCACAGCCTACATGCAGCTCAGCGGCCTAACATCTGAGGACTCTGCGGTCTATTTCT
GTGCAAGAAAGACCATTAGTTCGGTAGTAGATTTCTACTTTGACTACTGGGGCCAAGGGACCACGGTC
ACCGTCTCCTCAGGTGGAGGTGGATCAGGTGGAGGTGGATCTGGTGGAGGTGGATCTGACATTGAGCT
CACCCAGTCTCCAAAATTCATGTCCACATCAGTAGGAGACAGGGTCAGCGTCACCTGCAAGGCCAGTC
AGAATGTGGGTACTAATGTAGCCTGGTATCAACAGAAACCAGGACAATCTCCTAAACCACTGATTTAC
TCGGCAACCTACCGGAACAGTGGAGTCCCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTTCAC
TCTCACCATCACTAACGTGCAGTCTAAAGACTTGGCAGACTATTTCTGTCAACAATATAACAGGTATC
CGTACACGTCCGGAGGGGGGACCAAGCTGGAGATCAAACGGGCGGCCGCAATTGAGTTCATGTACCCT
CCGCCTTACCTAGACAACGAGAGGAGCAATGGAACTATTATTCACATAAAAGAGAAACATCTTTGTCA
TACTCAGTCATCTCCTAAGCTGTTTTGGGCACTGGTCGTGGTTGCTGGAGTCCTGTTTTGTTATGGCT
TGCTAGTGACAGTGGCTCTTTGTGTTATCTGGACAAGAGCAAAATTCAGCAGGAGTGCAGAGACTGCT
GCCAACCTGCAGGACCCCAACCAGCTCTACAATGAGCTCAATCTAGGGCGAAGAGAGGAATATGACGT
CTTGGAGAAGAAGCGGGCTCGGGATCCAGAGATGGGAGGCAAACAGCAGAGGAGGAGGAACCCCCAGG
AAGGCGTATACAATGCACTGCAGAAAGACAAGATGGCAGAAGCCTACAGTGAGATCGGCACAAAAGGC
GAGAGGCGGAGAGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGCACTGCCACCAAGGACACCTA
TGATGCCCTGCATATGCAGACCCTGGCCCCTCGCTAA [SEQ ID NO: 34]

In certain embodiments, a presently disclosed CAR comprises an extracellular antigen-binding domain that binds to CD19 (e.g., human CD19), a transmembrane domain comprising a CD28 polypeptide, and an intracellular signaling domain comprising a CD3ζ polypeptide (e.g., a human CD3ζ polypeptide), wherein the intracellular signaling domain does not comprise a co-stimulatory signaling region, namely, the CAR is a first generation CAR. In certain embodiments, the CAR is designated as "ah19hz". In certain embodiments, the CAR (e.g., ah19hz) comprises an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to the amino acid sequence set forth in SEQ ID NO: 35, which is provided below. SEQ ID NO: 35 includes a CD8 leader sequence at amino acids 1 to 18, and is able to bind to CD19 (e.g., human CD19).

MALPVTALLL PLALLLHAEV KLQQSGAELV RPGSSVKISC KASGYAFSSY WMNWVKQRPG
QGLEWIGQIY PGDGDTNYNG KFKGQATLTA DKSSSTAYMQ LSGLTSEDSA VYFCARKTIS
SVVDFYFDYW GQGTTVTVSS GGGGSGGGGS GGGGSDIELT QSPKFMSTSV GDRVSVTCKA
SQNVGTNVAW YQQKPGQSPK PLIYSATYRN SGVPDRFTGS GSGTDFTLTI TNVQSKDLAD
YFCQQYNRYP YTSGGGTKLE IKRAAAIEVM YPPPYLDNEK SNGTIIHVKG KHLCPSPLFP
GPSKPFWVLV VVGGVLACYS LLVTVAFIIF WVRVKFSRSA DAPAYQQGQN QLYNELNLGR
REEYDVLDKR RGRDPEMGGK PRRKNPQEGL YNELQKDKMA EAYSEIGMKG ERRRGKGHDG
LYQGLSTATK DTYDALHMQA LPPR [SEQ ID NO: 35]

An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 35 is set forth in SEQ ID NO: 36, which is provided below.

ATGGCTCTCCCAGTGACTGCCCTACTGCTTCCCCTAGCGCTTCTCCTGCATGCAGAGGTGAAGCTGCA
GCAGTCTGGGGCTGAGCTGGTGAGGCCTGGGTCCTCAGTGAAGATTTCCTGCAAGGCTTCTGGCTATG
CATTCAGTAGCTACTGGATGAACTGGGTGAAGCAGAGGCCTGGACAGGGTCTTGAGTGGATTGGACAG
ATTTATCCTGGAGATGGTGATACTAACTACAATGGAAAGTTCAAGGGTCAAGCCACACTGACTGCAGA
CAAATCCTCCAGCACAGCCTACATGCAGCTCAGCGGCCTAACATCTGAGGACTCTGCGGTCTATTTCT
GTGCAAGAAAGACCATTAGTTCGGTAGTAGATTTCTACTTTGACTACTGGGGCCAAGGGACCACGGTC
ACCGTCTCCTCAGGTGGAGGTGGATCAGGTGGAGGTGGATCTGGTGGAGGTGGATCTGACATTGAGCT
CACCCAGTCTCCAAAATTCATGTCCACATCAGTAGGAGACAGGGTCAGCGTCACCTGCAAGGCCAGTC
AGAATGTGGGTACTAATGTAGCCTGGTATCAACAGAAACCAGGACAATCTCCTAAACCACTGATTTAC
TCGGCAACCTACCGGAACAGTGGAGTCCCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTTCAC
TCTCACCATCACTAACGTGCAGTCTAAAGACTTGGCAGACTATTTCTGTCAACAATATAACAGGTATC
CGTACACGTCCGGAGGGGGGACCAAGCTGGAGATCAAACGGGCGGCCGCAATTGAAGTTATGTATCCT
CCTCCTTACCTAGACAATGAGAAGAGCAATGGAACCATTATCCATGTGAAAGGGAAACACCTTTGTCC
AAGTCCCCTATTTCCCGGACCTTCTAAGCCCTTTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTT
GCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGAGTGAAGTTCAGCAGGAGCGCA
GACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGA
GTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACC
CTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATG
AAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGA
CACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCTAG [SEQ ID NO: 36]

In certain embodiments, a presently disclosed CAR comprises an extracellular antigen-binding domain that binds to CD19 (e.g., human CD19), a transmembrane domain comprising a CD28 polypeptide, and an intracellular signaling domain comprising a CD3ζ polypeptide (e.g., a murine CD3ζ polypeptide) and a co-stimulatory signaling region comprising a CD28 polypeptide (e.g., a murine CD28 polypeptide). In certain embodiments, the CAR is designated as "ah19m28z". In certain embodiments, the CAR (e.g., ah19m28z) comprises an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to the amino acid sequence set forth in SEQ ID NO: 37, which is provided below. SEQ ID NO: 37 includes a CD8 leader sequence at amino acids 1 to 18, and is able to bind to CD19 (e.g., human CD19).

MALPVTALLL PLALLLHAEV KLQQSGAELV RPGSSVKISC KASGYAFSSY WMNWVKQRPG
QGLEWIGQIY PGDGDTNYNG KFKGQATLTA DKSSSTAYMQ LSGLTSEDSA VYFCARKTIS
SVVDFYFDYW GQGTTVTVSS GGGGSGGGGS GGGGSDIELT QSPKFMSTSV GDRVSVTCKA
SQNVGTNVAW YQQKPGQSPK PLIYSATYRN SGVPDRFTGS GSGTDFTLTI TNVQSKDLAD
YFCQQYNRYP YTSGGGTKLE IKRAAAIEFM YPPPYLDNER SNGTIIHIKE KHLCHTQSSP
KLFWALVVVA GVLFCYGLLV TVALCVIWTN SRRNRLLQSD YMNMTPRRPG LTRKPYQPYA

PARDFAAYRP RAKFSRSAET AANLQDPNQL YNELNLGRRE EYDVLEKKRA RDPEMGGKQQ RRRNPQEGVY NALQKDKMAE AYSEIGTKGE RRRGKGHDGL YQGLSTATKD TYDALHMQTL APR [SEQ ID NO: 37]

An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 37 is set forth in SEQ ID NO: 38, which is provided below.

ATGGCTCTCCCAGTGACTGCCCTACTGCTTCCCCTAGCGCTTCTCCTGCATGCAGAGGTGAAGCTGCA
GCAGTCTGGGGCTGAGCTGGTGAGGCCTGGGTCCTCAGTGAAGATTTCCTGCAAGGCTTCTGGCTATG
CATTCAGTAGCTACTGGATGAACTGGGTGAAGCAGAGGCCTGGACAGGGTCTTGAGTGGATTGGACAG
ATTTATCCTGGAGATGGTGATACTAACTACAATGGAAAGTTCAAGGGTCAAGCCACACTGACTGCAGA
CAAATCCTCCAGCACAGCCTACATGCAGCTCAGCGGCCTAACATCTGAGGACTCTGCGGTCTATTTCT
GTGCAAGAAAGACCATTAGTTCGGTAGTAGATTTCTACTTTGACTACTGGGGCCAAGGGACCACGGTC
ACCGTCTCCTCAGGTGGAGGTGGATCAGGTGGAGGTGGATCTGGTGGAGGTGGATCTGACATTGAGCT
CACCCAGTCTCCAAAATTCATGTCCACATCAGTAGGAGACAGGGTCAGCGTCACCTGCAAGGCCAGTC
AGAATGTGGGTACTAATGTAGCCTGGTATCAACAGAAACCAGGACAATCTCCTAAACCACTGATTTAC
TCGGCAACCTACCGGAACAGTGGAGTCCCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTTCAC
TCTCACCATCACTAACGTGCAGTCTAAAGACTTGGCAGACTATTTCTGTCAACAATATAACAGGTATC
CGTACACGTCCGGAGGGGGGACCAAGCTGGAGATCAAACGGGCGGCCGCAATTGAGTTCATGTACCCT
CCGCCTTACCTAGACAACGAGAGGAGCAATGGAACTATTATTCACATAAAAGAGAAACATCTTTGTCA
TACTCAGTCATCTCCTAAGCTGTTTTGGGCACTGGTCGTGGTTGCTGGAGTCCTGTTTTGTTATGGCT
TGCTAGTGACAGTGGCTCTTTGTGTTATCTGGACAAATAGTAGAAGGAACAGACTCCTTCAAAGTGAC
TACATGAACATGACTCCCCGGAGGCCTGGGCTCACTCGAAAGCCTTACCAGCCCTACGCCCCTGCCAG
AGACTTTGCAGCGTACCGCCCCAGAGCAAAATTCAGCAGGAGTGCAGAGACTGCTGCCAACCTGCAGG
ACCCCAACCAGCTCTACAATGAGCTCAATCTAGGGCGAAGAGAGGAATATGACGTCTTGGAGAAGAAG
CGGGCTCGGGATCCAGAGATGGGAGGCAAACAGCAGAGGAGGAGGAACCCCCAGGAAGGCGTATACAA
TGCACTGCAGAAAGACAAGATGGCAGAAGCCTACAGTGAGATCGGCACAAAAGGCGAGAGGCGGAGAG
GCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGCACTGCCACCAAGGACACCTATGATGCCCTGCAT
ATGCAGACCCTGGCCCCTCGCTGA [SEQ ID NO: 38]

In certain embodiments, a presently disclosed CAR comprises an extracellular antigen-binding domain that binds to CD19 (e.g., human CD19), a transmembrane domain comprising a CD28 polypeptide, and an intracellular signaling domain comprising a CD3ζ polypeptide (e.g., a human CD3ζ polypeptide) and a co-stimulatory signaling region comprising a CD28 polypeptide (e.g., a human CD28 polypeptide). In certain embodiments, the CAR is designated as "ah19h28z". In certain embodiments, the CAR (e.g., ah19h28z) comprises an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to the amino acid sequence set forth in SEQ ID NO: 39, which is provided

below. SEQ ID NO: 39 includes a CD8 leader sequence at amino acids 1 to 18, and is able to bind to CD19 (e.g., human CD19).

MALPVTALLL PLALLLHAEV KLQQSGAELV RPGSSVKISC KASGYAFSSY WMNWVKQRPG
QGLEWIGQIY PGDGDTNYNG KFKGQATLTA DKSSSTAYMQ LSGLTSEDSA VYFCARKTIS
SVVDFYFDYW GQGTTVTVSS GGGGSGGGGS GGGGSDIELT QSPKFMSTSV GDRVSVTCKA
SQNVGTNVAW YQQKPGQSPK PLIYSATYRN SGVPDRFTGS GSGTDFTLTI TNVQSKDLAD
YFCQQYNRYP YTSGGGTKLE IKRAAAIEVM YPPPYLDNEK SNGTIIHVKG KHLCPSPLFP
GPSKPFWVLV VVGGVLACYS LLVTVAFIIF WVRSKRSRLL HSDYMNMTPR RPGPTRKHYQ
PYAPPRDFAA YRSRVKFSRS ADAPAYQQGQ NQLYNELNLG RREEYDVLDK RRGRDPEMGG
KPRRKNPQEG LYNELQKDKM AEAYSEIGMK GERRRGKGHD GLYQGLSTAT KDTYDALHMQ
ALPPR [SEQ ID NO: 39]

An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 39 is set forth in SEQ ID NO: 40, which is provided below.

ATGGCTCTCCCAGTGACTGCCCTACTGCTTCCCCTAGCGCTTCTCCTGCATGCAGAGGTGAAGCTGCA
GCAGTCTGGGGCTGAGCTGGTGAGGCCTGGGTCCTCAGTGAAGATTTCCTGCAAGGCTTCTGGCTATG
CATTCAGTAGCTACTGGATGAACTGGGTGAAGCAGAGGCCTGGACAGGGTCTTGAGTGGATTGGACAG
ATTTATCCTGGAGATGGTGATACTAACTACAATGGAAAGTTCAAGGGTCAAGCCACACTGACTGCAGA
CAAATCCTCCAGCACAGCCTACATGCAGCTCAGCGGCCTAACATCTGAGGACTCTGCGGTCTATTTCT
GTGCAAGAAAGACCATTAGTTCGGTAGTAGATTTCTACTTTGACTACTGGGGCCAAGGGACCACGGTC
ACCGTCTCCTCAGGTGGAGGTGGATCAGGTGGAGGTGGATCTGGTGGAGGTGGATCTGACATTGAGCT
CACCCAGTCTCCAAAATTCATGTCCACATCAGTAGGAGACAGGGTCAGCGTCACCTGCAAGGCCAGTC
AGAATGTGGGTACTAATGTAGCCTGGTATCAACAGAAACCAGGACAATCTCCTAAACCACTGATTTAC
TCGGCAACCTACCGGAACAGTGGAGTCCCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTTCAC
TCTCACCATCACTAACGTGCAGTCTAAAGACTTGGCAGACTATTTCTGTCAACAATATAACAGGTATC
CGTACACGTCCGGAGGGGGGACCAAGCTGGAGATCAAACGGGCGGCCGCAATTGAAGTTATGTATCCT
CCTCCTTACCTAGACAATGAGAAGAGCAATGGAACCATTATCCATGTGAAAGGGAAACACCTTTGTCC
AAGTCCCCTATTTCCCGGACCTTCTAAGCCCTTTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTT
GCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTG
CACAGTGACTACATGAACATGACTCCCCGCCGCCCCGGGCCCACCCGCAAGCATTACCAGCCCTATGC
CCCACCACGCGACTTCGCAGCCTATCGCTCCAGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGT
ACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTG
GACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCT
GTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCC
GGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCC
CTTCACATGCAGGCCCTGCCCCCTCGCTAG [SEQ ID NO: 40]

In certain embodiments, a presently disclosed CAR comprises an extracellular antigen-binding domain that binds to CD19 (e.g., human CD19), a transmembrane domain comprising a CD28 polypeptide, and an intracellular signaling domain

comprising a CD3ζ polypeptide (e.g., a human CD3ζ polypeptide) and a co-stimulatory signaling region comprising a 4-1BB polypeptide (e.g., a human 4-1BB polypeptide). In certain embodiments, the CAR is designated as "ah19hBBz". In certain embodiments, the CAR (e.g., ah19hBBz) comprises an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to the amino acid sequence set forth in SEQ ID NO: 41, which is provided below. SEQ ID NO: 41 includes a CD8 leader sequence at amino acids 1 to 18, and is able to bind to CD19 (e.g., human CD19).

MALPVTALLL PLALLLHAEV KLQQSGAELV RPGSSVKISC KASGYAFSSY WMNWVKQRPG
QGLEWIGQIY PGDGDTNYNG KFKGQATLTA DKSSSTAYMQ LSGLTSEDSA VYFCARKTIS
SVVDFYFDYW GQGTTVTVSS GGGGSGGGGS GGGGSDIELT QSPKFMSTSV GDRVSVTCKA
SQNVGTNVAW YQQKPGQSPK PLIYSATYRN SGVPDRFTGS GSGTDFTLTI TNVQSKDLAD
YFCQQYNRYP YTSGGGTKLE IKRAAAIEVM YPPPYLDNEK SNGTIIHVKG KHLCPSPLFP
GPSKPFWVLV VVGGVLACYS LLVTVAFIIF WVKRGRKKLL YIFKQPFMRP VQTTQEEDGC
SCRFPEEEEG GCELRVKFSR SADAPAYQQG QNQLYNELNL GRREEYDVLD KRRGRDPEMG
GKPRRKNPQE GLYNELQKDK MAEAYSEIGM KGERRRGKGH DGLYQGLSTA TKDTYDALHM
QALPPR [SEQ ID NO: 41]

An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 41 is set forth in SEQ ID NO: 42, which is provided below.

ATGGCTCTCCCAGTGACTGCCCTACTGCTTCCCCTAGCGCTTCTCCTGCATGCAGAGGTGAAGCTGCA
GCAGTCTGGGGCTGAGCTGGTGAGGCCTGGGTCCTCAGTGAAGATTTCCTGCAAGGCTTCTGGCTATG
CATTCAGTAGCTACTGGATGAACTGGGTGAAGCAGAGGCCTGGACAGGGTCTTGAGTGGATTGGACAG
ATTTATCCTGGAGATGGTGATACTAACTACAATGGAAAGTTCAAGGGTCAAGCCACACTGACTGCAGA
CAAATCCTCCAGCACAGCCTACATGCAGCTCAGCGGCCTAACATCTGAGGACTCTGCGGTCTATTTCT
GTGCAAGAAAGACCATTAGTTCGGTAGTAGATTTCTACTTTGACTACTGGGGCCAAGGGACCACGGTC
ACCGTCTCCTCAGGTGGAGGTGGATCAGGTGGAGGTGGATCTGGTGGAGGTGGATCTGACATTGAGCT
CACCCAGTCTCCAAAATTCATGTCCACATCAGTAGGAGACAGGGTCAGCGTCACCTGCAAGGCCAGTC
AGAATGTGGGTACTAATGTAGCCTGGTATCAACAGAAACCAGGACAATCTCCTAAACCACTGATTTAC
TCGGCAACCTACCGGAACAGTGGAGTCCCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTTCAC
TCTCACCATCACTAACGTGCAGTCTAAAGACTTGGCAGACTATTTCTGTCAACAATATAACAGGTATC
CGTACACGTCCGGAGGGGGGACCAAGCTGGAGATCAAACGGGCGGCCGCAATTGAAGTTATGTATCCT
CCTCCTTACCTAGACAATGAGAAGAGCAATGGAACCATTATCCATGTGAAAGGGAAACACCTTTGTCC
AAGTCCCCTATTTCCCGGACCTTCTAAGCCCTTTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTT
GCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAAACGGGGCAGAAAGAAACTCCTG
TATATATTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCG
ATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCG
CGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTT

TTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGG
CCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGC
GCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGAC
GCCCTTCACATGCAGGCCCTGCCCCCTCGCTAG [SEQ ID NO: 42]

The presently disclosed subject matter also provides a nucleic acid composition comprising a first nucleic acid sequence encoding an antigen-recognizing receptor that binds to an antigen and a second nucleic acid sequence encoding an exogenous IL-36 polypeptide.

3. Immunoresponsive Cells

The presently disclosed subject matter provides immunoresponsive cells comprising (a) an antigen-recognizing receptor (e.g., CAR or TCR) that binds to an antigen, and (b) a secretable IL-36 polypeptide. In certain embodiments, the secretable IL-36 polypeptide is an exogenous IL-36 polypeptide. In certain embodiments, the antigen-recognizing receptor is capable of activating the immunoresponsive cell. In certain embodiments, the secretable IL-36 polypeptide (e.g., exogenous IL-36 polypeptide, such as a nucleic acid encoding an IL-36 polypeptide) is capable of promoting an anti-tumor effect of the immunoresponsive cell. The immunoresponsive cells can be transduced with an antigen-recognizing receptor and an exogenous IL-36 polypeptide such that the cells co-express the antigen-recognizing receptor and the exogenous IL-36 polypeptide.

The immunoresponsive cells of the presently disclosed subject matter can be cells of the lymphoid lineage. The lymphoid lineage, comprising B, T and natural killer (NK) cells, provides for the production of antibodies, regulation of the cellular immune system, detection of foreign agents in the blood, detection of cells foreign to the host, and the like. Non-limiting examples of immunoresponsive cells of the lymphoid lineage include T cells, Natural Killer (NK) cells, embryonic stem cells, and pluripotent stem cells (*e.g.*, those from which lymphoid cells may be differentiated). T cells can be lymphocytes that mature in the thymus and are chiefly responsible for cell-mediated immunity. T cells are involved in the adaptive immune system. The T cells of the presently disclosed subject matter can be any type of T cells, including, but not limited to, helper T cells, cytotoxic T cells, memory T cells (including central memory T cells, stem-cell-like memory T cells (or stem-like memory T cells), and two types of effector memory T cells: *e.g.*, T_{EM} cells and T_{EMRA} cells, Regulatory T cells (also known as suppressor T cells), Natural killer T cells, Mucosal associated invariant T cells, and γδ T

cells. Cytotoxic T cells (CTL or killer T cells) are a subset of T lymphocytes capable of inducing the death of infected somatic or tumor cells. A patient's own T cells may be genetically modified to target specific antigens through the introduction of an antigen-recognizing receptor, e.g., a CAR or a TCR. In certain embodiments, the immunoresponsive cell is a T cell. The T cell can be a $CD4^+$ T cell or a $CD8^+$ T cell. In certain embodiments, the T cell is a $CD4^+$ T cell. In certain embodiments, the T cell is a $CD8^+$ T cell.

Natural killer (NK) cells can be lymphocytes that are part of cell-mediated immunity and act during the innate immune response. NK cells do not require prior activation in order to perform their cytotoxic effect on target cells.

Types of human lymphocytes of the presently disclosed subject matter include, without limitation, peripheral donor lymphocytes, *e.g.*, those disclosed in Sadelain, M., *et al.* 2003 *Nat Rev Cancer* 3:35-45 (disclosing peripheral donor lymphocytes genetically modified to express CARs), in Morgan, R.A., *et al.* 2006 *Science* 314:126-129 (disclosing peripheral donor lymphocytes genetically modified to express a full-length tumor antigen-recognizing T cell receptor complex comprising the α and β heterodimer), in Panelli, M.C., *et al.* 2000 *J Immunol* 164:495-504; Panelli, M.C., *et al.* 2000 *J Immunol* 164:4382-4392 (disclosing lymphocyte cultures derived from tumor infiltrating lymphocytes (TILs) in tumor biopsies), and in Dupont, J., *et al.* 2005 *Cancer Res* 65:5417-5427; Papanicolaou, G.A., *et al.* 2003 *Blood* 102:2498-2505 (disclosing selectively *in vitro*-expanded antigen-specific peripheral blood leukocytes employing artificial antigen-presenting cells (AAPCs) or pulsed dendritic cells). The immunoresponsive cells (*e.g.*, T cells) can be autologous, non-autologous (*e.g.*, allogeneic), or derived *in vitro* from engineered progenitor or stem cells.

The presently disclosed immunoresponsive cells are capable of modulating the tumor microenvironment. Tumors have a microenvironment that is hostile to the host immune response involving a series of mechanisms by malignant cells to protect themselves from immune recognition and elimination. This "hostile tumor microenvironment" comprises a variety of immune suppressive factors including infiltrating regulatory $CD4^+$ T cells (Tregs), myeloid derived suppressor cells (MDSCs), tumor associated macrophages (TAMs), immune suppressive cytokines including TGF-β, and expression of ligands targeted to immune suppressive receptors expressed by activated T cells (CTLA-4 and PD-1). These mechanisms of immune suppression play a

role in the maintenance of tolerance and suppressing inappropriate immune responses, however within the tumor microenvironment these mechanisms prevent an effective anti-tumor immune response. Collectively these immune suppressive factors can induce either marked anergy or apoptosis of adoptively transferred CAR modified T cells upon encounter with targeted tumor cells.

In certain embodiments, the presently disclosed immunoresponsive cells have increased secretion of anti-tumor cytokines, including, but not limited to, IL-36, granulocyte macrophage colony-stimulating factor (GM-CSF), IFN-γ, IL-10, IL-6, IL-12, CXCL1, CCL1, IL-23, and CXCL10. In certain embodiments, the presently disclosed immunoresponsive cells have increased secretion of IL-36, GM-CSF, IFN-γ, IL-10, or a combination thereof.

Interleukin-36

Interleukin-36 cytokine family include fourIL-36α, IL-36β, IL-36γ, and IL-36Ra. IL-36α, IL-36β, and IL-36γ are agonists of IL-36 receptor (IL-36R), whereas IL-36Ra is an antagonist of IL-36 receptor.

Interleukin 36 alpha (IL-36 alpha) is also known as IL36A; FIL1; FIL1E; IL1F6; IL-1F6; IL1(EPSILON); FIL1(EPSILON). GenBank ID: 27179 (human), 54448 (mouse), 296541 (rat), 523429 (cattle), 100065063 (horse). The protein product of IL-36 alpha includes, but is not limited to, NCBI Reference Sequences NP_055255.1, XP_011509267.1, XP_005263696.1 and XP_016859295.1.

Interleukin 36 beta (IL-36 beta) is also known as IL36B; FIL1; FIL1H; IL1F8; IL1H2; IL-1F8; IL-1H2; IL1-ETA; FIL1-(ETA); FILI-(ETA). GenBank ID: 27177 (human), 69677 (mouse), 362076 (rat), 100297786 (cattle), 483068 (dog), 100065096 (horse). The protein product of IL-36 beta includes, but is not limited to, NCBI Reference Sequences NP_055253.2, NP_775270.1 and XP_011509264.1.

Interleukin 36 gamma (IL-36 gamma) is also known as IL36G; IL1E; IL1F9; IL1H1; IL-1F9; IL-1H1; IL1RP2; IL-1RP2. GenBank ID: 56300 (human), 215257 (mouse), 499744 (rat), 615762 (cattle), 100686137 (dog), 100065031 (horse). The protein product of IL-36 gamma includes, but is not limited to, NCBI Reference Sequences NP_001265497.1 and NP_062564.1.

Interleukin 36 receptor antagonist (IL-36RA) is also known as IL36RN, FIL1; FIL1D; IL1F5; IL1L1; PSORP; IL1HY1; IL1RP3; IL36RA; IL-36Ra; PSORS14; FIL1(DELTA). GenBank ID: 26525 (human), 54450 (mouse), 311783 (rat), 518514 (cattle), 611869 (dog), 100065154 (horse). The protein product of Interleukin 36

antagonist includes, but is not limited to, NCBI Reference Sequences NP_036407.1 and NP_775262.1.

IL-36 alpha, IL-36 beta and IL-36 gamma cytokines are produced by neutrophil, skin cells and other cells. They function by binding to the IL-36 receptor (IL-36R) and activate downstream signaling pathways. After stimulation with IL-36 alpha, IL-36 beta or IL-36 gamma, natural killer (NK) cells and certain T cells release other cytokines, such as IFN-γ, IL-10 and GM-CSF, which can further activate other types of immunoresponsive cells. After stimulation with IL-36 alpha, IL-36 beta or IL-36 gamma, dendritic cells can release IL-6, IL-12, CXCL1, CCL1, IL-23, and CXCL10, which can further modulate other types of immunoresponsive cells

In certain embodiments, the term "IL-36" or "IL-36 cytokine" refers to the bioactive form of IL-36 alpha, IL-36 beta and/or IL-36 gamma after secretion from a cell (e.g., a form where the signal peptide is cleaved off).

In certain embodiments, the IL-36 polypeptide is a human IL-36 polypeptide.

In certain embodiments, a human IL-36 alpha polypeptide comprises or has the amino acid sequence set forth in SEQ ID NO: 4, which is provided below. In certain embodiments, a human IL-36 alpha polypeptide comprises or has an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or at least about 100% homologous or identical to the sequence set forth in SEQ ID NO: 4.

KIDTPQQGSIQDINHRVWVLQDQTLIAVPRKDRMSPVTIALISCRHVETLEKDRGNPIYLGLNGLNLCLMC AKVGDQPTLQLKEKDIMDLYNQPEPVKSFLFYHSQSGRNSTFESVAFPGWFIAVSSEGGCPLILTQELGKA NTTDFGLTMLF (SEQ ID NO: 4)

In certain embodiments, a human IL-36 beta polypeptide comprises or has the amino acid sequence set forth in SEQ ID NO: 20, which is provided below. In certain embodiments, a human IL-36 beta polypeptide comprises or has an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or at least about 100% homologous or identical to the sequence set forth in SEQ ID NO: 20.

REAAPKSYAIRDSRQMVWVLSGNSLIAAPLSRSIKPVTLHLIACRDTEFSDKEKGNMVYLGIKGKDLCLFC AEIQGKPTLQLKLQGSQDNIGKDTCWKLVGIHTCINLDVRESCFMGTLDQWGIGVGRKKWKSSFQHHHLRK KDKDFSSMRTNIGMPGRM (SEQ ID NO: 20)

In certain embodiments, a human IL-36 gamma polypeptide comprises or has the following amino acid sequence set forth in SEQ ID NO: 21, which is provided below. In certain embodiments, a human IL-36 gamma polypeptide comprises or has an amino acid

sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or at least about 100% homologous or identical to the sequence set forth in SEQ ID NO: 21.

SMCKPITGTINDLNQQVWTLQGQNLVAVPRSDSVTPVTVAVITCKYPEALEQGRGDPIYLGIQNPEMCLYC
EKVGEQPTLQLKEQKIMDLYGQPEPVKPFLFYRAKTGRTSTLESVAFPDWFIASSKRDQPIILTSELGKSY
NTAFELNIND [SEQ ID NO: 21]

In certain embodiments, the IL-36 polypeptide is a murine IL-36 polypeptide.

In certain embodiments, a murine IL-36 alpha polypeptide comprises or has the amino acid sequence set forth in SEQ ID NO: 30, which is provided below. In certain embodiments, a murine IL-36 alpha polypeptide comprises or has an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or at least about 100% homologous or identical to the sequence set forth in SEQ ID NO: 30.

GRETPDFGEVFDLDQQVWIFRNQALVTVPRSHRVTPVSVTILPCKYPESLEQDKGIAIYLGIQNPDKCLFC
KEVNGHPTLLLKEEKILDLYHHPEPMKPFLFYHTRTGGTSTFESVAFPGHYIASSKTGNPIFLTSKKGEYY
NINFNLDIKS [SEQ ID NO: 30]

In certain embodiments, a murine IL-36 beta polypeptide comprises or has the amino acid sequence set forth in SEQ ID NO: 31, which is provided below. In certain embodiments, a murine IL-36 beta polypeptide comprises or has an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or at least about 100% homologous or identical to the sequence set forth in SEQ ID NO: 31.

SSQSPRNYRVHDSQQMVWVLTGNTLTAVPASNNVKPVILSLIACRDTEFQDVKKGNLVFLGIKNRNLCFCC
VEMEGKPTLQLKEVDIMNLYKERKAQKAFLFYHGIEGSTSVFQSVLYPGWFIATSSIERQTIILTHQRGKL
VNTNFYIESEK [SEQ ID NO: 31]

In certain embodiments, a murine IL-36 gamma polypeptide comprises or has the amino acid sequence set forth in SEQ ID NO: 32, which is provided below. In certain embodiments, a murine IL-36 gamma polypeptide comprises or has an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or at least about 100% homologous or identical to the sequence set forth in SEQ ID NO: 32.

GRETPDFGEVFDLDQQVWIFRNQALVTVPRSHRVTPVSVTILPCKYPESLEQDKGIAIYLGIQNPDKCLFC
KEVNGHPTLLLKEEKILDLYHHPEPMKPFLFYHTRTGGTSTFESVAFPGHYIASSKTGNPIFLTSKKGEYY
NINFNLDIKS[SEQ ID NO: 32]

In certain embodiments, the term "IL-36" or "IL-36 cytokine" refers to the bioactive form of IL-36 RA after secretion from a cell (e.g., a form where the signal peptide has been cleaved off).

In certain embodiments, a secretable IL-36 polypeptide refers to a polypeptide or a protein, the cytokine portion of which has at least about 80%, at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% homologous to the cytokine portion of the protein product of IL-36 alpha (GenBank ID: 27179 (human), 54448 (mouse), 296541 (rat), 523429 (cattle), 100065063 (horse)), IL-36 beta (GenBank ID: 27177 (human), 69677 (mouse), 362076 (rat), 100297786 (cattle), 483068 (dog), 100065096 (horse)), IL-36 gamma (GenBank ID: 56300 (human), 215257 (mouse), 499744 (rat), 615762 (cattle), 100686137 (dog), 100065031 (horse)), or a fragment thereof that has immunostimulatory activity. In certain non-limiting embodiments, the secretable IL-36 polypeptide comprises a cytokine portion and a signal peptide, optionally joined by a linker peptide. Non-limiting examples of secretable IL-36 polypeptides include NCBI Reference Sequences NP_055255.1, XP_011509267.1, XP_005263696.1, XP_016859295.1, NP_055253.2, NP_775270.1, XP_011509264.1, NP_001265497.1 and NP_062564.1.

In certain non-limiting embodiments, the secretable IL-36 polypeptide comprises a signal peptide, for example, an IL-2 signal peptide, a kappa leader sequence, a CD8 leader sequence or a peptide with essentially equivalent activity. In certain embodiments, the secretable IL-36 polypeptide comprises an IL-2 signal peptide. In certain embodiments, the IL-2 signal peptide comprises or has the amino acid sequence set forth in SEQ ID NO: 8

The unpurified source of CTLs may be any known in the art, such as the bone marrow, fetal, neonate or adult or other hematopoietic cell source, e.g., fetal liver, peripheral blood or umbilical cord blood. Various techniques can be employed to separate the cells. For instance, negative selection methods can remove non-CTLs initially. mAbs are particularly useful for identifying markers associated with particular cell lineages and/or stages of differentiation for both positive and negative selections.

A large proportion of terminally differentiated cells can be initially removed by a relatively crude separation. For example, magnetic bead separations can be used initially to remove large numbers of irrelevant cells. In certain embodiments, at least about 80%, usually at least 70% of the total hematopoietic cells will be removed prior to cell isolation.

Procedures for separation include, but are not limited to, density gradient centrifugation; resetting; coupling to particles that modify cell density; magnetic separation with antibody-coated magnetic beads; affinity chromatography; cytotoxic agents joined to or used in conjunction with a mAb, including, but not limited to, complement and cytotoxins; and panning with antibody attached to a solid matrix, e.g. plate, chip, elutriation or any other convenient technique.

Techniques for separation and analysis include, but are not limited to, flow cytometry, which can have varying degrees of sophistication, e.g., a plurality of color channels, low angle and obtuse light scattering detecting channels, impedance channels.

The cells can be selected against dead cells, by employing dyes associated with dead cells such as propidium iodide (PI). In certain embodiments, the cells are collected in a medium comprising 2% fetal calf serum (FCS) or 0.2% bovine serum albumin (BSA) or any other suitable, e.g., sterile, isotonic medium.

4. Vectors

Genetic modification of an immunoresponsive cell (e.g., a T cell or a NK cell) can be accomplished by transducing a substantially homogeneous cell composition with a recombinant DNA construct. In certain embodiments, a retroviral vector (either gamma-retroviral or lentiviral) is employed for the introduction of the DNA construct into the cell. For example, a polynucleotide encoding an antigen-recognizing receptor can be cloned into a retroviral vector and expression can be driven from its endogenous promoter, from the retroviral long terminal repeat, or from a promoter specific for a target cell type of interest. Non-viral vectors may be used as well.

For initial genetic modification of an immunoresponsive cell to include an antigen recognizing receptor (e.g., a CAR or TCR), a retroviral vector is generally employed for transduction, however any other suitable viral vector or non-viral delivery system can be used. The antigen-recognizing receptor and the IL-36 polypeptide can be constructed in a single, multicistronic expression cassette, in multiple expression cassettes of a single vector, or in multiple vectors. Examples of elements that create polycistronic expression cassette include, but is not limited to, various viral and non-viral Internal Ribosome Entry Sites (IRES, *e.g.*, FGF-1 IRES, FGF-2 IRES, VEGF IRES, IGF-II IRES, NF-κB IRES, RUNX1 IRES, p53 IRES, hepatitis A IRES, hepatitis C IRES, pestivirus IRES, aphthovirus IRES, picornavirus IRES, poliovirus IRES and encephalomyocarditis virus IRES) and cleavable linkers (*e.g.,* 2A peptides , *e.g.*, P2A, T2A, E2A and F2A peptides). Combinations of retroviral vector and an appropriate

packaging line are also suitable, where the capsid proteins will be functional for infecting human cells. Various amphotropic virus-producing cell lines are known, including, but not limited to, PA12 (Miller, *et al.* (1985) *Mol. Cell. Biol.* 5:431-437); PA317 (Miller, *et al.* (1986) *Mol. Cell. Biol.* 6:2895-2902); and CRIP (Danos, *et al.* (1988) *Proc. Natl. Acad. Sci.* USA 85:6460-6464). Non-amphotropic particles are suitable too, e.g., particles pseudotyped with VSVG, RD114 or GALV envelope and any other known in the art.

Possible methods of transduction also include direct co-culture of the cells with producer cells, e.g., by the method of Bregni, *et al.* (1992) *Blood* 80:1418-1422, or culturing with viral supernatant alone or concentrated vector stocks with or without appropriate growth factors and polycations, e.g., by the method of Xu, *et al.* (1994) *Exp. Hemat.* 22:223-230; and Hughes, *et al.* (1992) *J. Clin. Invest.* 89:1817.

Other transducing viral vectors can be used to modify an immunoresponsive cell. In certain embodiments, the chosen vector exhibits high efficiency of infection and stable integration and expression (see, e.g., Cayouette et al., Human Gene Therapy 8:423-430, 1997; Kido et al., Current Eye Research 15:833-844, 1996; Bloomer et al., Journal of Virology 71:6641-6649, 1997; Naldini et al., Science 272:263-267, 1996; and Miyoshi et al., Proc. Natl. Acad. Sci. U.S.A. 94:10319, 1997). Other viral vectors that can be used include, for example, adenoviral, lentiviral, and adena-associated viral vectors, vaccinia virus, a bovine papilloma virus, or a herpes virus, such as Epstein-Barr Virus (also see, for example, the vectors of Miller, Human Gene Therapy 15-14, 1990; Friedman, Science 244:1275-1281, 1989; Eglitis et al., BioTechniques 6:608-614, 1988; Tolstoshev et al., Current Opinion in Biotechnology 1:55-61, 1990; Sharp, The Lancet 337:1277-1278, 1991; Cornetta et al., Nucleic Acid Research and Molecular Biology 36:311-322, 1987; Anderson, Science 226:401-409, 1984; Moen, Blood Cells 17:407-416, 1991; Miller et al., Biotechnology 7:980-990, 1989; LeGal La Salle et al., Science 259:988-990, 1993; and Johnson, Chest 107:77S- 83S, 1995). Retroviral vectors are particularly well developed and have been used in clinical settings (Rosenberg et al., N. Engl. J. Med 323:370, 1990; Anderson et al., U.S. Pat. No. 5,399,346).

Non-viral approaches can also be employed for genetic modification of an immunoresponsive cell. For example, a nucleic acid molecule can be introduced into an immunoresponsive cell by administering the nucleic acid in the presence of lipofection (Feigner et al., Proc. Natl. Acad. Sci. U.S.A. 84:7413, 1987; Ono et al., Neuroscience Letters 17:259, 1990; Brigham et al., Am. J. Med. Sci. 298:278, 1989; Staubinger et al.,

Methods in Enzymology 101:512, 1983), asialoorosomucoid-polylysine conjugation (Wu et al., Journal of Biological Chemistry 263:14621, 1988; Wu et al., Journal of Biological Chemistry 264:16985, 1989), or by micro-injection under surgical conditions (Wolff et al., Science 247:1465, 1990). Other non-viral means for gene transfer include transfection *in vitro* using calcium phosphate, DEAE dextran, electroporation, and protoplast fusion. Liposomes can also be potentially beneficial for delivery of DNA into a cell. Transplantation of normal genes into the affected tissues of a subject can also be accomplished by transferring a normal nucleic acid into a cultivatable cell type *ex vivo* (e.g., an autologous or heterologous primary cell or progeny thereof), after which the cell (or its descendants) are injected into a targeted tissue or are injected systemically. Recombinant receptors can also be derived or obtained using transposases or targeted nucleases (e.g. Zinc finger nucleases, meganucleases, or TALE nucleases, CRISPR). Transient expression may be obtained by RNA electroporation.

Clustered regularly-interspaced short palindromic repeats (CRISPR) system is a genome editing tool discovered in prokaryotic cells. When utilized for genome editing, the system includes Cas9 (a protein able to modify DNA utilizing crRNA as its guide), CRISPR RNA (crRNA, contains the RNA used by Cas9 to guide it to the correct section of host DNA along with a region that binds to tracrRNA (generally in a hairpin loop form) forming an active complex with Cas9), trans-activating crRNA (tracrRNA, binds to crRNA and forms an active complex with Cas9), and an optional section of DNA repair template (DNA that guides the cellular repair process allowing insertion of a specific DNA sequence). CRISPR/Cas9 often employs a plasmid to transfect the target cells. The crRNA needs to be designed for each application as this is the sequence that Cas9 uses to identify and directly bind to the target DNA in a cell. The repair template carrying CAR expression cassette need also be designed for each application, as it must overlap with the sequences on either side of the cut and code for the insertion sequence. Multiple crRNA's and the tracrRNA can be packaged together to form a single-guide RNA (sgRNA). This sgRNA can be joined together with the Cas9 gene and made into a plasmid in order to be transfected into cells.

A zinc-finger nuclease (ZFN) is an artificial restriction enzyme, which is generated by combining a zinc finger DNA-binding domain with a DNA-cleavage domain. A zinc finger domain can be engineered to target specific DNA sequences which allows a zinc-finger nuclease to target desired sequences within genomes. The DNA-binding domains of individual ZFNs typically contain a plurality of individual zinc

finger repeats and can each recognize a plurality of basepairs. The most common method to generate new zinc-finger domain is to combine smaller zinc-finger "modules" of known specificity. The most common cleavage domain in ZFNs is the non-specific cleavage domain from the type IIs restriction endonuclease FokI. Using the endogenous homologous recombination (HR) machinery and a homologous DNA template carrying CAR expression cassette, ZFNs can be used to insert the CAR expression cassette into genome. When the targeted sequence is cleaved by ZFNs, the HR machinery searches for homology between the damaged chromosome and the homologous DNA template, and then copies the sequence of the template between the two broken ends of the chromosome, whereby the homologous DNA template is integrated into the genome.

Transcription activator-like effector nucleases (TALEN) are restriction enzymes that can be engineered to cut specific sequences of DNA. TALEN system operates on almost the same principle as ZFNs. They are generated by combining a transcription activator-like effectors DNA-binding domain with a DNA cleavage domain. Transcription activator-like effectors (TALEs) are composed of 33-34 amino acid repeating motifs with two variable positions that have a strong recognition for specific nucleotides. By assembling arrays of these TALEs, the TALE DNA-binding domain can be engineered to bind desired DNA sequence, and thereby guide the nuclease to cut at specific locations in genome.cDNA expression for use in polynucleotide therapy methods can be directed from any suitable promoter (e.g., the human cytomegalovirus (CMV), simian virus 40 (SV40), or metallothionein promoters), and regulated by any appropriate mammalian regulatory element or intron (e.g. the elongation factor 1a enhancer/promoter/intron structure). For example, if desired, enhancers known to preferentially direct gene expression in specific cell types can be used to direct the expression of a nucleic acid. The enhancers used can include, without limitation, those that are characterized as tissue- or cell-specific enhancers. Alternatively, if a genomic clone is used as a therapeutic construct, regulation can be mediated by the cognate regulatory sequences or, if desired, by regulatory sequences derived from a heterologous source, including any of the promoters or regulatory elements described above.

The resulting cells can be grown under conditions similar to those for unmodified cells, whereby the modified cells can be expanded and used for a variety of purposes.

5. Enhancing Endogenous IL-36 Gene Expression

Any targeted genome editing methods can be used to modify the promoter/enhancer region of an *IL-36* gene locus, and thereby enhancing the endogenous

expression of *IL-36* in an immunoresponsive cell. In certain embodiments, the modification comprises replacement of an endogenous promoter with a constitutive promoter or an inducible promoter, or insertion of a constitutive promoter or inducible promoter to the promoter region of an *IL-36* gene locus. In certain embodiments, a constitutive promoter is positioned on an *IL-36* gene locus to drive gene expression of the endogenous *IL-36* gene. Eligible constitutive promoters include, but are not limited to, a CMV promoter, an EF1a promoter, a SV40 promoter, a PGK1 promoter, a Ubc promoter, a beta-actin promoter, and a CAG promoter. Alternatively or additionally, a conditional or inducable promoter is positioned on an *IL-36* gene locus to drive gene expression of the endogenous *IL-36* gene. Non-limiting examples of conditional promoters include a tetracycline response element (TRE) promoter and an estrogen response element (ERE) promoter. In addition, enhancer elements can be placed in regions other than the promoter region.

6. Genome Editing Methods

Any targeted genome editing methods can be used to modify the promoter/enhancer region of an *IL-36* gene locus. In certain embodiments, a CRISPR system is used to modify the promoter/enhancer region of an *IL-36* gene locus. In certain embodiments, zinc-finger nucleases are used to modify the promoter/enhancer region of an *IL-36* gene locus. In certain embodiments, a TALEN system is used to modify the promoter/enhancer region of an *IL-36* gene locus.

Methods for delivering the genome editing agents/systems can vary depending on the need. In certain embodiments, the components of a selected genome editing method are delivered as DNA constructs in one or more plasmids. In certain embodiments, the components are delivered via viral vectors. Common delivery methods include but is not limited to, electroporation, microinjection, gene gun, impalefection, hydrostatic pressure, continuous infusion, sonication, magnetofection, adeno-associated viruses, envelope protein pseudotyping of viral vectors, replication-competent vectors cis and trans-acting elements, herpes simplex virus, and chemical vehicles (e.g., oligonucleotides, lipoplexes, polymersomes, polyplexes, dendrimers, inorganic Nanoparticles, and cell-penetrating peptides).

Modification can be made anywhere within an *IL-36* gene locus, or anywhere that can impact gene expression of an *IL-36* gene. In certain embodiments, the modification occurs upstream of the transcriptional start site of an *IL-36* gene. In certain embodiments, the modification occurs between the transcriptional start site and the protein coding

region of an *IL-36* gene. In certain embodiments, the modification occurs downstream of the protein coding region of an *IL-36* gene. In certain embodiments, the modification occurs upstream of the transcriptional start site of an *IL-36* gene, wherein the modification produces a new transcriptional start site.

7. Polypeptides and Analogs

Also included in the presently disclosed subject matter are a CD19, CD28, CD3ζ, and IL-36 polypeptides or fragments thereof that are modified in ways that enhance their anti-neoplastic activity when expressed in an immunoresponsive cell. The presently disclosed subject matter provides methods for optimizing an amino acid sequence or nucleic acid sequence by producing an alteration in the sequence. Such alterations may include certain mutations, deletions, insertions, or post-translational modifications. The presently disclosed subject matter further includes analogs of any naturally-occurring polypeptide disclosed herein (including, but not limited to, CD19, CD28, CD3ζ, and IL-36). Analogs can differ from a naturally-occurring polypeptide disclosed herein by amino acid sequence differences, by post-translational modifications, or by both. Analogs can exhibit at least about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more homologous to all or part of a naturally-occurring amino, acid sequence of the presently disclosed subject matter. The length of sequence comparison is at least 5, 10, 15 or 20 amino acid residues, e.g., at least 25, 50, or 75 amino acid residues, or more than 100 amino acid residues. Again, in an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence. Modifications include *in vivo* and *in vitro* chemical derivatization of polypeptides, e.g., acetylation, carboxylation, phosphorylation, or glycosylation; such modifications may occur during polypeptide synthesis or processing or following treatment with isolated modifying enzymes. Analogs can also differ from the naturally-occurring polypeptides by alterations in primary sequence. These include genetic variants, both natural and induced (for example, resulting from random mutagenesis by irradiation or exposure to ethanemethylsulfate or by site-specific mutagenesis as described in Sambrook, Fritsch and Maniatis, Molecular Cloning: A Laboratory Manual (2d ed.), CSH Press, 1989, or Ausubel et al., supra). Also included are cyclized peptides, molecules, and analogs which contain residues other than L-amina

acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., β or γ amino acids.

In addition to full-length polypeptides, the presently disclosed subject matter also provides fragments of any one of the polypeptides or peptide domains disclosed herein. As used herein, the term "a fragment" means at least 5, 10, 13, or 15 amino acids. In certain embodiments, a fragment comprises at least 20 contiguous amino acids, at least 30 contiguous amino acids, or at least 50 contiguous amino acids. In certain embodiments, a fragment comprises at least 60 to 80, 100, 200, 300 or more contiguous amino acids. Fragments can be generated by methods known to those skilled in the art or may result from normal protein processing (e.g., removal of amino acids from the nascent polypeptide that are not required for biological activity or removal of amino acids by alternative mRNA splicing or alternative protein processing events).

Non-protein analogs have a chemical structure designed to mimic the functional activity of a protein disclosed herein (e.g., IL-36). Such analogs may exceed the physiological activity of the original polypeptide. Methods of analog design are well known in the art, and synthesis of analogs can be carried out according to such methods by modifying the chemical structures such that the resultant analogs increase the anti-neoplastic activity of the original polypeptide when expressed in an immunoresponsive cell. These chemical modifications include, but are not limited to, substituting alternative R groups and varying the degree of saturation at specific carbon atoms of a reference polypeptide. In certain embodiments, the protein analogs are relatively resistant to *in vivo* degradation, resulting in a more prolonged therapeutic effect upon administration. Assays for measuring functional activity include, but are not limited to, those described in the Examples below.

8. Administration

Compositions comprising the presently disclosed immunoresponsive cells can be provided systemically or directly to a subject for inducing and/or enhancing an immune response to an antigen and/or treating and/or preventing a neoplasm, pathogen infection, or infectious disease. In certain embodiments, the presently disclosed immunoresponsive cells or compositions comprising thereof are directly injected into an organ of interest (e.g., an organ affected by a neoplasm). Alternatively, the presently disclosed immunoresponsive cells or compositions comprising thereof are provided indirectly to the organ of interest, for example, by administration into the circulatory

system (e.g., the tumor vasculature). Expansion and differentiation agents can be provided prior to, during or after administration of the cells or compositions to increase production of T cells, NK cells, or CTL cells *in vitro* or *in vivo*.

The presently disclosed immunoresponsive cells can be administered in any physiologically acceptable vehicle, normally intravascularly, although they may also be introduced into bone or other convenient site where the cells may find an appropriate site for regeneration and differentiation (e.g., thymus). Usually, at least about 1×10^5 cells will be administered, eventually reaching about 1×10^{10} or more. The presently disclosed immunoresponsive cells can comprise a purified population of cells. Those skilled in the art can readily determine the percentage of the presently disclosed immunoresponsive cells in a population using various well-known methods, such as fluorescence activated cell sorting (FACS). Suitable ranges of purity in populations comprising the presently disclosed immunoresponsive cells are about 50% to about 55%, about 5% to about 60%, and about 65% to about 70%. In certain embodiments, the purity is about 70% to about 75%, about 75% to about 80%, or about 80% to about 85%. In certain embodiments, the purity is about 85% to about 90%, about 90% to about 95%, and about 95% to about 100%. Dosages can be readily adjusted by those skilled in the art (e.g., a decrease in purity may require an increase in dosage). The cells can be introduced by injection, catheter, or the like.

The presently disclosed compositions can be pharmaceutical compositions comprising the presently disclosed immunoresponsive cells or their progenitors and a pharmaceutically acceptable carrier. Administration can be autologous or heterologous. For example, immunoresponsive cells, or progenitors can be obtained from one subject, and administered to the same subject or a different, compatible subject. Peripheral blood derived immunoresponsive cells or their progeny (e.g., *in vivo*, *ex vivo* or *in vitro* derived) can be administered via localized injection, including catheter administration, systemic injection, localized injection, intravenous injection, or parenteral administration. When administering a therapeutic composition of the presently disclosed subject matter (e.g., a pharmaceutical composition comprising a presently disclosed immunoresponsive cell), it can be formulated in a unit dosage injectable form (solution, suspension, emulsion).

9. Formulations

Compositions comprising the presently disclosed immunoresponsive cells can be conveniently provided as sterile liquid preparations, e.g., isotonic aqueous solutions,

suspensions, emulsions, dispersions, or viscous compositions, which may be buffered to a selected pH. Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues. Liquid or viscous compositions can comprise carriers, which can be a solvent or dispersing medium containing, for example, water, saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like) and suitable mixtures thereof.

Sterile injectable solutions can be prepared by incorporating the genetically modified immunoresponsive cells in the required amount of the appropriate solvent with various amounts of the other ingredients, as desired. Such compositions may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting, dispersing, or emulsifying agents (e.g., methylcellulose), pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts, such as "REMINGTON'S PHARMACEUTICAL SCIENCE", 17th edition, 1985, incorporated herein by reference, may be consulted to prepare suitable preparations, without undue experimentation.

Various additives which enhance the stability and sterility of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. According to the presently disclosed subject matter, however, any vehicle, diluent, or additive used would have to be compatible with the genetically modified immunoresponsive cells or their progenitors.

The compositions can be isotonic, i.e., they can have the same osmotic pressure as blood and lacrimal fluid. The desired isotonicity of the compositions may be accomplished using sodium chloride, or other pharmaceutically acceptable agents such

as dextrose, boric acid, sodium tartrate, propylene glycol or other inorganic or organic solutes. Sodium chloride can be particularly for buffers containing sodium ions.

Viscosity of the compositions, if desired, can be maintained at the selected level using a pharmaceutically acceptable thickening agent. For example, methylcellulose is readily and economically available and is easy to work with. Other suitable thickening agents include, for example, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, carbomer, and the like. The concentration of the thickener can depend upon the agent selected. The important point is to use an amount that will achieve the selected viscosity. Obviously, the choice of suitable carriers and other additives will depend on the exact route of administration and the nature of the particular dosage form, e.g., liquid dosage form (e.g., whether the composition is to be formulated into a solution, a suspension, gel or another liquid form, such as a time release form or liquid-filled form).

The quantity of cells to be administered will vary for the subject being treated. In a one embodiment, between about 10^4 and about 10^{10}, between about 10^5 and about 10^9, or between about 10^6 and about 10^8 of the presently disclosed immunoresponsive cells are administered to a human subject. More effective cells may be administered in even smaller numbers. In certain embodiments, at least about 1×10^8, about 2×10^8, about 3×10^8, about 4×10^8, or about 5×10^8 of the presently disclosed immunoresponsive cells are administered to a human subject. The precise determination of what would be considered an effective dose may be based on factors individual to each subject, including their size, age, sex, weight, and condition of the particular subject. Dosages can be readily ascertained by those skilled in the art from this disclosure and the knowledge in the art.

The skilled artisan can readily determine the amount of cells and optional additives, vehicles, and/or carrier in compositions and to be administered in methods. Typically, any additives (in addition to the active cell(s) and/or agent(s)) are present in an amount of 0.001 to 50% (weight) solution in phosphate buffered saline, and the active ingredient is present in the order of micrograms to milligrams, such as about 0.0001 to about 5 wt %, about 0.0001 to about 1 wt %, about 0.0001 to about 0.05 wt% or about 0.001 to about 20 wt %, about 0.01 to about 10 wt %, or about 0.05 to about 5 wt %. For any composition to be administered to an animal or human, the followings can be determined: toxicity such as by determining the lethal dose (LD) and LD50 in a suitable animal model e.g., rodent such as mouse; the dosage of the composition(s), concentration of components therein and timing of administering the composition(s), which elicit a

suitable response. Such determinations do not require undue experimentation from the knowledge of the skilled artisan, this disclosure and the documents cited herein. And, the time for sequential administrations can be ascertained without undue experimentation.

10. Methods of Treatment

The presently disclosed subject matter provides methods for inducing and/or increasing an immune response in a subject in need thereof. The presently disclosed immunoresponsive cells and compositions comprising thereof can be used for treating and/or preventing a neoplasm in a subject. The presently disclosed immunoresponsive cells and compositions comprising thereof can be used for prolonging the survival of a subject suffering from a neoplasm. The presently disclosed immunoresponsive cells and compositions comprising thereof can also be used for treating and/or preventing a pathogen infection or other infectious disease in a subject, such as an immunocompromised human subject. Such methods comprise administering the presently disclosed immunoresponsive cells in an amount effective or a composition (e.g., pharmaceutical composition) comprising thereof to achieve the desired effect, be it palliation of an existing condition or prevention of recurrence. For treatment, the amount administered is an amount effective in producing the desired effect. An effective amount can be provided in one or a series of administrations. An effective amount can be provided in a bolus or by continuous perfusion.

An "effective amount" (or, "therapeutically effective amount") is an amount sufficient to effect a beneficial or desired clinical result upon treatment. An effective amount can be administered to a subject in one or more doses. In terms of treatment, an effective amount is an amount that is sufficient to palliate, ameliorate, stabilize, reverse or slow the progression of the disease, or otherwise reduce the pathological consequences of the disease. The effective amount is generally determined by the physician on a case-by-case basis and is within the skill of one in the art. Several factors are typically taken into account when determining an appropriate dosage to achieve an effective amount. These factors include age, sex and weight of the subject, the condition being treated, the severity of the condition and the form and effective concentration of the immunoresponsive cells administered.

For adoptive immunotherapy using antigen-specific T cells, cell doses in the range of about 10^6-10^{10} (e.g., about 10^9) are typically infused. Upon administration of the presently disclosed cells into the host and subsequent differentiation, T cells are induced that are specifically directed against the specific antigen. The modified cells can be

administered by any method known in the art including, but not limited to, intravenous, subcutaneous, intranodal, intratumoral, intrathecal, intrapleural, intraperitoneal and directly to the thymus.

The presently disclosed subject matter provides methods for treating and/or preventing a neoplasm in a subject. The method can comprise administering an effective amount of the presently disclosed immunoresponsive cells or a composition comprising thereof to a subject having a neoplasm.

Non-limiting examples of neoplasia include blood cancers (e.g. leukemias, lymphomas, and myelomas), ovarian cancer, breast cancer, bladder cancer, brain cancer, colon cancer, intestinal cancer, liver cancer, lung cancer, pancreatic cancer, prostate cancer, skin cancer, stomach cancer, glioblastoma, throat cancer, melanoma, neuroblastoma, adenocarcinoma, glioma, soft tissue sarcoma, and various carcinomas (including prostate and small cell lung cancer). Suitable carcinomas further include any known in the field of oncology, including, but not limited to, astrocytoma, fibrosarcoma, myxosarcoma, liposarcoma, oligodendroglioma, ependymoma, medulloblastoma, primitive neural ectodermal tumor (PNET), chondrosarcoma, osteogenic sarcoma, pancreatic ductal adenocarcinoma, small and large cell lung adenocarcinomas, chordoma, angiosarcoma, endotheliosarcoma, squamous cell carcinoma, bronchoalveolar carcinoma, epithelial adenocarcinoma, and liver metastases thereof, lymphangiosarcoma, lymphangioendotheliosarcoma, hepatoma, cholangiocarcinoma, synovioma, mesothelioma, Ewing's tumor, rhabdomyosarcoma, colon carcinoma, basal cell carcinoma, sweat gland carcinoma, papillary carcinoma, sebaceous gland carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, testicular tumor, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma, retinoblastoma, leukemia, multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease, breast tumors such as ductal and lobular adenocarcinoma, squamous and adenocarcinomas of the uterine cervix, uterine and ovarian epithelial carcinomas, prostatic adenocarcinomas, transitional squamous cell carcinoma of the bladder, B and T cell lymphomas (nodular and diffuse) plasmacytoma, acute and chronic leukemias, malignant melanoma, soft tissue sarcomas and leiomyosarcomas. In certain embodiments, the neoplasm is selected from the group consisting of blood cancers (e.g. leukemias, lymphomas, and myelomas),

ovarian cancer, prostate cancer, breast cancer, bladder cancer, brain cancer, colon cancer, intestinal cancer, liver cancer, lung cancer, pancreatic cancer, prostate cancer, skin cancer, stomach cancer, glioblastoma, and throat cancer. In certain embodiments, the presently disclosed immunoresponsive cells and compositions comprising thereof can be used for treating and/or preventing blood cancers (e.g., leukemias, lymphomas, and myelomas) or ovarian cancer, which are not amenable to conventional therapeutic interventions.

The subjects can have an advanced form of disease, in which case the treatment objective can include mitigation or reversal of disease progression, and/or amelioration of side effects. The subjects can have a history of the condition, for which they have already been treated, in which case the therapeutic objective will typically include a decrease or delay in the risk of recurrence.

Suitable human subjects for therapy typically comprise two treatment groups that can be distinguished by clinical criteria. Subjects with "advanced disease" or "high tumor burden" are those who bear a clinically measurable tumor. A clinically measurable tumor is one that can be detected on the basis of tumor mass (e.g., by palpation, CAT scan, sonogram, mammogram or X-ray; positive biochemical or histopathologic markers on their own are insufficient to identify this population). A pharmaceutical composition is administered to these subjects to elicit an anti-tumor response, with the objective of palliating their condition. Ideally, reduction in tumor mass occurs as a result, but any clinical improvement constitutes a benefit. Clinical improvement includes decreased risk or rate of progression or reduction in pathological consequences of the tumor.

A second group of suitable subjects is known in the art as the "adjuvant group." These are individuals who have had a history of neoplasm, but have been responsive to another mode of therapy. The prior therapy can have included, but is not restricted to, surgical resection, radiotherapy, and traditional chemotherapy. As a result, these individuals have no clinically measurable tumor. However, they are suspected of being at risk for progression of the disease, either near the original tumor site, or by metastases. This group can be further subdivided into high-risk and low-risk individuals. The subdivision is made on the basis of features observed before or after the initial treatment. These features are known in the clinical arts, and are suitably defined for each different neoplasia. Features typical of high-risk subgroups are those in which the tumor has invaded neighboring tissues, or who show involvement of lymph nodes.

Another group have a genetic predisposition to neoplasia but have not yet evidenced clinical signs of neoplasia. For instance, women testing positive for a genetic mutation associated with breast cancer, but still of childbearing age, can wish to receive one or more of the immunoresponsive cells described herein in treatment prophylactically to prevent the occurrence of neoplasia until it is suitable to perform preventive surgery.

As a consequence of surface expression of an antigen-recognizing receptor that binds to a tumor antigen and a secretable IL-36 polypeptide (e.g., an exogenous IL-36 polypeptide) that enhances the anti-tumor effect of the immunoresponsive cell, adoptively transferred T or NK cells are endowed with augmented and selective cytolytic activity at the tumor site. Furthermore, subsequent to their localization to tumor or viral infection and their proliferation, the T cells turn the tumor or viral infection site into a highly conductive environment for a wide range of immune cells involved in the physiological anti-tumor or antiviral response (tumor infiltrating lymphocytes, NK-, NKT- cells, dendritic cells, and macrophages).

Additionally, the presently disclosed subject matter provides methods for treating and/or preventing a pathogen infection (e.g., viral infection, bacterial infection, fungal infection, parasite infection, or protozoal infection) in a subject, e.g., in an immunocompromised subject. The method can comprise administering an effective amount of the presently disclosed immunoresponsive cells or a composition comprising thereof to a subject having a pathogen infection. Exemplary viral infections susceptible to treatment include, but are not limited to, Cytomegalovirus (CMV), Epstein Barr Virus (EBV), Human Immunodeficiency Virus (HIV), and influenza virus infections.

Further modification can be introduced to the presently disclosed immunoresponsive cells (*e.g.*, T cells) to avert or minimize the risks of immunological complications (known as "malignant T-cell transformation"), *e.g.*, graft versus-host disease (GvHD), or when healthy tissues express the same target antigens as the tumor cells, leading to outcomes similar to GvHD. A potential solution to this problem is engineering a suicide gene into the presently disclosed immunoresponsive cells. Suitable suicide genes include, but are not limited to, Herpes simplex virus thymidine kinase (hsv-tk), inducible Caspase 9 Suicide gene (iCasp-9), and a truncated human epidermal growth factor receptor (EGFRt) polypeptide. In certain embodiments, the suicide gene is an EGFRt polypeptide. The EGFRt polypeptide can enable T cell elimination by administering anti-EGFR monoclonal antibody (*e.g.*, cetuximab). EGFRt can be

covalently joined to the upstream of the antigen-recognizing receptor of a presently disclosed CAR. The suicide gene can be included within the vector comprising nucleic acids encoding a presently disclosed CAR. In this way, administration of a prodrug designed to activate the suicide gene (*e.g.*, a prodrug (*e.g.*, AP1903 that can activate iCasp-9) during malignant T-cell transformation (*e.g.*, GVHD) triggers apoptosis in the suicide gene-activated CAR-expressing T cells. The incorporation of a suicide gene into the a presently disclosed CAR gives an added level of safety with the ability to eliminate the majority of CAR T cells within a very short time period. A presently disclosed immunoresponsive cell (*e.g.*, a T cell) incorporated with a suicide gene can be pre-emptively eliminated at a given timepoint post CAR T cell infusion, or eradicated at the earliest signs of toxicity.

11. Kits

The presently disclosed subject matter provides kits for inducing and/or enhancing an immune response and/or treating and/or preventing a neoplasm or a pathogen infection in a subject. In certain embodiments, the kit comprises an effective amount of presently disclosed immunoresponsive cells or a pharmaceutical composition comprising thereof. In certain embodiments, the kit comprises a sterile container; such containers can be boxes, ampules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments. In certain non-limiting embodiments, the kit includes an isolated nucleic acid molecule encoding an antigen-recognizing receptor (e.g., a CAR or a TCR) directed toward an antigen of interest and an isolated nucleic acid molecule encoding an IL-36 polypeptide in expressible (and secretable) form, which may optionally be comprised in the same or different vectors.

If desired, the immunoresponsive cells and/or nucleic acid molecules are provided together with instructions for administering the cells or nucleic acid molecules to a subject having or at risk of developing a neoplasm or pathogen or immune disorder. The instructions generally include information about the use of the composition for the treatment and/or prevention of a neoplasm or a pathogen infection. In certain embodiments, the instructions include at least one of the following: description of the therapeutic agent; dosage schedule and administration for treatment or prevention of a neoplasm, pathogen infection, or immune disorder or symptoms thereof; precautions; warnings; indications; counter-indications; over-dosage information; adverse reactions;

animal pharmacology; clinical studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container.

EXAMPLES

The practice of the present disclosure employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are well within the purview of the skilled artisan. Such techniques are explained fully in the literature, such as, "Molecular Cloning: A Laboratory Manual", second edition (Sambrook, 1989); "Oligonucleotide Synthesis" (Gait, 1984); "Animal Cell Culture" (Freshney, 1987); "Methods in Enzymology" "Handbook of Experimental Immunology" (Weir, 1996); "Gene Transfer Vectors for Mammalian Cells" (Miller and Calos, 1987); "Current Protocols in Molecular Biology" (Ausubel, 1987); "PCR: The Polymerase Chain Reaction", (Mullis, 1994); "Current Protocols in Immunology" (Coligan, 1991). These techniques are applicable to the production of the polynucleotides and polypeptides disclosed herein, and, as such, may be considered in making and practicing the presently disclosed subject matter. Particularly useful techniques for particular embodiments will be discussed in the sections that follow.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the presently disclosed cells and compositions, and are not intended to limit the scope of what the inventors regard as their invention.

Example 1 – Interleukin-36 (IL-36) secreting CAR-T cells

Introduction

Genetically modified T cells expressing a first-generation anti-CD19 CAR and a secretable IL-36 polypeptide were generated. The IL-36-secreting CAR-T cells presented improvements when compared to a control anti-CD19 CAR-T cells that do not comprise a secretable IL-36 polypeptide. The IL-36-secreting CAR-T cells exhibited prolonged survival curves in murine models.

Results

CAR constructs

Murine anti-CD19 CAR constructs with or without a secretable murine IL-36 polypeptide were produced/constructed in SFG retroviral vector backbone e.g., am19mZ and am19mZ_IL-36, as shown in Figure 1A. Figure 1B shows the construct of first-

generation anti-mouse CD 19 myc-tag CAR comprising a constitutively-secreted murine IL-36 alpha, wherein the murine IL-36 alpha has the amino acid sequence set forth in SEQ ID NO: 30. Figure 1C shows the construct of first-generation anti-mouse CD 19 myc-tag CAR incorporating constitutively-secreted murine IL-36 beta, wherein the murine IL-36 alpha has the amino acid sequence set forth in SEQ ID NO: 31. Figure 1D shows the construct of first-generation anti-mouse CD 19 myc-tag CAR incorporating constitutively-secreted murine IL-36 gamma, wherein the murine IL-36 alpha has the amino acid sequence set forth in SEQ ID NO: 32.

T cells were transduced with one of the above-noted various constructs.

Cell surface CAR expression

The CAR expression of these AR constructs on mouse T cells (5 days post harvesting from spleen, and 4 days post start of transduction) cell surface were confirmed via flow cytometry analyses, and the results are shown in Figure 2. Surface expressions of anti-CD19 myc-tag first generation CAR T cells secreting IL-36 beta (d5M19ZTSB) and gamma (d5M19ZTSG) are shown in the first and the second scatter plots in row 1, respectively. d5B6emp (non-transduced C57BL/6 mouse splenocytes) served as a negative control. M19z, which refers to T cells comprising a first generation anti-CD19 CAR without a secretable IL-36 polypeptide, was used as a positive control.

Increased Cytokine Secretion

The cytokine production/secretion by the modified T cells was measured by flow cytometry. As shown in Figure 3, the IL-36-secreting CAR-expressing T cells exhibited an increased secretion of murine GM-CSF in $CD19^+$ tumor cells (EL4Sm19) as compared to control cells, i.e., T cells expressing M19Z alone and not expressing a secretable IL-36 polypeptide.

As shown in Figure 4, the IL-36-secreting CAR-expressing T cells exhibited an increased secretion of murine Interferon gamma (mINF-gamma) in $CD19^+$ tumor cells (EL4Sm19) as compared to control cells, i.e., T cells expressing M19Z alone and not expressing a secretable IL-36 polypeptide

As shown in Figure 5, the IL-36-secreting CAR-expressing T cells exhibited an increased secretion of murine Interleukin 10 (IL-10) in $CD19^+$ tumor cells (EL4Sm19) as compared to control cells, i.e., T cells expressing M19Z alone and not expressing a secretable IL-36 polypeptide.

Survival of EL4SmCD19$^+$ tumor-bearing syngeneic immuno-competent mice

The modified T cells were studied in the context of syngeneic, immune competent models of disease wherein long-term survival of EL4SmCD19$^+$ tumor-bearing syngeneic immuno-competent mice were assessed. C57BL/8 mice were inoculated with 1 x 10^6 EL4Sm19tc (EL4 tumor cells from Sigma which ectopically expressed murine CD19). The mice were subsequently treated with 5 x 10^6 CAR T cells transduced with various CAR constructs one day after tumor inoculation, and survival was followed. Survival curves of all subjects are shown in Figure 6. As shown in Figure 6A, the IL-36-secreting CAR-expressing T cells (e.g., am19MTmZpIL36B and am19MTmZpIL36G) both induced long-term survival in tumor-bearing mice compared to untreated mice. Furthermore, comparison of am19MTmZ (M19Z) and am19MTmZpIL36B (M19Z_36B) (*see* Figure 6B) and comparison of am19MTmZ (M19Z) and am19MTmZpIL36G (M19Z_36G) (*see* Figure 6C) showed significant increase in survival of mice treated with IL36 beta and gamma secreting CAR-expressing T cells compared to CAR-T cells without IL-36 secretion.

Example 2

Syngeneic IL36-gamma Secreting Murine CAR T Cells Improved Survival in Tumor-bearing Mice.

8-12 week-old C57BL/6 mice were inoculated with 1 million mouse CD19+ EL4 tumor cells (EL4-CD19) via tail vein injection. On the following day, the mice received (without getting any pre-conditioning chemotherapy): no CAR T cells (untreated), 2,500,000 m19m28mz (syngeneic anti-mouse CD19 CD28-based second generation CAR T cells), 2,500,000 m19mz-IL36Y (syngeneic anti-mouse CD19 first-generation IL36-gamma secreting CAR T cells) or m19m28mz-IL36Y (syngeneic anti-mouse CD19 CD28-based second generation IL36-gamma secreting CAR T cells) the following day. Survival was followed as shown in Figure 7. The results demonstrated that both first-generation and second-generation IL36-gamma secreting CAR T cells significantly improved survival and induced long-term remissions as compared to non-IL36-gamma secreting CAR T cells. These results indicated that IL36-gamma secreting CAR T cells were more potent than their non-secreting counterparts.

Syngeneic IL36-gamma Secreting Murine CAR T Cells Elaborated Increased Pro-inflammatory Cytokine Secretion in Tumor-bearing Mice.

The mice treated with the CAR T cells described in this example were eye bled on day 7, and serum was collected and analyzed for cytokine levels using a Luminex bead-based multiplex assay. The results shown in Figure 8 demonstrated that mice

receiving either m19mz-IL36Y or m19m28mz-IL36Y had significantly higher serum levels of interferon gamma and TNF-alpha as compared to untreated mice or those that received the m19m28mz, indicating increased functionality of IL36-gamma secreting CAR T cells *in vivo*.

Syngeneic IL36-gamma Secreting Murine CAR T Cells Induce B-cell Aplasia in Tumor-bearing Mice.

The mice treated with the CAR T cells described in this example were eye bled on day 7. Post RBC lysis, the percentage of peripheral B-cells (CD19+ cells as a percentage of CD45+ cells) was determined utilizing flow cytometry. As shown in Figure 9, mice that received IL36-gamma secreting CAR T cells demonstrated significantly decreased levels of peripherally detectable B-cells as compared to untreated mice or those that received m19m28mz. The results indicated that IL36-gamma secreting CAR T cells were more potent than their non-secreting counterparts as they were able to induce deeper levels of B-cell aplasia, a surrogate marker of CAR T cell potency *in vivo* in the setting of CD19-targeted CAR T cell therapy.

Embodiments of the presently disclosed subject matter

From the foregoing description, it will be apparent that variations and modifications may be made to the presently disclosed subject matter to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or sub-combination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference.

2018367452 27 May 2024

Claims:

1. An isolated immunoresponsive cell comprising:
an antigen-recognizing receptor comprising an extracellular antigen-binding domain that binds to an antigen and an intracellular signaling domain that is capable of inducing cytotoxicity of the cell upon the binding of the extracellular antigen-binding domain to the antigen; and
(a) an exogenous IL-36 polypeptide or a fragment thereof that is constitutively expressed; or
(b) a modified promoter at an endogenous *IL-36* gene locus, wherein the modified promoter enhances gene expression of the endogenous *IL-36* gene, thereby the endogenous IL-36 gene is constitutively expressed.

2. The isolated immunoresponsive cell of claim 1, wherein the modification comprises replacement of an endogenous promoter with a constitutive promoter or insertion of a constitutive promoter to the promoter region of the endogenous *IL-36* gene locus.

3. The isolated immunoresponsive cell of claims 1 or 2, wherein the antigen is a tumor antigen or a pathogen antigen.

4. The isolated immunoresponsive cell of claim 1, wherein the exogenous IL-36 polypeptide is secreted and/or expressed from a vector.

5. The isolated immunoresponsive cell of any one of claims 1-4, wherein said antigen-recognizing receptor: (a) is a T cell receptor (TCR) or a chimeric antigen receptor (CAR); (b) is exogenous or endogenous; (c) is recombinantly expressed; and/or (d) is expressed from a vector.

6. The isolated immunoresponsive cell of any one of claims 1-5, wherein the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a human embryonic stem cell, and a pluripotent stem cell from which lymphoid cells may be differentiated, and/or said immunoresponsive cell is autologous.

7. The isolated immunoresponsive cell of any one of claims 1-6, wherein the antigen is selected from the group consisting of CD19, MUC16, MUC1, CAIX, CEA, CD8,

CD7, CD10, CD20, CD22, CD30, CLL1, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD133, CD138, EGP-2, EGP-40, EpCAM, erb-B2, erb-B3, erb-B4, FBP, Fetal acetylcholine receptor, folate receptor-a, GD2, GD3, HER-2, hTERT, IL-13R-a2, K-light chain, KDR, LeY, L1 cell adhesion molecule, MAGE-A1, Mesothelin, ERBB2, MAGEA3, p53, MART1,GP100, Proteinase3 (PR1), Tyrosinase, Survivin, hTERT, EphA2, NKG2D ligands, NY-ES0-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, WT-1, BCMA, CD123, CD44V6, NKCS1, EGF1R, EGFR-VIII, and CD99, CD70, ADGRE2, CCR1, LILRB2, PRAME, and ERBB.

8. The isolated immunoresponsive cell of any one of claims 1-7, wherein said IL-36 polypeptide comprises a heterologous signal sequence at the amino-terminus.

9. The isolated immunoresponsive cell of claim 8, wherein said heterologous signal sequence is selected from the group consisting of an IL-2 signal sequence, a kappa leader sequence, a CD8 leader sequence, and combinations thereof.

10. The isolated immunoresponsive cell of any one of claim 1-9, wherein the antigen-recognizing receptor is a CAR and wherein the CAR: (a) comprises an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain; (b) does not comprise a co-stimulatory signaling domain; and/or (c) is 19z.

11. The isolated immunoresponsive cell of any one of claims 1-10, wherein the IL-36 peptide: (a) is a mature form of IL-36 alpha, IL-36 beta, IL-36 gamma, or a functional fragment thereof; (b) comprises an amino acid sequence that is at least about 80% homologous to the sequence set forth in SEQ ID NO: 4, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 30, SEQ ID NO: 31, or SEQ ID NO: 32; or (c) comprises the amino acid sequence set forth in SEQ ID NO: 4, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 30, SEQ ID NO: 31, or SEQ ID NO: 32.

12. A pharmaceutical composition comprising an effective amount of an immunoresponsive cell of any one of claims 1-11 and a pharmaceutically acceptable excipient.

13. A method of reducing tumor burden, reducing number of tumor cells, reducing tumor size, and/or eradicating a tumor in a subject, treating and/or preventing a neoplasm, lengthening survival of a subject having a neoplasm, or increasing immune-activating

cytokine production in response to a tumor antigen or a pathogen antigen in a subject, the method comprising administering to the subject an effective amount of immunoresponsive cells of any one of claims 1-11 or a pharmaceutical composition of claim 12.

14. The method of claim 13, wherein the neoplasm is selected from the group consisting of blood cancer, B cell leukemia, multiple myeloma, lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, non-Hodgkin's lymphoma, and ovarian cancer; wherein the neoplasm is B cell leukemia, multiple myeloma, lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, or non-Hodgkin's lymphoma, and the antigen is CD19; and/or wherein the neoplasm is ovarian, and the antigen is MUC16.

15. The method of claim 13, wherein the immune-activating cytokine is selected from the group consisting of IL-10, GM-SCF and IFN-γ.

16. A method of treating blood cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of an immunoresponsive cell of any one of claims 1-11, wherein the immunoresponsive cell is a T cell, and wherein the antigen is CD19, and/or wherein the blood cancer is selected from the group consisting of B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, and non-Hodgkin's lymphoma.

17. A nucleic acid composition comprising (a) a first nucleic acid sequence encoding an antigen-recognizing receptor comprising an extracellular antigen-binding domain that that binds to an antigen and an intracellular signaling domain that is capable of inducing cytotoxicity of the cell upon the binding of the extracellular antigen-binding domain to the antigen; and (b) a second nucleic acid sequence encoding an exogenous IL-36 polypeptide or a fragment thereof that is constitutively expressed, each optionally operably linked to a promoter element.

18. A vector comprising the nucleic acid composition of claim 17.

19. Use of an immunoresponsive cell of any one of claims 1-11 or a pharmaceutical composition of claim 12 in the manufacture of a medicament for reducing tumor burden, treating and/or preventing a neoplasm, lengthening survival of a subject

having a neoplasm, and/or increasing immune-activating cytokine production in response to a tumor antigen or a pathogen antigen in a subject.

20. A method for producing an antigen-specific immunoresponsive cell, the method comprising introducing into an immunoresponsive cell the nucleic acid composition of claim 17 or the vector of claim 18.

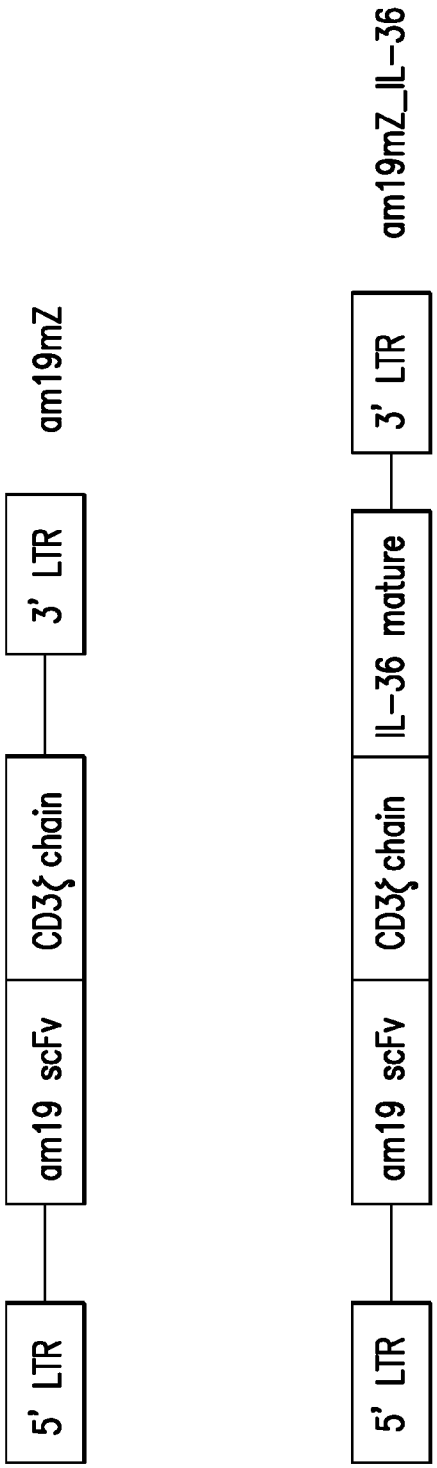

am19mZ
5' LTR
am19 scFv
CD3ζ chain
3' LTR
am19mZ_IL-36
5' LTR
am19 scFv
CD3ζ chain
IL-36 mature
3' LTR

FIG. 1A

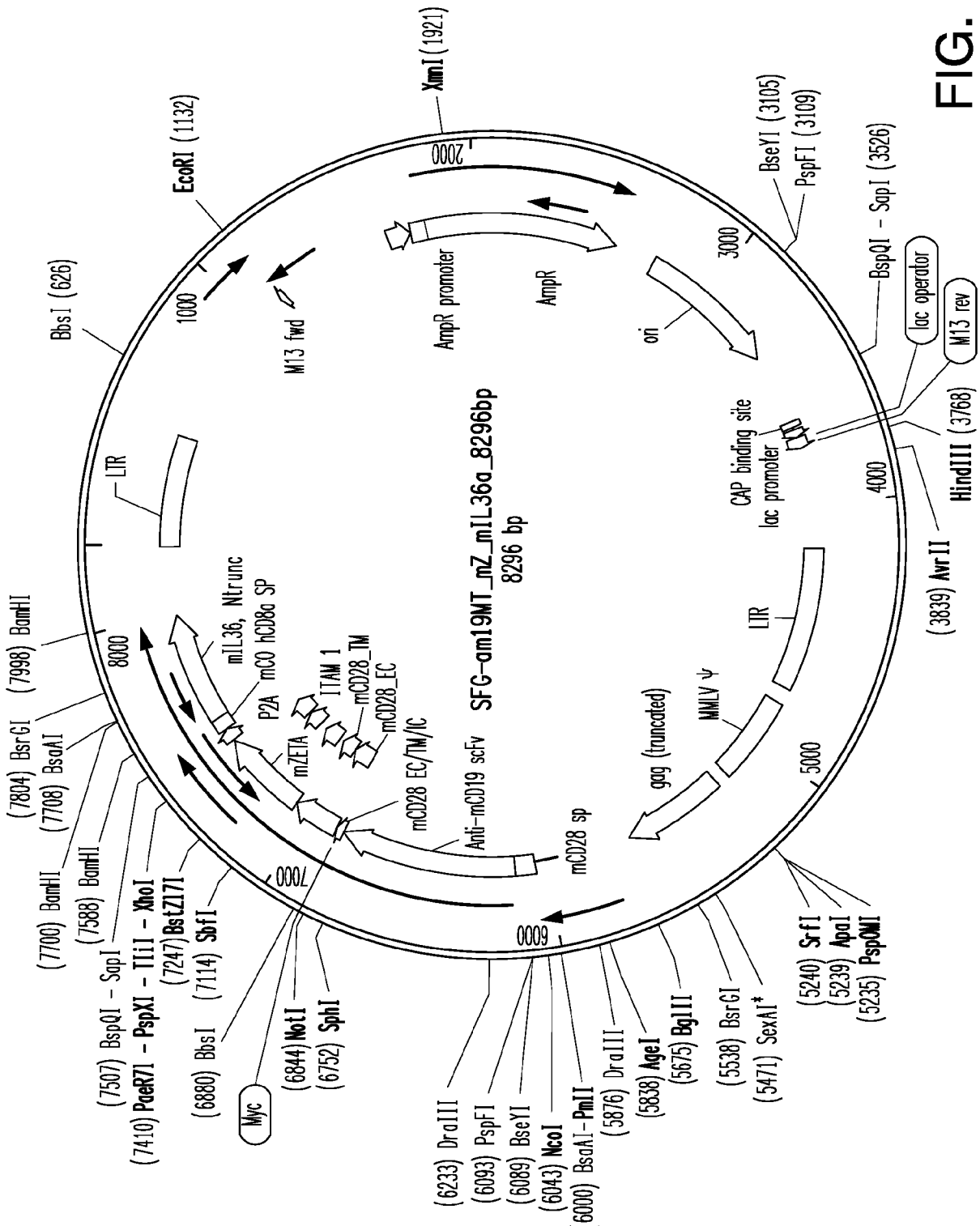

SFG-am19MT_mZ_mIL36a_8296bp
8296 bp
XmnI (1921)
EcoRI (1132)
BbsI (626)
BseYI (3105)
PspFI (3109)
BspQI - SapI (3526)
lac operator
M13 rev
HindIII (3768)
AvrII (3839)
CAP binding site
lac promoter
AmpR promoter
AmpR
ori
LTR
gag (truncated)
MMLV ψ
mCD28 sp
Anti-mCD19 scFv
mCD28 EC/TM/IC
mZETA
ITAM 1
mCD28_TM
mCD28_EC
P2A
mCO hCD8α SP
mIL36, Ntrunc
M13 fwd
Myc
(7804) BsrGI
(7998) BamHI
(7708) BsaAI
(7700) BamHI
(7588) BamHI
(7507) BspQI - SapI
(7410) PaeR7I - PspXI - TIi1 - XhoI
(7247) BstZ17I
(7114) SbfI
(6880) BbsI
(6844) NotI
(6752) SphI
(6233) DraIII
(6093) PspFI
(6089) BseYI
(6043) NcoI
(6000) BsaAI - PmlI
(5876) DraIII
(5838) AgeI
(5675) BglII
(5538) BsrGI
(5471) SexAI*
(5240) SrfI
(5239) ApaI
(5235) PspOMI

FIG. 1B

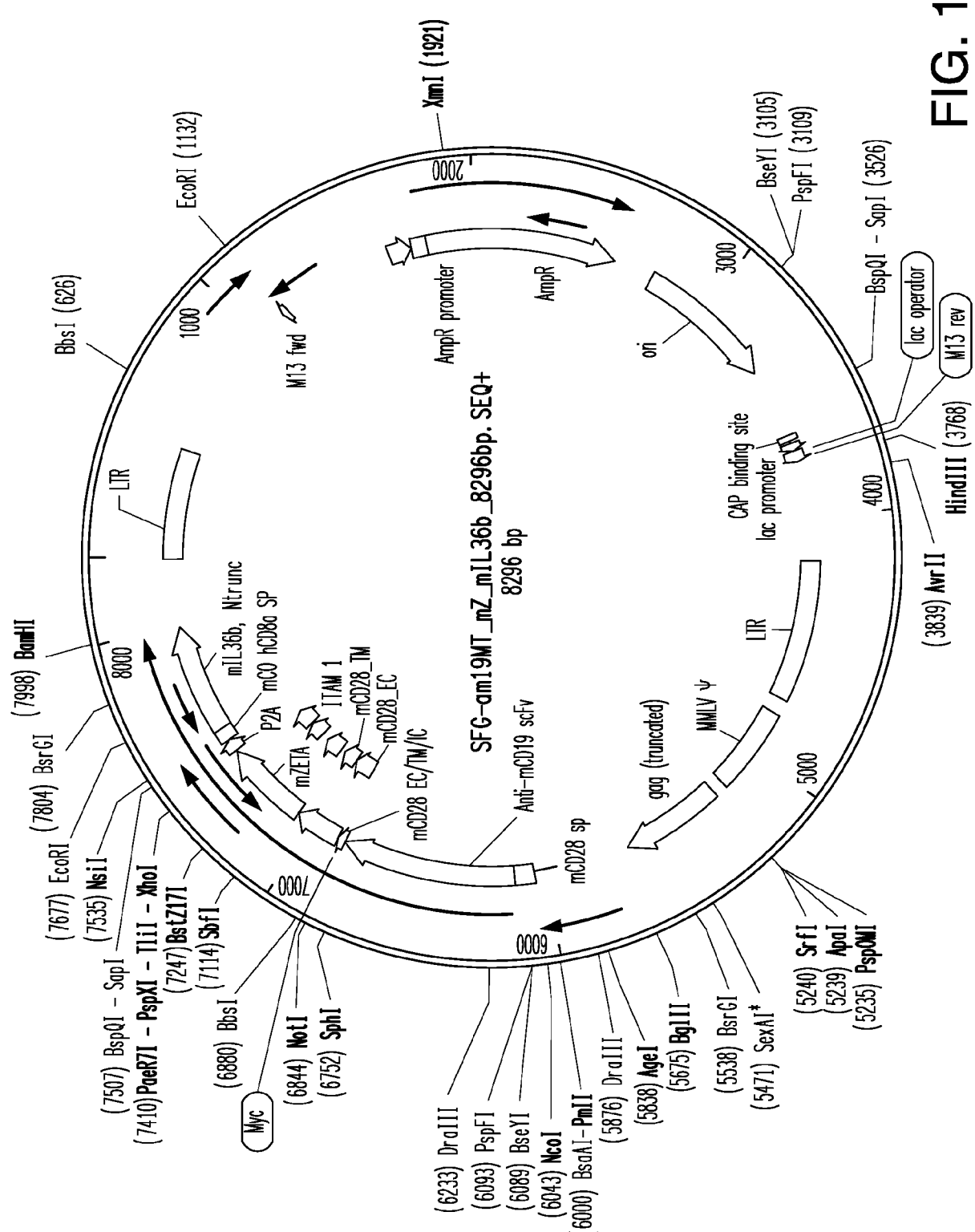

SFG-am19MT_mZ_mIL36b_8296bp. SEQ+
8296 bp
XmnI (1921)
EcoRI (1132)
BbsI (626)
BsrGI (7804)
BamHI (7998)
EcoRI (7677)
BspQI - SapI (7507)
NsiI (7535)
PaeR7I - PspXI - TliI - XhoI (7410)
BstZ17I (7247)
SbfI (7114)
BbsI (6880)
Myc
NotI (6844)
SphI (6752)
DraIII (6233)
PspFI (6093)
BseYI (6089)
NcoI (6043)
BsaAI - PmlI (6000)
DraIII (5876)
AgeI (5838)
BglII (5675)
BsrGI (5538)
SexAI* (5471)
SrfI (5240)
ApaI (5239)
PspOMI (5235)
BseYI (3105)
PspFI (3109)
BspQI - SapI (3526)
lac operator
M13 rev
HindIII (3768)
AvrII (3839)
M13 fwd
AmpR promoter
AmpR
ori
CAP binding site
lac promoter
LTR
mIL36b, Ntrunc
mCO hCD8α SP
P2A
mZETA
ITAM 1
mCD28_TM
mCD28_EC
mCD28 EC/TM/IC
Anti-mCD19 scFv
mCD28 sp
gag (truncated)
MMLV ψ
LTR
1000
2000
3000
4000
5000
6000
7000
8000

FIG. 1C

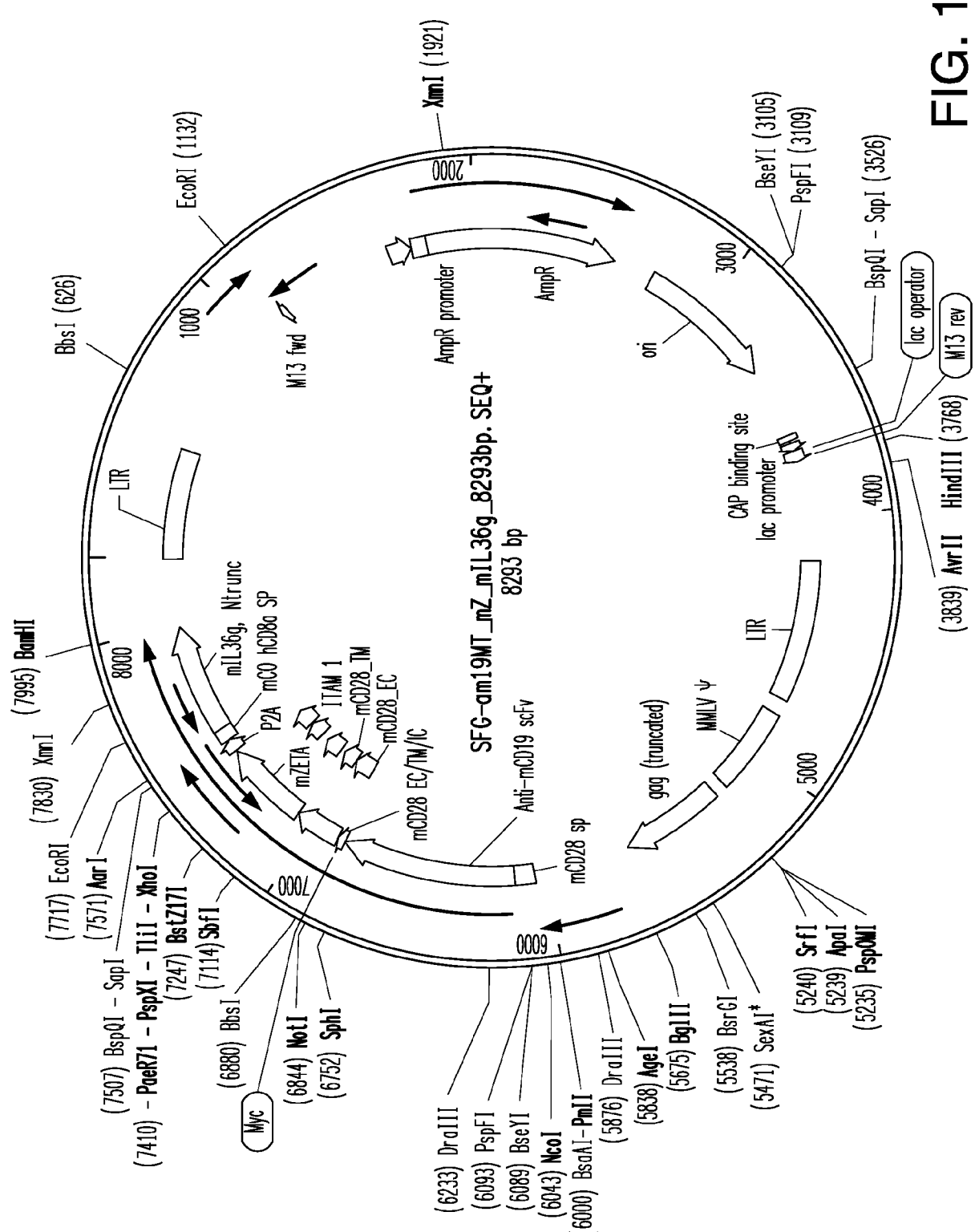

SFG-am19MT_mZ_mIL36g_8293bp. SEQ+
8293 bp
XmnI (1921)
EcoRI (1132)
BbsI (626)
BseYI (3105)
PspFI (3109)
BspQI - SapI (3526)
lac operator
M13 rev
HindIII (3768)
AvrII (3839)
CAP binding site
lac promoter
AmpR promoter
AmpR
ori
M13 fwd
LTR
mIL36g, Ntrunc
mCO hCD8α SP
P2A
ITAM 1
mCD28_TM
mCD28_EC
mZETA
mCD28 EC/TM/IC
Anti-mCD19 scFv
mCD28 sp
gag (truncated)
MMLV ψ
LTR
BamHI (7995)
XmnI (7830)
EcoRI (7717)
AvrII (7571)
BspQI - SapI (7507)
PaeR7I - PspXI - TliI - XhoI (7410)
BstZ17I (7247)
SbfI (7114)
BbsI (6880)
Myc
NotI (6844)
SphI (6752)
DraIII (6233)
PspFI (6093)
BseYI (6089)
NcoI (6043)
BsaAI - PmlI (6000)
DraIII (5876)
AgeI (5838)
BglII (5675)
BsrGI (5538)
SexAI* (5471)
SrfI (5240)
ApaI (5239)
PspOMI (5235)
2000
3000
4000
5000
6000
7000
8000
1000

FIG. 1D

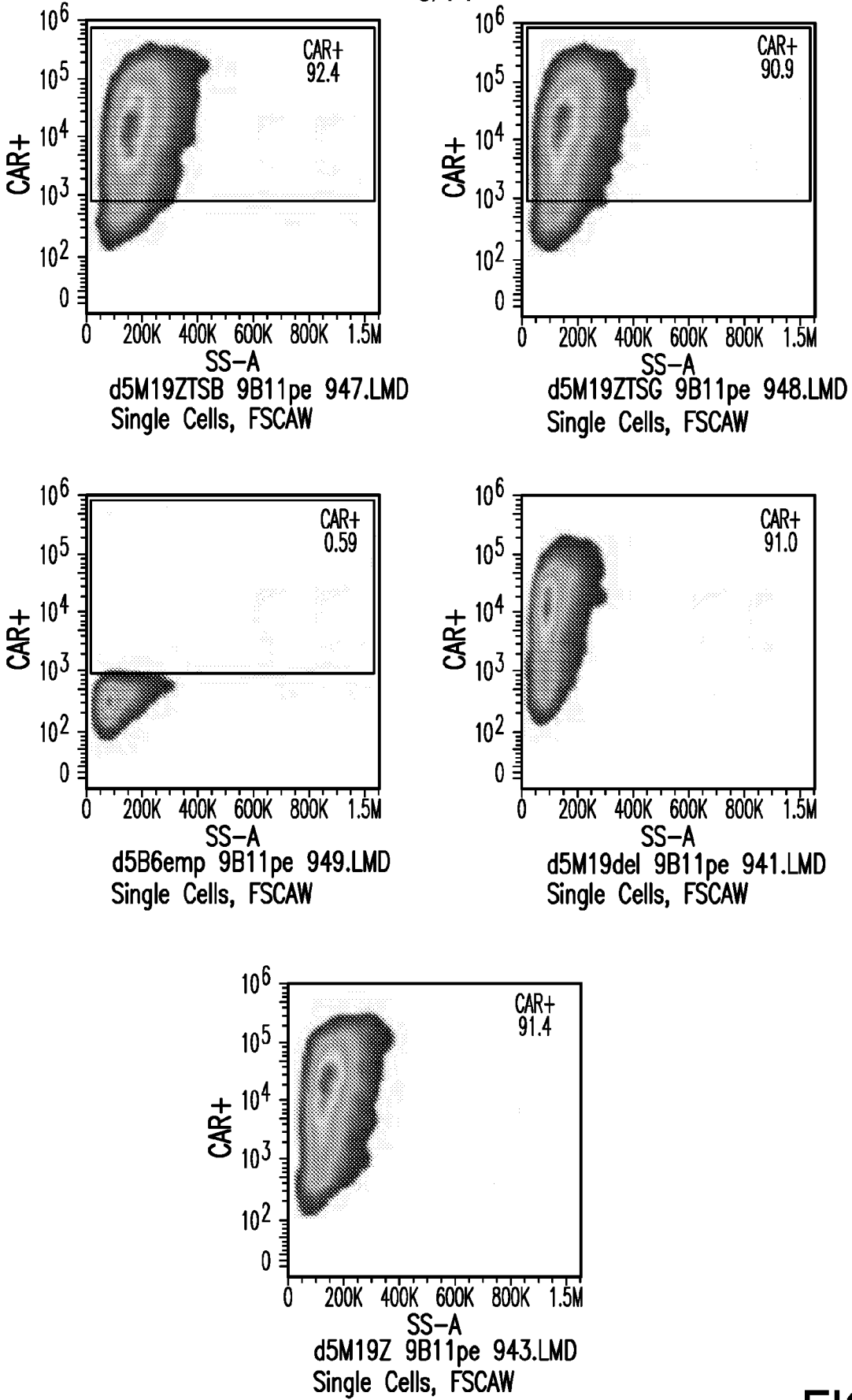
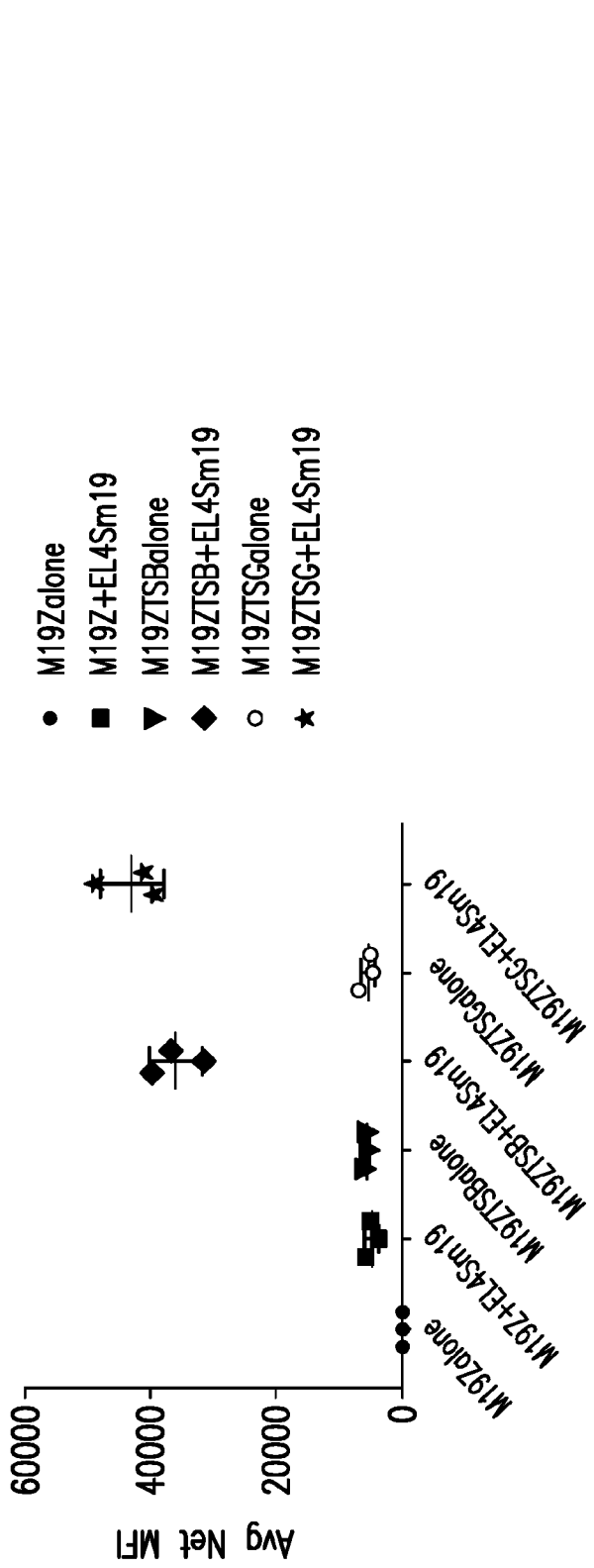

CAR+
92.4
CAR+
SS-A
d5M19ZTSB 9B11pe 947.LMD
Single Cells, FSCAW
CAR+
90.9
CAR+
SS-A
d5M19ZTSG 9B11pe 948.LMD
Single Cells, FSCAW
CAR+
0.59
CAR+
SS-A
d5B6emp 9B11pe 949.LMD
Single Cells, FSCAW
CAR+
91.0
CAR+
SS-A
d5M19del 9B11pe 941.LMD
Single Cells, FSCAW
CAR+
91.4
CAR+
SS-A
d5M19Z 9B11pe 943.LMD
Single Cells, FSCAW

FIG. 2



Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	B-?	
M19Z+EL4Sm19 vs M19Zalone	4440	-2067 to 10947	No	ns	0.2328	A	M19Zalone
M19Z+EL4Sm19 vs. M19ZTBalone	-786.7	-7294 to 5720	No	ns	0.9958	C	M19ZTSBalone
M19Z+EL4Sm19 vs. M19ZTSB+EL4Sm19	-31264	-37771 to -24757	Yes	****	0.0001	D	M19ZTSB+EL4Sm19
M19Z+EL4Sm19 vs. M19ZTSGalone	-5772	-7084 to 5930	No	ns	0.9986	E	M19ZTSGalone
M19Z+EL4Sm19 vs. M19ZTSG+EL4Sm19	-38359	-44866 to -31852	Yes	****	0.0001	F	M19ZTSG+EL4Sm19
Test details	Mean 1	Mean 2	Mean Diff	SE of diff.	n1	n2	q
M19Z+EL4Sm19 vs. M19Zalone	4643	203	4440	2243	3	3	1.98
M19Z+EL4Sm19 vs. M19ZTSBalone	4643	5430	-7867	2243	3	3	0.3508

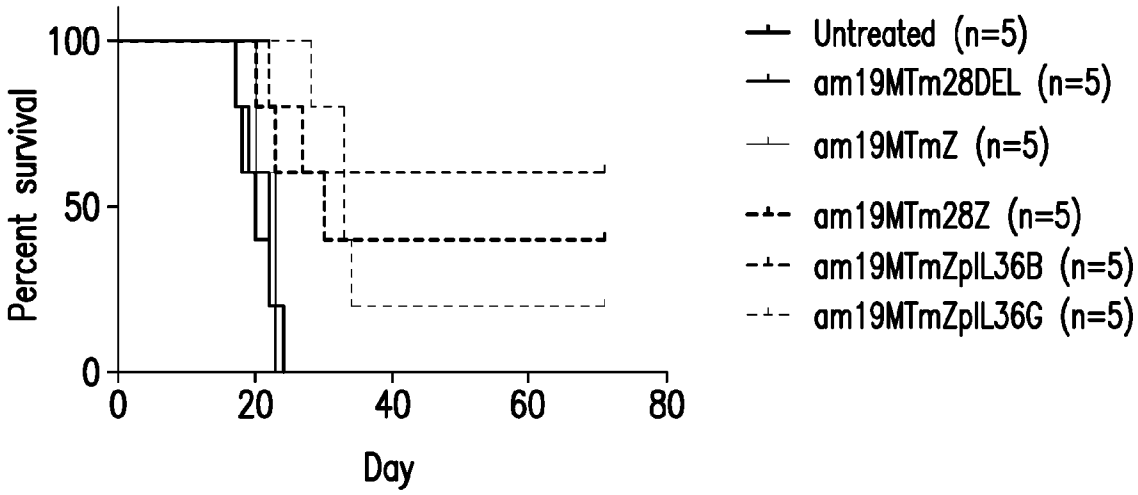
FIG. 3

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	B-?	
M19Z+EL4Sm19 vs M19Zalone	125163	112682 to 137643	Yes	****	0.0001	A	M19Zalone
M19Z+EL4Sm19 vs. M19ZTSBalone	106232	93752 to 118712	Yes	****	0.0001	C	M19ZTSBalone
M19Z+EL4Sm19 vs. M19ZTSB+EL4Sm19	-31172	-43652 to -18692	Yes	****	0.0001	D	M19ZTSB+EL4Sm19
M19Z+EL4Sm19 vs. M19ZTSGalone	99811	87331 to 112291	Yes	****	0.0001	E	M19ZTSGalone
M19Z+EL4Sm19 vs. M19ZTSG+EL4Sm19	-48790	-61271 to -36310	Yes	****	0.0001	F	M19ZTSG+EL4Sm19
Test details	Mean 1	Mean 2	Mean Diff	SE of diff.	n1	n2	q
M19Z+EL4Sm19 vs. M19Zalone	128067	2905	125163	4302	3	3	29.1
M19Z+EL4Sm19 vs. M19ZTSBalone	128067	21835	106232	4302	3	3	24.7

FIG. 4

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	B-?	
M19Z+EL4Sm19 vs M19Zalone	772.2	-748.8 to 2293	No	ns	0.4744	A	M19Zalone
M19Z+EL4Sm19 vs. M19ZTSBalone	359.7	-1161 to 1181	No	ns	0.9322	C	M19ZTSBalone
M19Z+EL4Sm19 vs. M19ZTSB+EL4Sm19	-9269	-10790 to -7748	Yes	****	0.0001	D	M19ZTSB+EL4Sm19
M19Z+EL4Sm19 vs. M19ZTSGalone	570.5	-950.5 to 2092	No	ns	0.7192	E	M19ZTSGalone
M19Z+EL4Sm19 vs. M19ZTSG+EL4Sm19	-3622	-5143 to -2101	Yes	****	0.0001	F	M19ZTSG+EL4Sm19
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q
M19Z+EL4Sm19 vs. M19Zalone	904.9	132.8	772.2	524.2	3	3	1.473
M19Z+EL4Sm19 vs. M19ZTSBalone	904.9	545.3	359.7	524.2	3	3	0.6861

FIG. 5



	UNtreated (n=5)	am19MTm28DEL (n=5)	am19MTmZ (n=5)	am19MTm28Z (n=5)	am19MTmZp IL36B (n=5)	am19MTmZp IL36G (n=5)
Median survival	22	20	23	30	Undefined	33

FIG. 6A

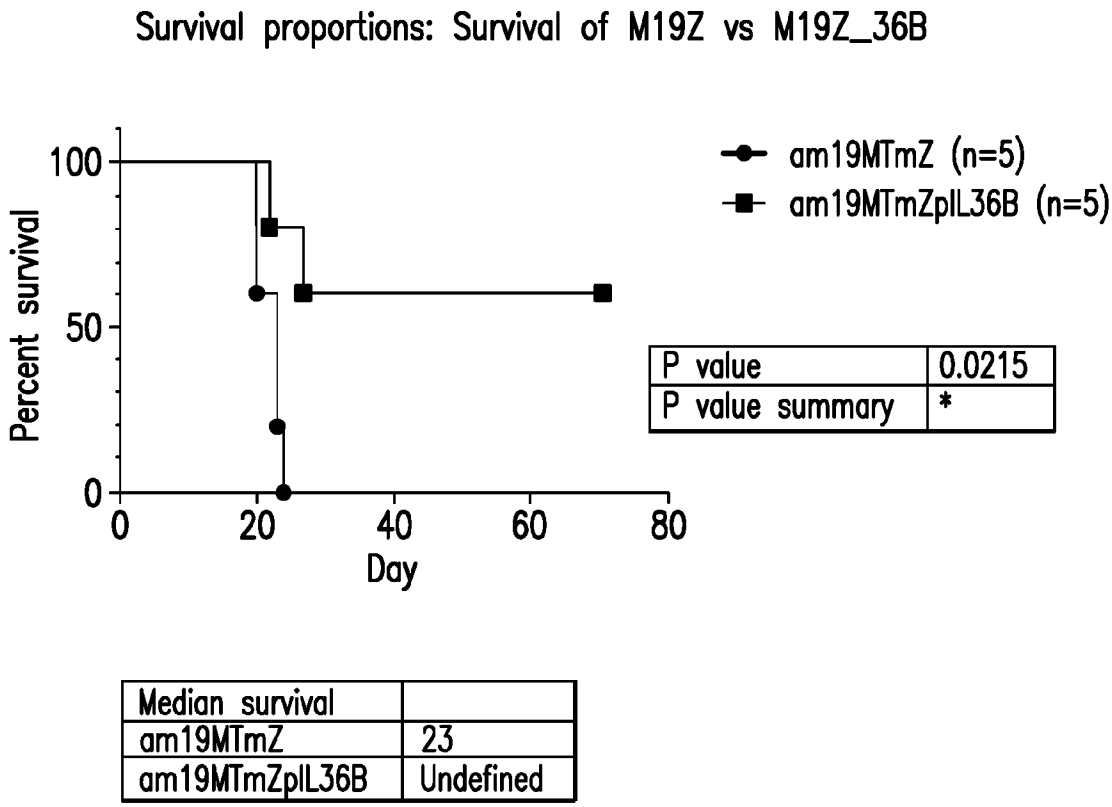
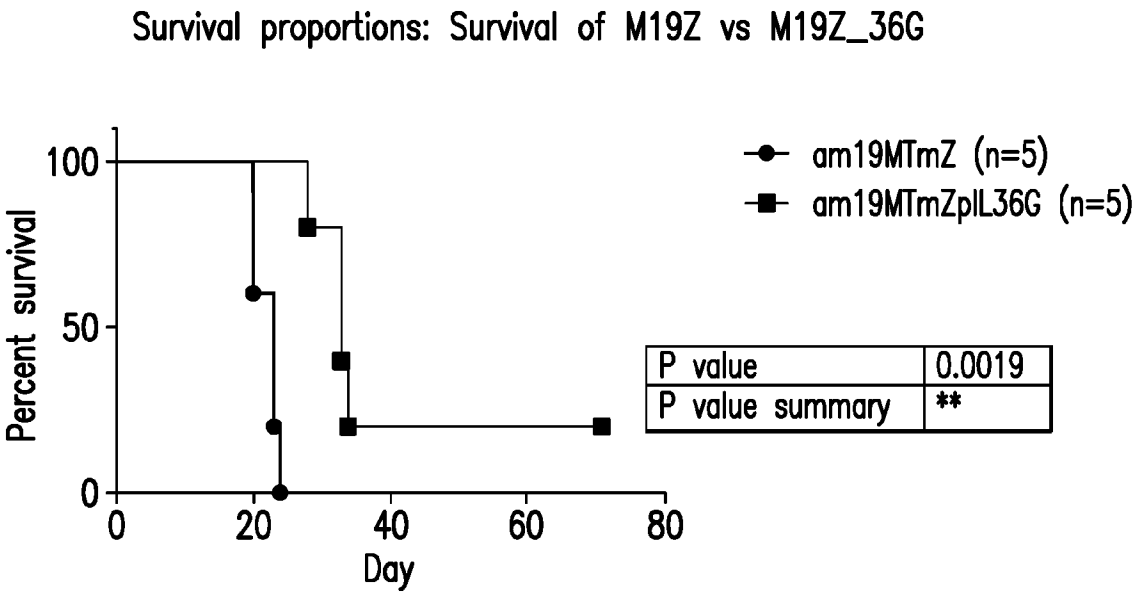

Survival proportions: Survival of M19Z vs M19Z_36B
Percent survival
100
50
0
0
20
40
60
80
Day
am19MTmZ (n=5)
am19MTmZpIL36B (n=5)
P value 0.0215
P value summary *
Median survival
am19MTmZ 23
am19MTmZpIL36B Undefined

FIG. 6B



Median survival		
am19MTmZ (n=5)	23	
am19MTmZpIL36G	33	
Ratio (and its reciprocal)	0.697	1.435
95% CI of ratio	0.1872 to 2.596	0.3853 to 5.343

FIG. 6C

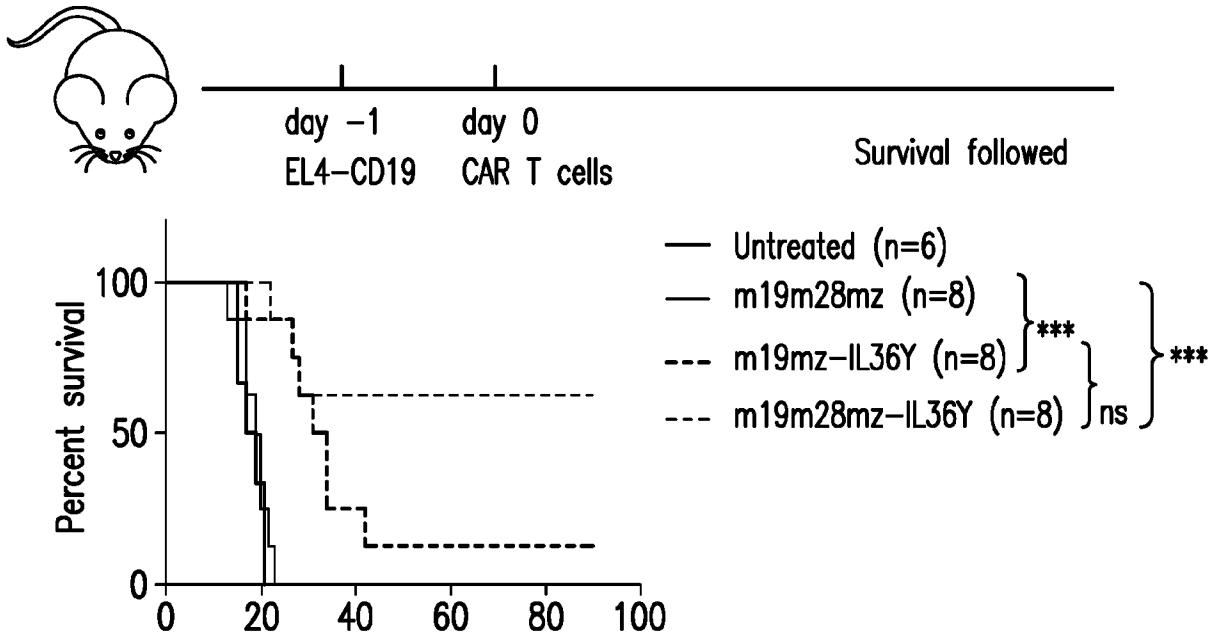

day −1
EL4−CD19
day 0
CAR T cells
Survival followed
Percent survival
100
50
0
0
20
40
60
80
100
Untreated (n=6)
m19m28mz (n=8)
m19mz−IL36Y (n=8)
m19m28mz−IL36Y (n=8)
***
ns
***

FIG. 7

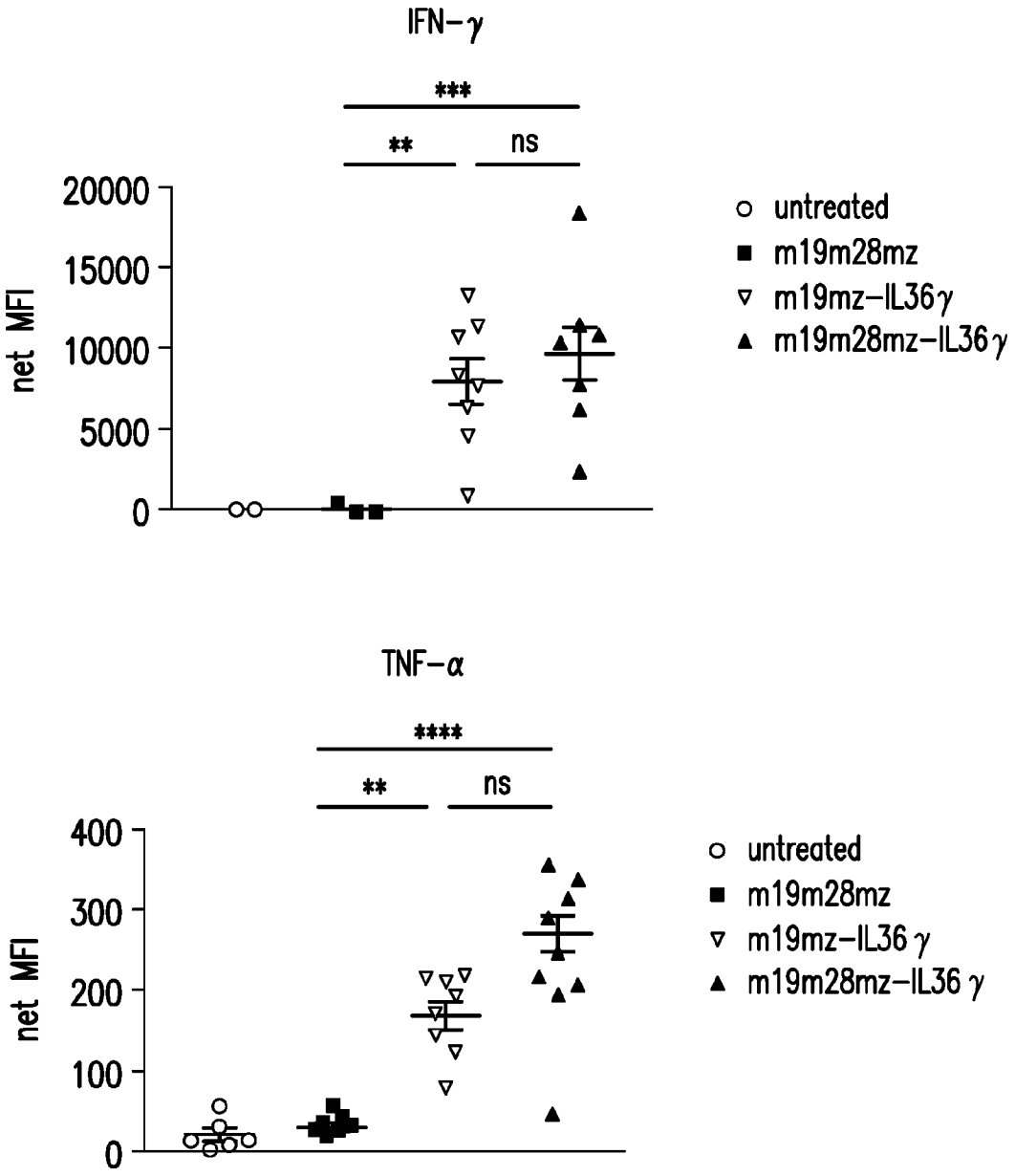

IFN-γ
***
**
ns
20000
15000
10000
5000
0
net MFI
untreated
m19m28mz
m19mz-IL36γ
m19m28mz-IL36γ
TNF-α
****
**
ns
400
300
200
100
0
net MFI
untreated
m19m28mz
m19mz-IL36γ
m19m28mz-IL36γ

FIG. 8

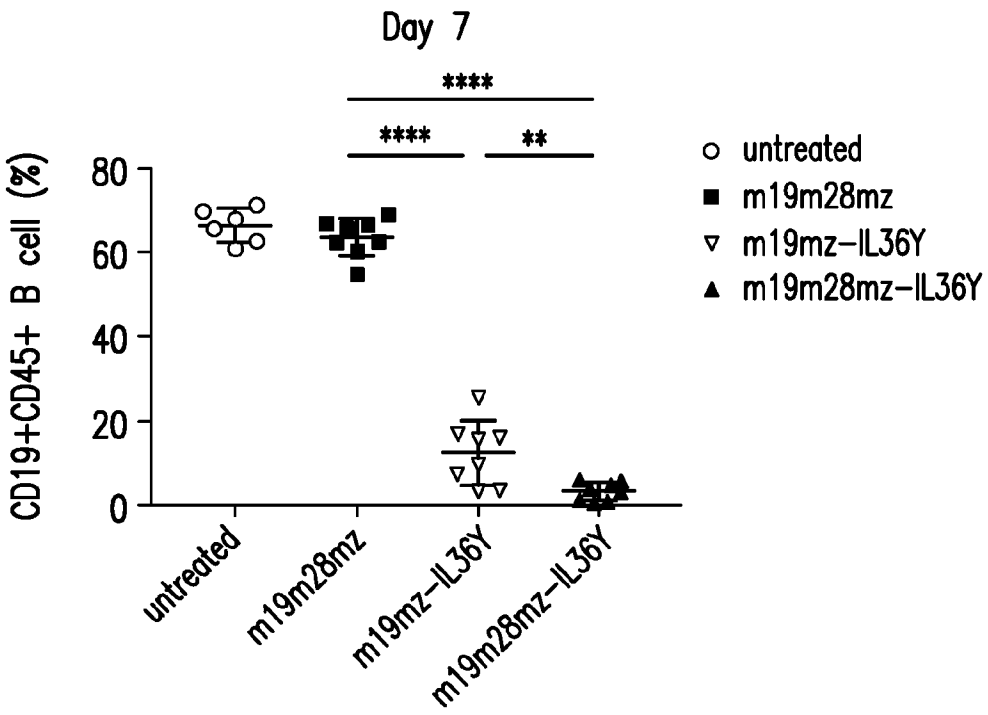

Day 7
****
****
**
CD19+CD45+ B cell (%)
80
60
40
20
0
untreated
m19m28mz
m19mz-IL36Y
m19m28mz-IL36Y
untreated
m19m28mz
m19mz-IL36Y
m19m28mz-IL36Y

FIG. 9

SEQUENCE LISTING

<110> MEMORIAL SLOAN-KETTERING CANCER CENTER

<120> IL-36 SECRETING IMMUNORESPONSIVE CELLS AND USES THEREOF

<130> 072734.0799

<140> PCT/US2018/061003
<141> 2018-11-14

<150> 62/585,879
<151> 2017-11-14

<160> 80

<170> PatentIn version 3.5

<210> 1
<211> 164
<212> PRT
<213> Homo sapiens

<400> 1

```
Met Lys Trp Lys Ala Leu Phe Thr Ala Ala Ile Leu Gln Ala Gln Leu
1               5                  10                  15

Pro Ile Thr Glu Ala Gln Ser Phe Gly Leu Leu Asp Pro Lys Leu Cys
            20                  25                  30

Tyr Leu Leu Asp Gly Ile Leu Phe Ile Tyr Gly Val Ile Leu Thr Ala
        35                  40                  45

Leu Phe Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr
    50                  55                  60

Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg
65                  70                  75                  80

Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met
```

85 90 95

Gly Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn
100 105 110

Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met
115 120 125

Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly
130 135 140

Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala
145 150 155 160

Leu Pro Pro Arg

<210> 2
<211> 220
<212> PRT
<213> Homo sapiens

<400> 2
Met Leu Arg Leu Leu Leu Ala Leu Asn Leu Phe Pro Ser Ile Gln Val
1 5 10 15

Thr Gly Asn Lys Ile Leu Val Lys Gln Ser Pro Met Leu Val Ala Tyr
20 25 30

Asp Asn Ala Val Asn Leu Ser Cys Lys Tyr Ser Tyr Asn Leu Phe Ser
35 40 45

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

Val Cys Val Val Tyr Gly Asn Tyr Ser Gln Gln Leu Gln Val Tyr Ser
65 70 75 80

Lys Thr Gly Phe Asn Cys Asp Gly Lys Leu Gly Asn Glu Ser Val Thr
85 90 95

Phe Tyr Leu Gln Asn Leu Tyr Val Asn Gln Thr Asp Ile Tyr Phe Cys
100 105 110

Lys Ile Glu Val Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Lys Ser
115 120 125

Asn Gly Thr Ile Ile His Val Lys Gly Lys His Leu Cys Pro Ser Pro
130 135 140

Leu Phe Pro Gly Pro Ser Lys Pro Phe Trp Val Leu Val Val Val Gly
145 150 155 160

Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile
165 170 175

Phe Trp Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met
180 185 190

Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro
195 200 205

Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser
210 215 220

<210> 3
<211> 255
<212> PRT
<213> Homo sapiens

<400> 3

```
Met Gly Asn Ser Cys Tyr Asn Ile Val Ala Thr Leu Leu Leu Val Leu
1               5                  10                  15

Asn Phe Glu Arg Thr Arg Ser Leu Gln Asp Pro Cys Ser Asn Cys Pro
            20                  25                  30

Ala Gly Thr Phe Cys Asp Asn Asn Arg Asn Gln Ile Cys Ser Pro Cys
        35                  40                  45

Pro Pro Asn Ser Phe Ser Ser Ala Gly Gly Gln Arg Thr Cys Asp Ile
    50                  55                  60

Cys Arg Gln Cys Lys Gly Val Phe Arg Thr Arg Lys Glu Cys Ser Ser
65                  70                  75                  80

Thr Ser Asn Ala Glu Cys Asp Cys Thr Pro Gly Phe His Cys Leu Gly
                85                  90                  95

Ala Gly Cys Ser Met Cys Glu Gln Asp Cys Lys Gln Gly Gln Glu Leu
            100                 105                 110

Thr Lys Lys Gly Cys Lys Asp Cys Cys Phe Gly Thr Phe Asn Asp Gln
        115                 120                 125

Lys Arg Gly Ile Cys Arg Pro Trp Thr Asn Cys Ser Leu Asp Gly Lys
    130                 135                 140

Ser Val Leu Val Asn Gly Thr Lys Glu Arg Asp Val Val Cys Gly Pro
145                 150                 155                 160

Ser Pro Ala Asp Leu Ser Pro Gly Ala Ser Ser Val Thr Pro Pro Ala
                165                 170                 175
```

Pro Ala Arg Glu Pro Gly His Ser Pro Gln Ile Ile Ser Phe Phe Leu
180 185 190

Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu
195 200 205

Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe
210 215 220

Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly
225 230 235 240

Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
245 250 255

<210> 4
<211> 153
<212> PRT
<213> Homo sapiens

<400> 4
Lys Ile Asp Thr Pro Gln Gln Gly Ser Ile Gln Asp Ile Asn His Arg
1 5 10 15

Val Trp Val Leu Gln Asp Gln Thr Leu Ile Ala Val Pro Arg Lys Asp
20 25 30

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

Thr Leu Glu Lys Asp Arg Gly Asn Pro Ile Tyr Leu Gly Leu Asn Gly
50 55 60

Leu Asn Leu Cys Leu Met Cys Ala Lys Val Gly Asp Gln Pro Thr Leu
65 70 75 80

Gln Leu Lys Glu Lys Asp Ile Met Asp Leu Tyr Asn Gln Pro Glu Pro
85 90 95

Val Lys Ser Phe Leu Phe Tyr His Ser Gln Ser Gly Arg Asn Ser Thr
100 105 110

Phe Glu Ser Val Ala Phe Pro Gly Trp Phe Ile Ala Val Ser Ser Glu
115 120 125

Gly Gly Cys Pro Leu Ile Leu Thr Gln Glu Leu Gly Lys Ala Asn Thr
130 135 140

Thr Asp Phe Gly Leu Thr Met Leu Phe
145 150

<210> 5
<211> 455
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 5
Met Ala Ser Pro Leu Thr Arg Phe Leu Ser Leu Asn Leu Leu Leu Leu
1 5 10 15

Gly Glu Ser Ile Ile Leu Gly Ser Gly Glu Ala Glu Val Gln Leu Gln
20 25 30

Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Thr Ser Val Lys Leu Ser
35 40 45

Cys Lys Val Ser Gly Asp Thr Ile Thr Phe Tyr Tyr Met His Phe Val
50 55 60

Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Arg Ile Asp Pro
65 70 75 80

Glu Asp Glu Ser Thr Lys Tyr Ser Glu Lys Phe Lys Asn Lys Ala Thr
85 90 95

Leu Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr Leu Lys Leu Ser Ser
100 105 110

Leu Thr Ser Glu Asp Thr Ala Thr Tyr Phe Cys Ile Tyr Gly Gly Tyr
115 120 125

Tyr Phe Asp Tyr Trp Gly Gln Gly Val Met Val Thr Val Ser Ser Gly
130 135 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile
145 150 155 160

Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Thr Ser Leu Gly Glu Thr
165 170 175

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

Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Gln Leu Leu Ile Tyr Gly
195 200 205

Ala Ser Asp Leu Gln Asp Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
210 215 220

Ser Gly Thr Gln Tyr Ser Leu Lys Ile Thr Ser Met Gln Thr Glu Asp
225 230 235 240

```
Glu Gly Val Tyr Phe Cys Gln Gln Gly Leu Thr Tyr Pro Arg Thr Phe
                245                 250                 255

Gly Gly Gly Thr Lys Leu Glu Leu Lys Arg Ala Ala Ala Glu Gln Lys
            260                 265                 270

Leu Ile Ser Glu Glu Asp Leu Ile Glu Phe Met Tyr Pro Pro Pro Tyr
        275                 280                 285

Leu Asp Asn Glu Arg Ser Asn Gly Thr Ile Ile His Ile Lys Glu Lys
    290                 295                 300

His Leu Cys His Thr Gln Ser Ser Pro Lys Leu Phe Trp Ala Leu Val
305                 310                 315                 320

Val Val Ala Gly Val Leu Phe Cys Tyr Gly Leu Leu Val Thr Val Ala
                325                 330                 335

Leu Cys Val Ile Trp Thr Arg Ala Lys Phe Ser Arg Ser Ala Glu Thr
            340                 345                 350

Ala Ala Asn Leu Gln Asp Pro Asn Gln Leu Tyr Asn Glu Leu Asn Leu
        355                 360                 365

Gly Arg Arg Glu Glu Tyr Asp Val Leu Glu Lys Lys Arg Ala Arg Asp
    370                 375                 380

Pro Glu Met Gly Gly Lys Gln Gln Arg Arg Arg Asn Pro Gln Glu Gly
385                 390                 395                 400

Val Tyr Asn Ala Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu
                405                 410                 415
```

Ile Gly Thr Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu
420 425 430

Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His
435 440 445

Met Gln Thr Leu Ala Pro Arg
450 455

<210> 6
<211> 496
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 6
Met Ala Ser Pro Leu Thr Arg Phe Leu Ser Leu Asn Leu Leu Leu Leu
1 5 10 15

Gly Glu Ser Ile Ile Leu Gly Ser Gly Glu Ala Glu Val Gln Leu Gln
20 25 30

Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Thr Ser Val Lys Leu Ser
35 40 45

Cys Lys Val Ser Gly Asp Thr Ile Thr Phe Tyr Tyr Met His Phe Val
50 55 60

Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Arg Ile Asp Pro
65 70 75 80

Glu Asp Glu Ser Thr Lys Tyr Ser Glu Lys Phe Lys Asn Lys Ala Thr
85 90 95

Leu Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr Leu Lys Leu Ser Ser
100 105 110

Leu Thr Ser Glu Asp Thr Ala Thr Tyr Phe Cys Ile Tyr Gly Gly Tyr
115 120 125

Tyr Phe Asp Tyr Trp Gly Gln Gly Val Met Val Thr Val Ser Ser Gly
130 135 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile
145 150 155 160

Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Thr Ser Leu Gly Glu Thr
165 170 175

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

Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Gln Leu Leu Ile Tyr Gly
195 200 205

Ala Ser Asp Leu Gln Asp Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
210 215 220

Ser Gly Thr Gln Tyr Ser Leu Lys Ile Thr Ser Met Gln Thr Glu Asp
225 230 235 240

Glu Gly Val Tyr Phe Cys Gln Gln Gly Leu Thr Tyr Pro Arg Thr Phe
245 250 255

Gly Gly Gly Thr Lys Leu Glu Leu Lys Arg Ala Ala Ala Glu Gln Lys
260 265 270

Leu Ile Ser Glu Glu Asp Leu Ile Glu Phe Met Tyr Pro Pro Pro Tyr
275 280 285

Leu Asp Asn Glu Arg Ser Asn Gly Thr Ile Ile His Ile Lys Glu Lys
290 295 300

His Leu Cys His Thr Gln Ser Ser Pro Lys Leu Phe Trp Ala Leu Val
305 310 315 320

Val Val Ala Gly Val Leu Phe Cys Tyr Gly Leu Leu Val Thr Val Ala
325 330 335

Leu Cys Val Ile Trp Thr Asn Ser Arg Arg Asn Arg Leu Leu Gln Ser
340 345 350

Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly Leu Thr Arg Lys Pro
355 360 365

Tyr Gln Pro Tyr Ala Pro Ala Arg Asp Phe Ala Ala Tyr Arg Pro Arg
370 375 380

Ala Lys Phe Ser Arg Ser Ala Glu Thr Ala Ala Asn Leu Gln Asp Pro
385 390 395 400

Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp
405 410 415

Val Leu Glu Lys Lys Arg Ala Arg Asp Pro Glu Met Gly Gly Lys Gln
420 425 430

Gln Arg Arg Arg Asn Pro Gln Glu Gly Val Tyr Asn Ala Leu Gln Lys
435 440 445

Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Thr Lys Gly Glu Arg

450 455 460

Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala
465 470 475 480

Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Thr Leu Ala Pro Arg
485 490 495

<210> 7
<211> 1491
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 7
atggcctcac cgttgacccg ctttctgtcg ctgaacctgc tgctgctggg tgagtcgatt 60

atcctgggga gtggagaagc tgaagtccag ctgcagcagt ctggggctga gcttgtgaga 120

cctgggacct ctgtgaagtt atcttgcaaa gtttctggcg ataccattac attttactac 180

atgcactttg tgaagcaaag gcctggacag ggtctggaat ggataggaag gattgatcct 240

gaggatgaaa gtactaaata ttctgagaag ttcaaaaaca aggcgacact cactgcagat 300

acatcttcca acacagccta cctgaagctc agcagcctga cctctgagga cactgcaacc 360

tatttttgta tctacggagg atactacttt gattactggg gccaaggggt catggtcaca 420

gtctcctcag gtggaggtgg atcaggtgga ggtggatctg gtggaggtgg atctgacatc 480

cagatgacac agtctccagc ttccctgtct acatctctgg gagaaactgt caccatccaa
540

tgtcaagcaa gtgaggacat ttacagtggt ttagcgtggt atcagcagaa gccagggaaa
600

tctcctcagc tcctgatcta tggtgcaagt gacttacaag acggcgtccc atcacgattc
660

agtggcagtg gatctggcac acagtattct ctcaagatca ccagcatgca aactgaagat
720

gaaggggttt atttctgtca acagggttta acgtatcctc ggacgttcgg tggcggcacc
780

aagctggaat tgaaacgggc ggccgcagaa cagaaactga tctctgaaga agacctgatt
840

gagttcatgt accctccgcc ttacctagac aacgagagga gcaatggaac tattattcac
900

ataaaagaga aacatctttg tcatactcag tcatctccta agctgttttg ggcactggtc
960

gtggttgctg gagtcctgtt ttgttatggc ttgctagtga cagtggctct ttgtgttatc
1020

tggacaaata gtagaaggaa cagactcctt caaagtgact acatgaacat gactccccgg
1080

aggcctgggc tcactcgaaa gccttaccag ccctacgccc ctgccagaga ctttgcagcg
1140

taccgcccca gagcaaaatt cagcaggagt gcagagactg ctgccaacct gcaggacccc
1200

aaccagctct acaatgagct caatctaggg cgaagagagg aatatgacgt cttggagaag
1260

aagcgggctc gggatccaga gatgggaggc aaacagcaga ggaggaggaa cccccaggaa
1320

ggcgtataca atgcactgca gaaagacaag atggcagaag cctacagtga gatcggcaca
1380

aaaggcgaga ggcggagagg caaggggcac gatggccttt accagggtct cagcactgcc 1440

accaaggaca cctatgatgc cctgcatatg cagaccctgg cccctcgcta a 1491

<210> 8
<211> 20
<212> PRT
<213> Homo sapiens

<400> 8
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser
20

<210> 9
<211> 235
<212> PRT
<213> Homo sapiens

<400> 9
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Ser Gln Phe Arg Val Ser Pro Leu Asp Arg Thr
20 25 30

Trp Asn Leu Gly Glu Thr Val Glu Leu Lys Cys Gln Val Leu Leu Ser
35 40 45

Asn Pro Thr Ser Gly Cys Ser Trp Leu Phe Gln Pro Arg Gly Ala Ala
50 55 60

Ala Ser Pro Thr Phe Leu Leu Tyr Leu Ser Gln Asn Lys Pro Lys Ala
65 70 75 80

Ala Glu Gly Leu Asp Thr Gln Arg Phe Ser Gly Lys Arg Leu Gly Asp
85 90 95

Thr Phe Val Leu Thr Leu Ser Asp Phe Arg Arg Glu Asn Glu Gly Tyr
100 105 110

Tyr Phe Cys Ser Ala Leu Ser Asn Ser Ile Met Tyr Phe Ser His Phe
115 120 125

Val Pro Val Phe Leu Pro Ala Lys Pro Thr Thr Thr Pro Ala Pro Arg
130 135 140

Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg
145 150 155 160

Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly
165 170 175

Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr
180 185 190

Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Asn His
195 200 205

Arg Asn Arg Arg Arg Val Cys Lys Cys Pro Arg Pro Val Val Lys Ser
210 215 220

Gly Asp Lys Pro Ser Leu Ser Ala Arg Tyr Val
225 230 235

<210> 10
<211> 247
<212> PRT

<213> Mus musculus

<400> 10

```
Met Ala Ser Pro Leu Thr Arg Phe Leu Ser Leu Asn Leu Leu Leu Met
1               5                  10                  15

Gly Glu Ser Ile Ile Leu Gly Ser Gly Glu Ala Lys Pro Gln Ala Pro
            20                  25                  30

Glu Leu Arg Ile Phe Pro Lys Lys Met Asp Ala Glu Leu Gly Gln Lys
        35                  40                  45

Val Asp Leu Val Cys Glu Val Leu Gly Ser Val Ser Gln Gly Cys Ser
    50                  55                  60

Trp Leu Phe Gln Asn Ser Ser Ser Lys Leu Pro Gln Pro Thr Phe Val
65                  70                  75                  80

Val Tyr Met Ala Ser Ser His Asn Lys Ile Thr Trp Asp Glu Lys Leu
                85                  90                  95

Asn Ser Ser Lys Leu Phe Ser Ala Val Arg Asp Thr Asn Asn Lys Tyr
            100                 105                 110

Val Leu Thr Leu Asn Lys Phe Ser Lys Glu Asn Glu Gly Tyr Tyr Phe
        115                 120                 125

Cys Ser Val Ile Ser Asn Ser Val Met Tyr Phe Ser Ser Val Val Pro
    130                 135                 140

Val Leu Gln Lys Val Asn Ser Thr Thr Thr Lys Pro Val Leu Arg Thr
145                 150                 155                 160

Pro Ser Pro Val His Pro Thr Gly Thr Ser Gln Pro Gln Arg Pro Glu
                165                 170                 175
```

Asp Cys Arg Pro Arg Gly Ser Val Lys Gly Thr Gly Leu Asp Phe Ala
180 185 190

Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Ile Cys Val Ala Pro
195 200 205

Leu Leu Ser Leu Ile Ile Thr Leu Ile Cys Tyr His Arg Ser Arg Lys
210 215 220

Arg Val Cys Lys Cys Pro Arg Pro Leu Val Arg Gln Glu Gly Lys Pro
225 230 235 240

Arg Pro Ser Glu Lys Ile Val
245

<210> 11
<211> 69
<212> PRT
<213> Mus musculus

<400> 11
Ser Thr Thr Thr Lys Pro Val Leu Arg Thr Pro Ser Pro Val His Pro
1 5 10 15

Thr Gly Thr Ser Gln Pro Gln Arg Pro Glu Asp Cys Arg Pro Arg Gly
20 25 30

Ser Val Lys Gly Thr Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp
35 40 45

Ala Pro Leu Ala Gly Ile Cys Val Ala Leu Leu Leu Ser Leu Ile Ile
50 55 60

Thr Leu Ile Cys Tyr

<210> 12
<211> 207
<212> DNA
<213> Mus musculus

<400> 12
tctactacta ccaagccagt gctgcgaact ccctcacctg tgcaccctac cgggacatct 60

cagccccaga gaccagaaga ttgtcggccc cgtggctcag tgaaggggac cggattggac 120

ttcgcctgtg atatttacat ctgggcaccc ttggccggaa tctgcgtggc ccttctgctg 180

t c c t t g a t c a t c a c t c t c a t c t g c t a c 207

<210> 13
<211> 188
<212> PRT
<213> Mus musculus

<400> 13
Met Lys Trp Lys Val Ser Val Leu Ala Cys Ile Leu His Val Arg Phe
1 5 10 15

Pro Gly Ala Glu Ala Gln Ser Phe Gly Leu Leu Asp Pro Lys Leu Cys
            20 25 30

Tyr Leu Leu Asp Gly Ile Leu Phe Ile Tyr Gly Val Ile Ile Thr Ala
        35 40 45

Leu Tyr Leu Arg Ala Lys Phe Ser Arg Ser Ala Glu Thr Ala Ala Asn
    50 55 60

Leu Gln Asp Pro Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg

65 70 75 80

Glu Glu Tyr Asp Val Leu Glu Lys Lys Arg Ala Arg Asp Pro Glu Met
85 90 95

Gly Gly Lys Gln Arg Arg Arg Asn Pro Gln Glu Gly Val Tyr Asn Ala
100 105 110

Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Thr Lys
115 120 125

Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Asp Ser
130 135 140

His Phe Gln Ala Val Gln Phe Gly Asn Arg Arg Glu Arg Glu Gly Ser
145 150 155 160

Glu Leu Thr Arg Thr Leu Gly Leu Arg Ala Arg Pro Lys Ala Cys Arg
165 170 175

His Lys Lys Pro Leu Ser Leu Pro Ala Ala Val Ser
180 185

<210> 14
<211> 113
<212> PRT
<213> Mus musculus

<400> 14
Arg Ala Lys Phe Ser Arg Ser Ala Glu Thr Ala Ala Asn Leu Gln Asp
1 5 10 15

Pro Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
20 25 30

Asp Val Leu Glu Lys Lys Arg Ala Arg Asp Pro Glu Met Gly Gly Lys
35 40 45

Gln Gln Arg Arg Arg Asn Pro Gln Glu Gly Val Tyr Asn Ala Leu Gln
50 55 60

Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Thr Lys Gly Glu
65 70 75 80

Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr
85 90 95

Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Thr Leu Ala Pro
100 105 110

Arg

<210> 15
<211> 342
<212> DNA
<213> Mus musculus

<400> 15
agagcaaaat tcagcaggag tgcagagact gctgccaacc tgcaggaccc caaccagctc 60

tacaatgagc tcaatctagg gcgaagagag gaatatgacg tcttggagaa gaagcgggct 120

cgggatccag agatgggagg caaacagcag aggaggagga acccccagga aggcgtatac 180

aatgcactgc agaaagacaa gatggcagaa gcctacagtg agatcggcac aaaaggcgag 240

aggcggagag gcaaggggca cgatggcctt taccagggtc tcagcactgc caccaaggac 300

acctatgatg ccctgcatat gcagaccctg gcccctcgct aa
342

<210> 16
<211> 218
<212> PRT
<213> Mus musculus

<400> 16
Met Thr Leu Arg Leu Leu Phe Leu Ala Leu Asn Phe Phe Ser Val Gln
1 5 10 15

Val Thr Glu Asn Lys Ile Leu Val Lys Gln Ser Pro Leu Leu Val Val
20 25 30

Asp Ser Asn Glu Val Ser Leu Ser Cys Arg Tyr Ser Tyr Asn Leu Leu
35 40 45

Ala Lys Glu Phe Arg Ala Ser Leu Tyr Lys Gly Val Asn Ser Asp Val
50 55 60

Glu Val Cys Val Gly Asn Gly Asn Phe Thr Tyr Gln Pro Gln Phe Arg
65 70 75 80

Ser Asn Ala Glu Phe Asn Cys Asp Gly Asp Phe Asp Asn Glu Thr Val
85 90 95

Thr Phe Arg Leu Trp Asn Leu His Val Asn His Thr Asp Ile Tyr Phe
100 105 110

Cys Lys Ile Glu Phe Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Arg
115 120 125

Ser Asn Gly Thr Ile Ile His Ile Lys Glu Lys His Leu Cys His Thr
130 135 140

Gln Ser Ser Pro Lys Leu Phe Trp Ala Leu Val Val Val Ala Gly Val
145 150 155 160

Leu Phe Cys Tyr Gly Leu Leu Val Thr Val Ala Leu Cys Val Ile Trp
165 170 175

Thr Asn Ser Arg Arg Asn Arg Leu Leu Gln Ser Asp Tyr Met Asn Met
180 185 190

Thr Pro Arg Arg Pro Gly Leu Thr Arg Lys Pro Tyr Gln Pro Tyr Ala
195 200 205

Pro Ala Arg Asp Phe Ala Ala Tyr Arg Pro
210 215

<210> 17
<211> 123
<212> DNA
<213> Mus musculus

<400> 17
aatagtagaa ggaacagact ccttcaaagt gactacatga acatgactcc ccggaggcct
60

gggctcactc gaaagcctta ccagccctac gcccctgcca gagactttgc agcgtaccgc
120

c c c
123

<210> 18
<211> 277
<212> PRT
<213> Homo sapiens

<400> 18
Met Cys Val Gly Ala Arg Arg Leu Gly Arg Gly Pro Cys Ala Ala Leu
1 5 10 15

Leu Leu Leu Gly Leu Gly Leu Ser Thr Val Thr Gly Leu His Cys Val
20 25 30

Gly Asp Thr Tyr Pro Ser Asn Asp Arg Cys Cys His Glu Cys Arg Pro
35 40 45

Gly Asn Gly Met Val Ser Arg Cys Ser Arg Ser Gln Asn Thr Val Cys
50 55 60

Arg Pro Cys Gly Pro Gly Phe Tyr Asn Asp Val Val Ser Ser Lys Pro
65 70 75 80

Cys Lys Pro Cys Thr Trp Cys Asn Leu Arg Ser Gly Ser Glu Arg Lys
85 90 95

Gln Leu Cys Thr Ala Thr Gln Asp Thr Val Cys Arg Cys Arg Ala Gly
100 105 110

Thr Gln Pro Leu Asp Ser Tyr Lys Pro Gly Val Asp Cys Ala Pro Cys
115 120 125

Pro Pro Gly His Phe Ser Pro Gly Asp Asn Gln Ala Cys Lys Pro Trp
130 135 140

Thr Asn Cys Thr Leu Ala Gly Lys His Thr Leu Gln Pro Ala Ser Asn
145 150 155 160

Ser Ser Asp Ala Ile Cys Glu Asp Arg Asp Pro Pro Ala Thr Gln Pro
165 170 175

Gln Glu Thr Gln Gly Pro Pro Ala Arg Pro Ile Thr Val Gln Pro Thr
180 185 190

Glu Ala Trp Pro Arg Thr Ser Gln Gly Pro Ser Thr Arg Pro Val Glu
195 200 205

Val Pro Gly Gly Arg Ala Val Ala Ala Ile Leu Gly Leu Gly Leu Val
210 215 220

Leu Gly Leu Leu Gly Pro Leu Ala Ile Leu Leu Ala Leu Tyr Leu Leu
225 230 235 240

Arg Arg Asp Gln Arg Leu Pro Pro Asp Ala His Lys Pro Pro Gly Gly
245 250 255

Gly Ser Phe Arg Thr Pro Ile Gln Glu Glu Gln Ala Asp Ala His Ser
260 265 270

Thr Leu Ala Lys Ile
275

<210> 19
<211> 199
<212> PRT
<213> Homo sapiens

<400> 19
Met Lys Ser Gly Leu Trp Tyr Phe Phe Leu Phe Cys Leu Arg Ile Lys
1 5 10 15

Val Leu Thr Gly Glu Ile Asn Gly Ser Ala Asn Tyr Glu Met Phe Ile
20 25 30

Phe His Asn Gly Gly Val Gln Ile Leu Cys Lys Tyr Pro Asp Ile Val
35 40 45

Gln Gln Phe Lys Met Gln Leu Leu Lys Gly Gly Gln Ile Leu Cys Asp
50 55 60

Leu Thr Lys Thr Lys Gly Ser Gly Asn Thr Val Ser Ile Lys Ser Leu
65 70 75 80

Lys Phe Cys His Ser Gln Leu Ser Asn Asn Ser Val Ser Phe Phe Leu
85 90 95

Tyr Asn Leu Asp His Ser His Ala Asn Tyr Tyr Phe Cys Asn Leu Ser
100 105 110

Ile Phe Asp Pro Pro Pro Phe Lys Val Thr Leu Thr Gly Gly Tyr Leu
115 120 125

His Ile Tyr Glu Ser Gln Leu Cys Cys Gln Leu Lys Phe Trp Leu Pro
130 135 140

Ile Gly Cys Ala Ala Phe Val Val Val Cys Ile Leu Gly Cys Ile Leu
145 150 155 160

Ile Cys Trp Leu Thr Lys Lys Lys Tyr Ser Ser Ser Val His Asp Pro
165 170 175

Asn Gly Glu Tyr Met Phe Met Arg Ala Val Asn Thr Ala Lys Lys Ser
180 185 190

Arg Leu Thr Asp Val Thr Leu
195

<210> 20
<211> 160
<212> PRT
<213> Homo sapiens

<400> 20
Arg Glu Ala Ala Pro Lys Ser Tyr Ala Ile Arg Asp Ser Arg Gln Met

1 5 10 15

Val Trp Val Leu Ser Gly Asn Ser Leu Ile Ala Ala Pro Leu Ser Arg
20 25 30

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

Phe Ser Asp Lys Glu Lys Gly Asn Met Val Tyr Leu Gly Ile Lys Gly
50 55 60

Lys Asp Leu Cys Leu Phe Cys Ala Glu Ile Gln Gly Lys Pro Thr Leu
65 70 75 80

Gln Leu Lys Leu Gln Gly Ser Gln Asp Asn Ile Gly Lys Asp Thr Cys
85 90 95

Trp Lys Leu Val Gly Ile His Thr Cys Ile Asn Leu Asp Val Arg Glu
100 105 110

Ser Cys Phe Met Gly Thr Leu Asp Gln Trp Gly Ile Gly Val Gly Arg
115 120 125

Lys Lys Trp Lys Ser Ser Phe Gln His His His Leu Arg Lys Lys Asp
130 135 140

Lys Asp Phe Ser Ser Met Arg Thr Asn Ile Gly Met Pro Gly Arg Met
145 150 155 160

<210> 21
<211> 152
<212> PRT
<213> Homo sapiens

<400> 21

Ser Met Cys Lys Pro Ile Thr Gly Thr Ile Asn Asp Leu Asn Gln Gln
1 5 10 15

Val Trp Thr Leu Gln Gly Gln Asn Leu Val Ala Val Pro Arg Ser Asp
20 25 30

Ser Val Thr Pro Val Thr Val Ala Val Ile Thr Cys Lys Tyr Pro Glu
35 40 45

Ala Leu Glu Gln Gly Arg Gly Asp Pro Ile Tyr Leu Gly Ile Gln Asn
50 55 60

Pro Glu Met Cys Leu Tyr Cys Glu Lys Val Gly Glu Gln Pro Thr Leu
65 70 75 80

Gln Leu Lys Glu Gln Lys Ile Met Asp Leu Tyr Gly Gln Pro Glu Pro
85 90 95

Val Lys Pro Phe Leu Phe Tyr Arg Ala Lys Thr Gly Arg Thr Ser Thr
100 105 110

Leu Glu Ser Val Ala Phe Pro Asp Trp Phe Ile Ala Ser Ser Lys Arg
115 120 125

Asp Gln Pro Ile Ile Leu Thr Ser Glu Leu Gly Lys Ser Tyr Asn Thr
130 135 140

Ala Phe Glu Leu Asn Ile Asn Asp
145 150

<210> 22
<211> 81
<212> DNA
<213> Homo sapiens

<400> 22
ttttgggtgc tggtggtggt tggtggagtc ctggcttgct atagcttgct agtaacagtg
60

g c c t t t a t t a t t t t c t g g g t g
81

<210> 23
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 23
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> 24
<211> 20
<212> PRT
<213> Mus musculus

<400> 24
Met Tyr Ser Met Gln Leu Ala Ser Cys Val Thr Leu Thr Leu Val Leu
1 5 10 15

Leu Val Asn Ser
20

<210> 25
<211> 20
<212> PRT
<213> Homo sapiens

<400> 25
Met Glu Thr Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly
20

<210> 26
<211> 20
<212> PRT
<213> Mus musculus

<400> 26
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly
20

<210> 27
<211> 21
<212> PRT
<213> Homo sapiens

<400> 27
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro
20

<210> 28
<211> 16
<212> PRT
<213> Homo sapiens

<400> 28
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Ser Ser Ala Tyr Ser
1 5 10 15

<210> 29
<211> 30

<212> PRT
<213> Homo sapiens

<400> 29
Met Asp Ser Lys Gly Ser Ser Gln Lys Gly Ser Arg Leu Leu Leu Leu
1 5 10 15

Leu Val Val Ser Asn Leu Leu Leu Cys Gln Gly Val Val Ser
20 25 30

<210> 30
<211> 152
<212> PRT
<213> Mus musculus

<400> 30
Gly Arg Glu Thr Pro Asp Phe Gly Glu Val Phe Asp Leu Asp Gln Gln
1 5 10 15

Val Trp Ile Phe Arg Asn Gln Ala Leu Val Thr Val Pro Arg Ser His
20 25 30

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

Ser Leu Glu Gln Asp Lys Gly Ile Ala Ile Tyr Leu Gly Ile Gln Asn
50 55 60

Pro Asp Lys Cys Leu Phe Cys Lys Glu Val Asn Gly His Pro Thr Leu
65 70 75 80

Leu Leu Lys Glu Glu Lys Ile Leu Asp Leu Tyr His His Pro Glu Pro
85 90 95

Met Lys Pro Phe Leu Phe Tyr His Thr Arg Thr Gly Gly Thr Ser Thr
100 105 110

Phe Glu Ser Val Ala Phe Pro Gly His Tyr Ile Ala Ser Ser Lys Thr
        115   120   125

Gly Asn Pro Ile Phe Leu Thr Ser Lys Lys Gly Glu Tyr Tyr Asn Ile
    130   135   140

Asn Phe Asn Leu Asp Ile Lys Ser
145   150

<210> 31
<211> 153
<212> PRT
<213> Mus musculus

<400> 31
Ser Ser Gln Ser Pro Arg Asn Tyr Arg Val His Asp Ser Gln Gln Met
1   5   10   15

Val Trp Val Leu Thr Gly Asn Thr Leu Thr Ala Val Pro Ala Ser Asn
            20   25   30

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

Phe Gln Asp Val Lys Lys Gly Asn Leu Val Phe Leu Gly Ile Lys Asn
    50   55   60

Arg Asn Leu Cys Phe Cys Cys Val Glu Met Glu Gly Lys Pro Thr Leu
65   70   75   80

Gln Leu Lys Glu Val Asp Ile Met Asn Leu Tyr Lys Glu Arg Lys Ala
                85   90   95

Gln Lys Ala Phe Leu Phe Tyr His Gly Ile Glu Gly Ser Thr Ser Val
            100   105   110

Phe Gln Ser Val Leu Tyr Pro Gly Trp Phe Ile Ala Thr Ser Ser Ile
115 120 125

Glu Arg Gln Thr Ile Ile Leu Thr His Gln Arg Gly Lys Leu Val Asn
130 135 140

Thr Asn Phe Tyr Ile Glu Ser Glu Lys
145 150

<210> 32
<211> 152
<212> PRT
<213> Mus musculus

<400> 32
Gly Arg Glu Thr Pro Asp Phe Gly Glu Val Phe Asp Leu Asp Gln Gln
1 5 10 15

Val Trp Ile Phe Arg Asn Gln Ala Leu Val Thr Val Pro Arg Ser His
20 25 30

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

Ser Leu Glu Gln Asp Lys Gly Ile Ala Ile Tyr Leu Gly Ile Gln Asn
50 55 60

Pro Asp Lys Cys Leu Phe Cys Lys Glu Val Asn Gly His Pro Thr Leu
65 70 75 80

Leu Leu Lys Glu Glu Lys Ile Leu Asp Leu Tyr His His Pro Glu Pro
85 90 95

Met Lys Pro Phe Leu Phe Tyr His Thr Arg Thr Gly Gly Thr Ser Thr

100 105 110

Phe Glu Ser Val Ala Phe Pro Gly His Tyr Ile Ala Ser Ser Lys Thr
115 120 125

Gly Asn Pro Ile Phe Leu Thr Ser Lys Lys Gly Glu Tyr Tyr Asn Ile
130 135 140

Asn Phe Asn Leu Asp Ile Lys Ser
145 150

<210> 33
<211> 442
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 33
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Glu Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro
20 25 30

Gly Ser Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser
35 40 45

Ser Tyr Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu
50 55 60

Trp Ile Gly Gln Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly
65 70 75 80

Lys Phe Lys Gly Gln Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr
85 90 95

Ala Tyr Met Gln Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr
100 105 110

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

Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly
130 135 140

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr
145 150 155 160

Gln Ser Pro Lys Phe Met Ser Thr Ser Val Gly Asp Arg Val Ser Val
165 170 175

Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn Val Ala Trp Tyr Gln
180 185 190

Gln Lys Pro Gly Gln Ser Pro Lys Pro Leu Ile Tyr Ser Ala Thr Tyr
195 200 205

Arg Asn Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
210 215 220

Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser Lys Asp Leu Ala Asp
225 230 235 240

Tyr Phe Cys Gln Gln Tyr Asn Arg Tyr Pro Tyr Thr Ser Gly Gly Gly
245 250 255

Thr Lys Leu Glu Ile Lys Arg Ala Ala Ala Ile Glu Phe Met Tyr Pro

260 265 270

Pro Pro Tyr Leu Asp Asn Glu Arg Ser Asn Gly Thr Ile Ile His Ile
275 280 285

Lys Glu Lys His Leu Cys His Thr Gln Ser Ser Pro Lys Leu Phe Trp
290 295 300

Ala Leu Val Val Val Ala Gly Val Leu Phe Cys Tyr Gly Leu Leu Val
305 310 315 320

Thr Val Ala Leu Cys Val Ile Trp Thr Arg Ala Lys Phe Ser Arg Ser
325 330 335

Ala Glu Thr Ala Ala Asn Leu Gln Asp Pro Asn Gln Leu Tyr Asn Glu
340 345 350

Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Glu Lys Lys Arg
355 360 365

Ala Arg Asp Pro Glu Met Gly Gly Lys Gln Gln Arg Arg Arg Asn Pro
370 375 380

Gln Glu Gly Val Tyr Asn Ala Leu Gln Lys Asp Lys Met Ala Glu Ala
385 390 395 400

Tyr Ser Glu Ile Gly Thr Lys Gly Glu Arg Arg Arg Gly Lys Gly His
405 410 415

Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
420 425 430

Ala Leu His Met Gln Thr Leu Ala Pro Arg
435 440

<210> 34
<211> 1329
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 34
atggctctcc cagtgactgc cctactgctt cccctagcgc ttctcctgca tgcagaggtg
60

aagctgcagc agtctggggc tgagctggtg aggcctgggt cctcagtgaa gatttcctgc
120

aaggcttctg gctatgcatt cagtagctac tggatgaact gggtgaagca gaggcctgga
180

cagggtcttg agtggattgg acagatttat cctggagatg gtgatactaa ctacaatgga
240

aagttcaagg gtcaagccac actgactgca gacaaatcct ccagcacagc ctacatgcag
300

ctcagcggcc taacatctga ggactctgcg gtctatttct gtgcaagaaa gaccattagt
360

tcggtagtag atttctactt tgactactgg ggccaaggga ccacggtcac cgtctcctca
420

ggtggaggtg gatcaggtgg aggtggatct ggtggaggtg gatctgacat tgagctcacc
480

cagtctccaa aattcatgtc cacatcagta ggagacaggg tcagcgtcac ctgcaaggcc
540

agtcagaatg tgggtactaa tgtagcctgg tatcaacaga aaccaggaca atctcctaaa
600

ccactgattt actcggcaac ctaccggaac agtggagtcc ctgatcgctt cacaggcagt
660

ggatctggga cagatttcac tctcaccatc actaacgtgc agtctaaaga cttggcagac
720

tatttctgtc aacaatataa caggtatccg tacacgtccg gaggggggac caagctggag
780

atcaaacggg cggccgcaat tgagttcatg taccctccgc cttacctaga caacgagagg
840

agcaatggaa ctattattca cataaaagag aaacatcttt gtcatactca gtcatctcct
900

aagctgtttt gggcactggt cgtggttgct ggagtcctgt tttgttatgg cttgctagtg
960

acagtggctc tttgtgttat ctggacaaga gcaaaattca gcaggagtgc agagactgct
1020

gccaacctgc aggaccccaa ccagctctac aatgagctca atctagggcg aagagaggaa
1080

tatgacgtct tggagaagaa gcgggctcgg gatccagaga tgggaggcaa acagcagagg
1140

aggaggaacc cccaggaagg cgtatacaat gcactgcaga aagacaagat ggcagaagcc
1200

tacagtgaga tcggcacaaa aggcgagagg cggagaggca aggggcacga tggcctttac
1260

cagggtctca gcactgccac caaggacacc tatgatgccc tgcatatgca gaccctggcc
1320

c c t c g c t a a
1329

<210> 35
<211> 444
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 35

```
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                  10                  15

His Ala Glu Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro
            20                  25                  30

Gly Ser Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser
        35                  40                  45

Ser Tyr Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu
    50                  55                  60

Trp Ile Gly Gln Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly
65                  70                  75                  80

Lys Phe Lys Gly Gln Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr
                85                  90                  95

Ala Tyr Met Gln Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr
            100                 105                 110

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

Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly
    130                 135                 140

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr
145                 150                 155                 160

Gln Ser Pro Lys Phe Met Ser Thr Ser Val Gly Asp Arg Val Ser Val
                165                 170                 175
```

Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn Val Ala Trp Tyr Gln
180 185 190

Gln Lys Pro Gly Gln Ser Pro Lys Pro Leu Ile Tyr Ser Ala Thr Tyr
195 200 205

Arg Asn Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
210 215 220

Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser Lys Asp Leu Ala Asp
225 230 235 240

Tyr Phe Cys Gln Gln Tyr Asn Arg Tyr Pro Tyr Thr Ser Gly Gly Gly
245 250 255

Thr Lys Leu Glu Ile Lys Arg Ala Ala Ala Ile Glu Val Met Tyr Pro
260 265 270

Pro Pro Tyr Leu Asp Asn Glu Lys Ser Asn Gly Thr Ile Ile His Val
275 280 285

Lys Gly Lys His Leu Cys Pro Ser Pro Leu Phe Pro Gly Pro Ser Lys
290 295 300

Pro Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser
305 310 315 320

Leu Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Val Lys Phe
325 330 335

Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu
340 345 350

Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp
355 360 365

Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys
370 375 380

Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala
385 390 395 400

Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys
405 410 415

Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr
420 425 430

Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
435 440

<210> 36
<211> 1335
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 36
atggctctcc cagtgactgc cctactgctt cccctagcgc ttctcctgca tgcagaggtg 60

aagctgcagc agtctggggc tgagctggtg aggcctgggt cctcagtgaa gatttcctgc 120

aaggcttctg gctatgcatt cagtagctac tggatgaact gggtgaagca gaggcctgga 180

cagggtcttg agtggattgg acagatttat cctggagatg gtgatactaa ctacaatgga 240

aagttcaagg gtcaagccac actgactgca gacaaatcct ccagcacagc ctacatgcag
300

ctcagcggcc taacatctga ggactctgcg gtctatttct gtgcaagaaa gaccattagt
360

tcggtagtag atttctactt tgactactgg ggccaaggga ccacggtcac cgtctcctca
420

ggtggaggtg gatcaggtgg aggtggatct ggtggaggtg gatctgacat tgagctcacc
480

cagtctccaa aattcatgtc cacatcagta ggagacaggg tcagcgtcac ctgcaaggcc
540

agtcagaatg tgggtactaa tgtagcctgg tatcaacaga aaccaggaca atctcctaaa
600

ccactgattt actcggcaac ctaccggaac agtggagtcc ctgatcgctt cacaggcagt
660

ggatctggga cagatttcac tctcaccatc actaacgtgc agtctaaaga cttggcagac
720

tatttctgtc aacaatataa caggtatccg tacacgtccg gagggggggac caagctggag
780

atcaaacggg cggccgcaat tgaagttatg tatcctcctc cttacctaga caatgagaag
840

agcaatggaa ccattatcca tgtgaaaggg aaacaccttt gtccaagtcc cctatttccc
900

ggaccttcta agccctttg ggtgctggtg gtggttggtg gagtcctggc ttgctatagc
960

ttgctagtaa cagtggcctt tattattttc tgggtgagag tgaagttcag caggagcgca
1020

gacgcccccg cgtaccagca gggccagaac cagctctata acgagctcaa tctaggacga
1080

agagaggagt acgatgtttt ggacaagaga cgtggccggg accctgagat ggggggaaag
1140

ccgagaagga agaaccctca ggaaggcctg tacaatgaac tgcagaaaga taagatggcg 1200

gaggcctaca gtgagattgg gatgaaaggc gagcgccgga ggggcaaggg gcacgatggc 1260

ctttaccagg gtctcagtac agccaccaag gacacctacg acgcccttca catgcaggcc 1320

c t g c c c c t c g c t a g 1335

<210> 37
<211> 483
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 37
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Glu Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro
20 25 30

Gly Ser Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser
35 40 45

Ser Tyr Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu
50 55 60

Trp Ile Gly Gln Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly
65 70 75 80

Lys Phe Lys Gly Gln Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr

85 90 95

Ala Tyr Met Gln Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr
100 105 110

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

Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly
130 135 140

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr
145 150 155 160

Gln Ser Pro Lys Phe Met Ser Thr Ser Val Gly Asp Arg Val Ser Val
165 170 175

Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn Val Ala Trp Tyr Gln
180 185 190

Gln Lys Pro Gly Gln Ser Pro Lys Pro Leu Ile Tyr Ser Ala Thr Tyr
195 200 205

Arg Asn Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
210 215 220

Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser Lys Asp Leu Ala Asp
225 230 235 240

Tyr Phe Cys Gln Gln Tyr Asn Arg Tyr Pro Tyr Thr Ser Gly Gly Gly
245 250 255

Thr Lys Leu Glu Ile Lys Arg Ala Ala Ala Ile Glu Phe Met Tyr Pro
260 265 270

Pro Pro Tyr Leu Asp Asn Glu Arg Ser Asn Gly Thr Ile Ile His Ile
275 280 285

Lys Glu Lys His Leu Cys His Thr Gln Ser Ser Pro Lys Leu Phe Trp
290 295 300

Ala Leu Val Val Val Ala Gly Val Leu Phe Cys Tyr Gly Leu Leu Val
305 310 315 320

Thr Val Ala Leu Cys Val Ile Trp Thr Asn Ser Arg Arg Asn Arg Leu
325 330 335

Leu Gln Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly Leu Thr
340 345 350

Arg Lys Pro Tyr Gln Pro Tyr Ala Pro Ala Arg Asp Phe Ala Ala Tyr
355 360 365

Arg Pro Arg Ala Lys Phe Ser Arg Ser Ala Glu Thr Ala Ala Asn Leu
370 375 380

Gln Asp Pro Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu
385 390 395 400

Glu Tyr Asp Val Leu Glu Lys Lys Arg Ala Arg Asp Pro Glu Met Gly
405 410 415

Gly Lys Gln Gln Arg Arg Arg Asn Pro Gln Glu Gly Val Tyr Asn Ala
420 425 430

Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Thr Lys
435 440 445

Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu
450 455 460

Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Thr Leu
465 470 475 480

Ala Pro Arg

<210> 38
<211> 1452
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 38
atggctctcc cagtgactgc cctactgctt cccctagcgc ttctcctgca tgcagaggtg 60

aagctgcagc agtctggggc tgagctggtg aggcctgggt cctcagtgaa gatttcctgc 120

aaggcttctg gctatgcatt cagtagctac tggatgaact gggtgaagca gaggcctgga 180

cagggtcttg agtggattgg acagatttat cctggagatg gtgatactaa ctacaatgga 240

aagttcaagg gtcaagccac actgactgca gacaaatcct ccagcacagc ctacatgcag 300

ctcagcggcc taacatctga ggactctgcg gtctatttct gtgcaagaaa gaccattagt 360

tcggtagtag atttctactt tgactactgg ggccaaggga ccacggtcac cgtctcctca 420

ggtggaggtg gatcaggtgg aggtggatct ggtggaggtg gatctgacat tgagctcacc 480

cagtctccaa aattcatgtc cacatcagta ggagacaggg tcagcgtcac ctgcaaggcc
540

agtcagaatg tgggtactaa tgtagcctgg tatcaacaga aaccaggaca atctcctaaa
600

ccactgattt actcggcaac ctaccggaac agtggagtcc ctgatcgctt cacaggcagt
660

ggatctggga cagatttcac tctcaccatc actaacgtgc agtctaaaga cttggcagac
720

tatttctgtc aacaatataa caggtatccg tacacgtccg gagggggggac caagctggag
780

atcaaacggg cggccgcaat tgagttcatg taccctccgc cttacctaga caacgagagg
840

agcaatggaa ctattattca cataaaagag aaacatcttt gtcatactca gtcatctcct
900

aagctgtttt gggcactggt cgtggttgct ggagtcctgt tttgttatgg cttgctagtg
960

acagtggctc tttgtgttat ctggacaaat agtagaagga acagactcct tcaaagtgac
1020

tacatgaaca tgactccccg gaggcctggg ctcactcgaa agccttacca gccctacgcc
1080

cctgccagag actttgcagc gtaccgcccc agagcaaaat tcagcaggag tgcagagact
1140

gctgccaacc tgcaggaccc caaccagctc tacaatgagc tcaatctagg gcgaagagag
1200

gaatatgacg tcttggagaa gaagcgggct cgggatccag agatgggagg caaacagcag
1260

aggaggagga acccccagga aggcgtatac aatgcactgc agaaagacaa gatggcagaa
1320

gcctacagtg agatcggcac aaaaggcgag aggcggagag gcaaggggca cgatggcctt
1380

taccagggtc tcagcactgc caccaaggac acctatgatg ccctgcatat gcagaccctg 1440

g c c c c t c g c t g a 1452

<210> 39
<211> 485
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 39
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Glu Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro
20 25 30

Gly Ser Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser
35 40 45

Ser Tyr Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu
50 55 60

Trp Ile Gly Gln Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly
65 70 75 80

Lys Phe Lys Gly Gln Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr
85 90 95

Ala Tyr Met Gln Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr
100 105 110

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

Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly
130 135 140

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr
145 150 155 160

Gln Ser Pro Lys Phe Met Ser Thr Ser Val Gly Asp Arg Val Ser Val
165 170 175

Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn Val Ala Trp Tyr Gln
180 185 190

Gln Lys Pro Gly Gln Ser Pro Lys Pro Leu Ile Tyr Ser Ala Thr Tyr
195 200 205

Arg Asn Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
210 215 220

Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser Lys Asp Leu Ala Asp
225 230 235 240

Tyr Phe Cys Gln Gln Tyr Asn Arg Tyr Pro Tyr Thr Ser Gly Gly Gly
245 250 255

Thr Lys Leu Glu Ile Lys Arg Ala Ala Ala Ile Glu Val Met Tyr Pro
260 265 270

Pro Pro Tyr Leu Asp Asn Glu Lys Ser Asn Gly Thr Ile Ile His Val
275 280 285

Lys Gly Lys His Leu Cys Pro Ser Pro Leu Phe Pro Gly Pro Ser Lys
290 295 300

Pro Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser
305 310 315 320

Leu Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg
325 330 335

Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro
340 345 350

Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe
355 360 365

Ala Ala Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro
370 375 380

Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly
385 390 395 400

Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro
405 410 415

Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr
420 425 430

Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly
435 440 445

Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln
450 455 460

Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln

465 470 475 480

Ala Leu Pro Pro Arg
485

<210> 40
<211> 1458
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 40
atggctctcc cagtgactgc cctactgctt cccctagcgc ttctcctgca tgcagaggtg 60

aagctgcagc agtctggggc tgagctggtg aggcctgggt cctcagtgaa gatttcctgc 120

aaggcttctg gctatgcatt cagtagctac tggatgaact gggtgaagca gaggcctgga 180

cagggtcttg agtggattgg acagatttat cctggagatg gtgatactaa ctacaatgga 240

aagttcaagg gtcaagccac actgactgca gacaaatcct ccagcacagc ctacatgcag 300

ctcagcggcc taacatctga ggactctgcg gtctatttct gtgcaagaaa gaccattagt 360

tcggtagtag atttctactt tgactactgg ggccaaggga ccacggtcac cgtctcctca 420

ggtggaggtg gatcaggtgg aggtggatct ggtggaggtg gatctgacat tgagctcacc 480

cagtctccaa aattcatgtc cacatcagta ggagacaggg tcagcgtcac ctgcaaggcc 540

agtcagaatg tgggtactaa tgtagcctgg tatcaacaga aaccaggaca atctcctaaa 600

ccactgattt actcggcaac ctaccggaac agtggagtcc ctgatcgctt cacaggcagt
660

ggatctggga cagatttcac tctcaccatc actaacgtgc agtctaaaga cttggcagac
720

tatttctgtc aacaatataa caggtatccg tacacgtccg gagggggac caagctggag
780

atcaaacggg cggccgcaat tgaagttatg tatcctcctc cttacctaga caatgagaag
840

agcaatggaa ccattatcca tgtgaaaggg aaacaccttt gtccaagtcc cctatttccc
900

ggaccttcta agccctttg ggtgctggtg gtggttggtg gagtcctggc ttgctatagc
960

ttgctagtaa cagtggcctt tattattttc tgggtgagga gtaagaggag caggctcctg
1020

cacagtgact acatgaacat gactccccgc cgccccgggc ccacccgcaa gcattaccag
1080

ccctatgccc caccacgcga cttcgcagcc tatcgctcca gagtgaagtt cagcaggagc
1140

gcagacgccc ccgcgtacca gcagggccag aaccagctct ataacgagct caatctagga
1200

cgaagagagg agtacgatgt tttggacaag agacgtggcc gggaccctga gatgggggga
1260

aagccgagaa ggaagaaccc tcaggaaggc ctgtacaatg aactgcagaa agataagatg
1320

gcggaggcct acagtgagat tgggatgaaa ggcgagcgcc ggaggggcaa ggggcacgat
1380

ggcctttacc agggtctcag tacagccacc aaggacacct acgacgccct tcacatgcag
1440

g c c c t g c c c c c t c g c t a g
1458

<210> 41
<211> 486
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 41
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Glu Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro
20 25 30

Gly Ser Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser
35 40 45

Ser Tyr Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu
50 55 60

Trp Ile Gly Gln Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly
65 70 75 80

Lys Phe Lys Gly Gln Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr
85 90 95

Ala Tyr Met Gln Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr
100 105 110

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

Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly

130 135 140

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr
145 150 155 160

Gln Ser Pro Lys Phe Met Ser Thr Ser Val Gly Asp Arg Val Ser Val
165 170 175

Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn Val Ala Trp Tyr Gln
180 185 190

Gln Lys Pro Gly Gln Ser Pro Lys Pro Leu Ile Tyr Ser Ala Thr Tyr
195 200 205

Arg Asn Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
210 215 220

Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser Lys Asp Leu Ala Asp
225 230 235 240

Tyr Phe Cys Gln Gln Tyr Asn Arg Tyr Pro Tyr Thr Ser Gly Gly Gly
245 250 255

Thr Lys Leu Glu Ile Lys Arg Ala Ala Ala Ile Glu Val Met Tyr Pro
260 265 270

Pro Pro Tyr Leu Asp Asn Glu Lys Ser Asn Gly Thr Ile Ile His Val
275 280 285

Lys Gly Lys His Leu Cys Pro Ser Pro Leu Phe Pro Gly Pro Ser Lys
290 295 300

Pro Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser
305 310 315 320

Leu Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Lys Arg Gly Arg
325 330 335

Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln
340 345 350

Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu
355 360 365

Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala
370 375 380

Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu
385 390 395 400

Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp
405 410 415

Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu
420 425 430

Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile
435 440 445

Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr
450 455 460

Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met
465 470 475 480

Gln Ala Leu Pro Pro Arg
485

<210> 42
<211> 1461
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 42
atggctctcc cagtgactgc cctactgctt cccctagcgc ttctcctgca tgcagaggtg 60

aagctgcagc agtctggggc tgagctggtg aggcctgggt cctcagtgaa gatttcctgc 120

aaggcttctg gctatgcatt cagtagctac tggatgaact gggtgaagca gaggcctgga 180

cagggtcttg agtggattgg acagatttat cctggagatg gtgatactaa ctacaatgga 240

aagttcaagg gtcaagccac actgactgca gacaaatcct ccagcacagc ctacatgcag 300

ctcagcggcc taacatctga ggactctgcg gtctatttct gtgcaagaaa gaccattagt 360

tcggtagtag atttctactt tgactactgg ggccaaggga ccacggtcac cgtctcctca 420

ggtggaggtg gatcaggtgg aggtggatct ggtggaggtg gatctgacat tgagctcacc 480

cagtctccaa aattcatgtc cacatcagta ggagacaggg tcagcgtcac ctgcaaggcc 540

agtcagaatg tgggtactaa tgtagcctgg tatcaacaga aaccaggaca atctcctaaa 600

ccactgattt actcggcaac ctaccggaac agtggagtcc ctgatcgctt cacaggcagt 660

ggatctggga cagatttcac tctcaccatc actaacgtgc agtctaaaga cttggcagac 720

tatttctgtc aacaatataa caggtatccg tacacgtccg gaggggggac caagctggag
780

atcaaacggg cggccgcaat tgaagttatg tatcctcctc cttacctaga caatgagaag
840

agcaatggaa ccattatcca tgtgaaaggg aaacaccttt gtccaagtcc cctatttccc
900

ggaccttcta agccctttg ggtgctggtg gtggttggtg gagtcctggc ttgctatagc
960

ttgctagtaa cagtggcctt tattattttc tgggtgaaac ggggcagaaa gaaactcctg
1020

tatatattca aacaaccatt tatgagacca gtacaaacta ctcaagagga agatggctgt
1080

agctgccgat ttccagaaga agaagaagga ggatgtgaac tgagagtgaa gttcagcagg
1140

agcgcagacg cccccgcgta ccagcagggc cagaaccagc tctataacga gctcaatcta
1200

ggacgaagag aggagtacga tgttttggac aagagacgtg gccgggaccc tgagatgggg
1260

ggaaagccga gaaggaagaa ccctcaggaa ggcctgtaca atgaactgca gaaagataag
1320

atggcggagg cctacagtga gattgggatg aaaggcgagc gccggagggg caaggggcac
1380

gatggccttt accagggtct cagtacagcc accaaggaca cctacgacgc ccttcacatg
1440

c a g g c c c t g c c c c c t c g c t a g
1461

<210> 43
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 43
Phe Tyr Tyr Met His
1 5

<210> 44
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 44
Arg Ile Asp Pro Glu Asp Glu Ser Thr Lys Tyr Ser Glu Lys Phe Lys
1 5 10 15

Asn

<210> 45
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 45
Gly Gly Tyr Tyr Phe Asp Tyr
1 5

<210> 46
<211> 11
<212> PRT

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 46
Gln Ala Ser Glu Asp Ile Tyr Ser Gly Leu Ala
1 5 10

<210> 47
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 47
Gly Ala Ser Asp Leu Gln Asp
1 5

<210> 48
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 48
Gln Gln Gly Leu Thr Tyr Pro Arg Thr
1 5

<210> 49
<211> 116
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 49

Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Thr
1				5				10						15	

Ser	Val	Lys	Leu	Ser	Cys	Lys	Val	Ser	Gly	Asp	Thr	Ile	Thr	Phe	Tyr
			20					25					30		

Tyr	Met	His	Phe	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
		35					40					45			

Gly	Arg	Ile	Asp	Pro	Glu	Asp	Glu	Ser	Thr	Lys	Tyr	Ser	Glu	Lys	Phe
	50					55					60				

Lys	Asn	Lys	Ala	Thr	Leu	Thr	Ala	Asp	Thr	Ser	Ser	Asn	Thr	Ala	Tyr
65					70					75					80

Leu	Lys	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Thr	Ala	Thr	Tyr	Phe	Cys
				85					90					95	

Ile	Tyr	Gly	Gly	Tyr	Tyr	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Val	Met	Val
			100					105					110		

Thr	Val	Ser	Ser
		115	

<210> 50
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 50
Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Thr Ser Leu Gly
1 5 10 15

Glu Thr Val Thr Ile Gln Cys Gln Ala Ser Glu Asp Ile Tyr Ser Gly
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Gln Leu Leu Ile
35 40 45

Tyr Gly Ala Ser Asp Leu Gln Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Thr Ser Met Gln Thr
65 70 75 80

Glu Asp Glu Gly Val Tyr Phe Cys Gln Gln Gly Leu Thr Tyr Pro Arg
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu Lys Arg
100 105

<210> 51
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 51
Gly Tyr Ala Phe Ser Ser
1 5

<210> 52
<211> 6

<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 52
Tyr Pro Gly Asp Gly Asp
1 5

<210> 53
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 53
Lys Thr Ile Ser Ser Val Val Asp Phe
1 5

<210> 54
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 54
Asn Val Gly Thr Asn Val Ala
1 5

<210> 55
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 55
Ser Ala Thr Tyr Arg Asn
1 5

<210> 56
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 56
Phe Cys Gln Gln Tyr Asn Arg Tyr
1 5

<210> 57
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 57
Glu Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr
20 25 30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Gln Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Gln Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Lys Thr Ile Ser Ser Val Val Asp Phe Tyr Phe Asp Tyr Trp
100 105 110

Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 58
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 58
Asp Ile Glu Leu Thr Gln Ser Pro Lys Phe Met Ser Thr Ser Val Gly
1 5 10 15

Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Pro Leu Ile
35 40 45

Tyr Ser Ala Thr Tyr Arg Asn Ser Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser
65 70 75 80

Lys Asp Leu Ala Asp Tyr Phe Cys Gln Gln Tyr Asn Arg Tyr Pro Tyr
85 90 95

Thr Ser Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 59
<211> 266
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 59
Met Ala Ser Pro Leu Thr Arg Phe Leu Ser Leu Asn Leu Leu Leu Leu
1 5 10 15

Gly Glu Ser Ile Ile Leu Gly Ser Gly Glu Ala Glu Val Gln Leu Gln
20 25 30

Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Thr Ser Val Lys Leu Ser
35 40 45

Cys Lys Val Ser Gly Asp Thr Ile Thr Phe Tyr Tyr Met His Phe Val
50 55 60

Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Arg Ile Asp Pro
65 70 75 80

Glu Asp Glu Ser Thr Lys Tyr Ser Glu Lys Phe Lys Asn Lys Ala Thr
85 90 95

Leu Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr Leu Lys Leu Ser Ser
100 105 110

Leu Thr Ser Glu Asp Thr Ala Thr Tyr Phe Cys Ile Tyr Gly Gly Tyr
115 120 125

Tyr Phe Asp Tyr Trp Gly Gln Gly Val Met Val Thr Val Ser Ser Gly
130 135 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile
145 150 155 160

Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Thr Ser Leu Gly Glu Thr
165 170 175

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

Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Gln Leu Leu Ile Tyr Gly
195 200 205

Ala Ser Asp Leu Gln Asp Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
210 215 220

Ser Gly Thr Gln Tyr Ser Leu Lys Ile Thr Ser Met Gln Thr Glu Asp
225 230 235 240

Glu Gly Val Tyr Phe Cys Gln Gln Gly Leu Thr Tyr Pro Arg Thr Phe
245 250 255

Gly Gly Gly Thr Lys Leu Glu Leu Lys Arg
260 265

<210> 60
<211> 798
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 60
atggcctcac cgttgacccg ctttctgtcg ctgaacctgc tgctgctggg tgagtcgatt 60

atcctgggga gtggagaagc tgaagtccag ctgcagcagt ctggggctga gcttgtgaga 120

cctgggacct ctgtgaagtt atcttgcaaa gtttctggcg ataccattac attttactac 180

atgcactttg tgaagcaaag gcctggacag ggtctggaat ggataggaag gattgatcct 240

gaggatgaaa gtactaaata ttctgagaag ttcaaaaaca aggcgacact cactgcagat 300

acatcttcca acacagccta cctgaagctc agcagcctga cctctgagga cactgcaacc 360

tatttttgta tctacggagg atactacttt gattactggg gccaaggggt catggtcaca 420

gtctcctcag gtggaggtgg atcaggtgga ggtggatctg gtggaggtgg atctgacatc 480

cagatgacac agtctccagc ttccctgtct acatctctgg gagaaactgt caccatccaa 540

tgtcaagcaa gtgaggacat ttacagtggt ttagcgtggt atcagcagaa gccagggaaa 600

tctcctcagc tcctgatcta tggtgcaagt gacttacaag acggcgtccc atcacgattc 660

agtggcagtg gatctggcac acagtattct ctcaagatca ccagcatgca aactgaagat 720

gaaggggttt atttctgtca acagggttta acgtatcctc ggacgttcgg tggcggcacc
780

a a g c t g g a a t t g a a a c g g
798

<210> 61
<211> 269
<212> PRT
<213> Mus musculus

<400> 61
Arg Pro Gln Lys Ser Leu Leu Val Glu Val Glu Glu Gly Gly Asn Val
1 5 10 15

Val Leu Pro Cys Leu Pro Asp Ser Ser Pro Val Ser Ser Glu Lys Leu
20 25 30

Ala Trp Tyr Arg Gly Asn Gln Ser Thr Pro Phe Leu Glu Leu Ser Pro
35 40 45

Gly Ser Pro Gly Leu Gly Leu His Val Gly Ser Leu Gly Ile Leu Leu
50 55 60

Val Ile Val Asn Val Ser Asp His Met Gly Gly Phe Tyr Leu Cys Gln
65 70 75 80

Lys Arg Pro Pro Phe Lys Asp Ile Trp Gln Pro Ala Trp Thr Val Asn
85 90 95

Val Glu Asp Ser Gly Glu Met Phe Arg Trp Asn Ala Ser Asp Val Arg
100 105 110

Asp Leu Asp Cys Asp Leu Arg Asn Arg Ser Ser Gly Ser His Arg Ser
115 120 125

Thr Ser Gly Ser Gln Leu Tyr Val Trp Ala Lys Asp His Pro Lys Val
130 135 140

Trp Gly Thr Lys Pro Val Cys Ala Pro Arg Gly Ser Ser Leu Asn Gln
145 150 155 160

Ser Leu Ile Asn Gln Asp Leu Thr Val Ala Pro Gly Ser Thr Leu Trp
165 170 175

Leu Ser Cys Gly Val Pro Pro Val Pro Val Ala Lys Gly Ser Ile Ser
180 185 190

Trp Thr His Val His Pro Arg Arg Pro Asn Val Ser Leu Leu Ser Leu
195 200 205

Ser Leu Gly Gly Glu His Pro Val Arg Glu Met Trp Val Trp Gly Ser
210 215 220

Leu Leu Leu Leu Pro Gln Ala Thr Ala Leu Asp Glu Gly Thr Tyr Tyr
225 230 235 240

Cys Leu Arg Gly Asn Leu Thr Ile Glu Arg His Val Lys Val Ile Ala
245 250 255

Arg Ser Ala Val Trp Leu Trp Leu Leu Arg Thr Gly Gly
260 265

<210> 62
<211> 272
<212> PRT
<213> Homo sapiens

<400> 62
Pro Glu Glu Pro Leu Val Val Lys Val Glu Glu Gly Asp Asn Ala Val
1 5 10 15

Leu Gln Cys Leu Lys Gly Thr Ser Asp Gly Pro Thr Gln Gln Leu Thr
20 25 30

Trp Ser Arg Glu Ser Pro Leu Lys Pro Phe Leu Lys Leu Ser Leu Gly
35 40 45

Leu Pro Gly Leu Gly Ile His Met Arg Pro Leu Ala Ile Trp Leu Phe
50 55 60

Ile Phe Asn Val Ser Gln Gln Met Gly Gly Phe Tyr Leu Cys Gln Pro
65 70 75 80

Gly Pro Pro Ser Glu Lys Ala Trp Gln Pro Gly Trp Thr Val Asn Val
85 90 95

Glu Gly Ser Gly Glu Leu Phe Arg Trp Asn Val Ser Asp Leu Gly Gly
100 105 110

Leu Gly Cys Gly Leu Lys Asn Arg Ser Ser Glu Gly Pro Ser Ser Pro
115 120 125

Ser Gly Lys Leu Met Ser Pro Lys Leu Tyr Val Trp Ala Lys Asp Arg
130 135 140

Pro Glu Ile Trp Glu Gly Glu Pro Pro Cys Leu Pro Pro Arg Asp Ser
145 150 155 160

Leu Asn Gln Ser Leu Ser Gln Asp Leu Thr Met Ala Pro Gly Ser Thr
165 170 175

Leu Trp Leu Ser Cys Gly Val Pro Pro Asp Ser Val Ser Arg Gly Pro
180 185 190

Leu Ser Trp Thr His Val His Pro Lys Gly Pro Lys Ser Leu Leu Ser
195 200 205

Leu Glu Leu Lys Asp Asp Arg Pro Ala Arg Asp Met Trp Val Met Glu
210 215 220

Thr Gly Leu Leu Leu Pro Arg Ala Thr Ala Gln Asp Ala Gly Lys Tyr
225 230 235 240

Tyr Cys His Arg Gly Asn Leu Thr Met Ser Phe His Leu Glu Ile Thr
245 250 255

Ala Arg Pro Val Leu Trp His Trp Leu Leu Arg Thr Gly Gly Trp Lys
260 265 270

<210> 63
<211> 263
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 63
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Glu Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro
20 25 30

Gly Ser Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser
35 40 45

Ser Tyr Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu
50 55 60

Trp Ile Gly Gln Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly
65 70 75 80

Lys Phe Lys Gly Gln Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr
85 90 95

Ala Tyr Met Gln Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr
100 105 110

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

Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly
130 135 140

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr
145 150 155 160

Gln Ser Pro Lys Phe Met Ser Thr Ser Val Gly Asp Arg Val Ser Val
165 170 175

Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn Val Ala Trp Tyr Gln
180 185 190

Gln Lys Pro Gly Gln Ser Pro Lys Pro Leu Ile Tyr Ser Ala Thr Tyr
195 200 205

Arg Asn Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
210 215 220

Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser Lys Asp Leu Ala Asp
225 230 235 240

Tyr Phe Cys Gln Gln Tyr Asn Arg Tyr Pro Tyr Thr Ser Gly Gly Gly
245 250 255

Thr Lys Leu Glu Ile Lys Arg
260

<210> 64
<211> 789
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 64
atggctctcc cagtgactgc cctactgctt cccctagcgc ttctcctgca tgcagaggtg 60

aagctgcagc agtctggggc tgagctggtg aggcctgggt cctcagtgaa gatttcctgc 120

aaggcttctg gctatgcatt cagtagctac tggatgaact gggtgaagca gaggcctgga 180

cagggtcttg agtggattgg acagatttat cctggagatg gtgatactaa ctacaatgga 240

aagttcaagg gtcaagccac actgactgca gacaaatcct ccagcacagc ctacatgcag 300

ctcagcggcc taacatctga ggactctgcg gtctatttct gtgcaagaaa gaccattagt 360

tcggtagtag atttctactt tgactactgg ggccaaggga ccacggtcac cgtctcctca 420

ggtggaggtg gatcaggtgg aggtggatct ggtggaggtg gatctgacat tgagctcacc 480

cagtctccaa aattcatgtc cacatcagta ggagacaggg tcagcgtcac ctgcaaggcc 540

agtcagaatg tgggtactaa tgtagcctgg tatcaacaga aaccaggaca atctcctaaa 600

ccactgattt actcggcaac ctaccggaac agtggagtcc ctgatcgctt cacaggcagt 660

ggatctggga cagatttcac tctcaccatc actaacgtgc agtctaaaga cttggcagac 720

tatttctgtc aacaatataa caggtatccg tacacgtccg gagggggggac caagctggag 780

a t c a a a c g g 789

<210> 65
<211> 1368
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 65
atggcctcac cgttgacccg ctttctgtcg ctgaacctgc tgctgctggg tgagtcgatt 60

atcctgggga gtggagaagc tgaagtccag ctgcagcagt ctggggctga gcttgtgaga 120

cctgggacct ctgtgaagtt atcttgcaaa gtttctggcg ataccattac attttactac 180

atgcactttg tgaagcaaag gcctggacag ggtctggaat ggataggaag gattgatcct 240

gaggatgaaa gtactaaata ttctgagaag ttcaaaaaca aggcgacact cactgcagat 300

acatcttcca acacagccta cctgaagctc agcagcctga cctctgagga cactgcaacc 360

tatttttgta tctacggagg atactacttt gattactggg gccaaggggt catggtcaca
420

gtctcctcag gtggaggtgg atcaggtgga ggtggatctg gtggaggtgg atctgacatc
480

cagatgacac agtctccagc ttccctgtct acatctctgg gagaaactgt caccatccaa
540

tgtcaagcaa gtgaggacat ttacagtggt ttagcgtggt atcagcagaa gccagggaaa
600

tctcctcagc tcctgatcta tggtgcaagt gacttacaag acggcgtccc atcacgattc
660

agtggcagtg gatctggcac acagtattct ctcaagatca ccagcatgca aactgaagat
720

gaaggggttt atttctgtca acagggttta acgtatcctc ggacgttcgg tggcggcacc
780

aagctggaat tgaaacgggc ggccgcagaa cagaaactga tctctgaaga agacctgatt
840

gagttcatgt accctccgcc ttacctagac aacgagagga gcaatggaac tattattcac
900

ataaaagaga aacatctttg tcatactcag tcatctccta agctgttttg ggcactggtc
960

gtggttgctg gagtcctgtt ttgttatggc ttgctagtga cagtggctct ttgtgttatc
1020

tggacaagag caaaattcag caggagtgca gagactgctg ccaacctgca ggaccccaac
1080

cagctctaca atgagctcaa tctagggcga agagaggaat atgacgtctt ggagaagaag
1140

cgggctcggg atccagagat gggaggcaaa cagcagagga ggaggaaccc ccaggaaggc
1200

gtatacaatg cactgcagaa agacaagatg gcagaagcct acagtgagat cggcacaaaa
1260

ggcgagaggc ggagaggcaa ggggcacgat ggcctttacc agggtctcag cactgccacc 1320

aaggacacct atgatgccct gcatatgcag accctggccc ctcgctaa 1368

<210> 66
<211> 42
<212> PRT
<213> Homo sapiens

<400> 66
Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met
1 5 10 15

Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe
20 25 30

Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
35 40

<210> 67
<211> 126
<212> DNA
<213> Homo sapiens

<400> 67
aaacggggca gaaagaaact cctgtatata ttcaaacaac catttatgag accagtacaa 60

actactcaag aggaagatgg ctgtagctgc cgatttccag aagaagaaga aggaggatgt 120

g a a c t g 126

<210> 68
<211> 41
<212> PRT
<213> Mus musculus

<400> 68
Asn Ser Arg Arg Asn Arg Leu Leu Gln Ser Asp Tyr Met Asn Met Thr
1 5 10 15

Pro Arg Arg Pro Gly Leu Thr Arg Lys Pro Tyr Gln Pro Tyr Ala Pro
20 25 30

Ala Arg Asp Phe Ala Ala Tyr Arg Pro
35 40

<210> 69
<211> 123
<212> DNA
<213> Mus musculus

<400> 69
aatagtagaa ggaacagact ccttcaaagt gactacatga acatgactcc ccggaggcct
60

gggctcactc gaaagcctta ccagccctac gcccctgcca gagactttgc agcgtaccgc
120

c c c
123

<210> 70
<211> 41
<212> PRT
<213> Homo sapiens

<400> 70
Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr
1 5 10 15

Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro
20 25 30

Pro Arg Asp Phe Ala Ala Tyr Arg Ser

35 40

<210> 71
<211> 123
<212> DNA
<213> Homo sapiens

<400> 71
aggagtaaga ggagcaggct cctgcacagt gactacatga acatgactcc ccgccgcccc
60

gggcccaccc gcaagcatta ccagccctat gccccaccac gcgacttcgc agcctatcgc
120

t c c
123

<210> 72
<211> 113
<212> PRT
<213> Mus musculus

<400> 72
Arg Ala Lys Phe Ser Arg Ser Ala Glu Thr Ala Ala Asn Leu Gln Asp
1 5 10 15

Pro Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
20 25 30

Asp Val Leu Glu Lys Lys Arg Ala Arg Asp Pro Glu Met Gly Gly Lys
35 40 45

Gln Gln Arg Arg Arg Asn Pro Gln Glu Gly Val Tyr Asn Ala Leu Gln
50 55 60

Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Thr Lys Gly Glu
65 70 75 80

Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr
85 90 95

Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Thr Leu Ala Pro
100 105 110

Arg

<210> 73
<211> 339
<212> DNA
<213> Mus musculus

<400> 73
agagcaaaat tcagcaggag tgcagagact gctgccaacc tgcaggaccc caaccagctc 60

tacaatgagc tcaatctagg gcgaagagag gaatatgacg tcttggagaa gaagcgggct 120

cgggatccag agatgggagg caaacagcag aggaggagga acccccagga aggcgtatac 180

aatgcactgc agaaagacaa gatggcagaa gcctacagtg agatcggcac aaaaggcgag 240

aggcggagag gcaaggggca cgatggcctt taccagggtc tcagcactgc caccaaggac 300

acctatgatg ccctgcatat gcagaccctg gcccctcgc 339

<210> 74
<211> 112
<212> PRT
<213> Homo sapiens

<400> 74
Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly
1 5 10 15

Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
20 25 30

Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys
35 40 45

Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys
50 55 60

Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg
65 70 75 80

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

Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
100 105 110

<210> 75
<211> 336
<212> DNA
<213> Homo sapiens

<400> 75
agagtgaagt tcagcaggag cgcagacgcc cccgcgtacc agcagggcca gaaccagctc
60

tataacgagc tcaatctagg acgaagagag gagtacgatg ttttggacaa gagacgtggc
120

cgggaccctg agatgggggg aaagccgaga aggaagaacc ctcaggaagg cctgtacaat
180

gaactgcaga aagataagat ggcggaggcc tacagtgaga ttgggatgaa aggcgagcgc
240

cggaggggca aggggcacga tggcctttac cagggtctca gtacagccac caaggacacc
300

t a c g a c g c c c t t c a c a t g c a g g c c c t g c c c c c t c g c
336

<210> 76
<211> 27
<212> PRT
<213> Mus musculus

<400> 76
Phe Trp Ala Leu Val Val Val Ala Gly Val Leu Phe Cys Tyr Gly Leu
1 5 10 15

Leu Val Thr Val Ala Leu Cys Val Ile Trp Thr
            20 25

<210> 77
<211> 81
<212> DNA
<213> Mus musculus

<400> 77
ttttgggcac tggtcgtggt tgctggagtc ctgttttgtt atggcttgct agtgacagtg
60

g c t c t t t g t g t t a t c t g g a c a
81

<210> 78
<211> 27
<212> PRT
<213> Homo sapiens

<400> 78
Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
1 5 10 15

Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val
            20 25

<210> 79
<211> 81
<212> DNA
<213> Homo sapiens

<400> 79
ttttgggtgc tggtggtggt tggtggagtc ctggcttgct atagcttgct agtaacagtg
60

g c c t t t a t t a t t t t c t g g g t g
81

<210> 80
<211> 18
<212> PRT
<213> Homo sapiens

<400> 80
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala